*SOMATIC ASSOCIATION IN TRITICUM AESTIVUM**

By M. Feldman, T. Mello-Sampayo,† and E. R. Sears‡

DEPARTMENT OF FIELD CROPS, UNIVERSITY OF MISSOURI, COLUMBIA

Communicated August 29, 1966

A recent study[1] on the effect of chromosome 5B on meiotic pairing in common wheat, *Triticum aestivum* L., indicated that with the normal two doses of 5B, homologous chromosomes are associated before meiosis begins. Although such somatic association could conceivably be limited to the stage immediately preceding meiosis, a number of investigators[2-4] have maintained that homologues are associated throughout the life of the organism. The present work was undertaken to determine whether homologues in wheat are associated in dividing cells of root tips.

Material and Methods.—For the observations on somatic association, telocentric chromosomes were used. Having terminal centromeres, these chromosomes are easily distinguished from the normal chromosomes of *T. aestivum,* all of which have median or submedian centromeres (Fig. 1). Some plants had two doses of the same arm, and others had one dose each of two different arms. All telocentrics used were derived from wheat chromosomes except one, which consisted of the short arm of the hairy-neck chromosome of rye, and which is designated $5R^S$. The wheat variety used was "Chinese Spring."

Seeds were allowed to germinate at room temperature. When the seminal roots were 0.5–1.5-cm long, their tips were severed and immersed in tap water at 0°C for 18–24 hr to shorten the chromosomes and suppress spindle development.[5] Squashes were prepared by means of either the Feulgen or the aceto-carmine staining technique. Only those cells which were reasonably flat were used. Nearly all chromosome groups were approximately circular.

Distances between the mid-points of the two telocentrics concerned were measured with an ocular micrometer. To minimize differences due to degree of squashing of cells, each distance between telocentrics was divided by the distance between the two chromosomes farthest apart in the cell concerned. Throughout the paper this corrected distance will be referred to simply as the distance between telocentrics.

In order to determine whether or not two telocentrics were associated, it was necessary to know the frequencies with which two chromosomes located at random would lie at various distances from each other. By assuming that the chromosomes of a squashed cell are distributed uniformly in a circular area, it was possible to treat the two telocentrics as a random pair of points uniformly distributed throughout a circle. Following Hammersley[6] and Lord,[7] who extended the work of Deltheil,[8] the distance, X, between such a pair of points has the following probability density function for a circle of radius 0.50:

$$fX^{(x)} = \frac{16\chi}{\pi}\,[\cos^{-1}\chi - \chi\,(1 - \chi^2)^{1/2}].$$

It was only necessary to substitute for X a series of values between 0 and 1 to obtain the frequencies of cells in which the telocentrics lay at progressively greater distances from each other. When this was done (Fig. 2*A*), the mean value of X proved to be 0.4527, with a variance of 0.0451.

For comparison of the random distribution with that observed for various homologous and nonhomologous telocentrics, the frequencies at the different distances were accumulated. This permitted use of the Kolmogorov-Smirnov One-Sample or Two-Sample Tests of goodness of fit, which take into account differences in the shape of the distribution curve as well as in its mean.[9]

In one group of cells where there were three telocentric chromosomes, two of which were homologous and the other nonhomologous, a direct comparison could be made of distance between homologues with distance between nonhomologues. For this comparison the Wilcoxon Matched-Pairs Signed-Ranks Test[9] was used.

Results.—Nonhomologous telocentrics: In their distribution with respect to each other, neither pair of nonhomologous telocentrics ($6B^L$-$1D^L$ and $3B^L$-$5R^S$) differed

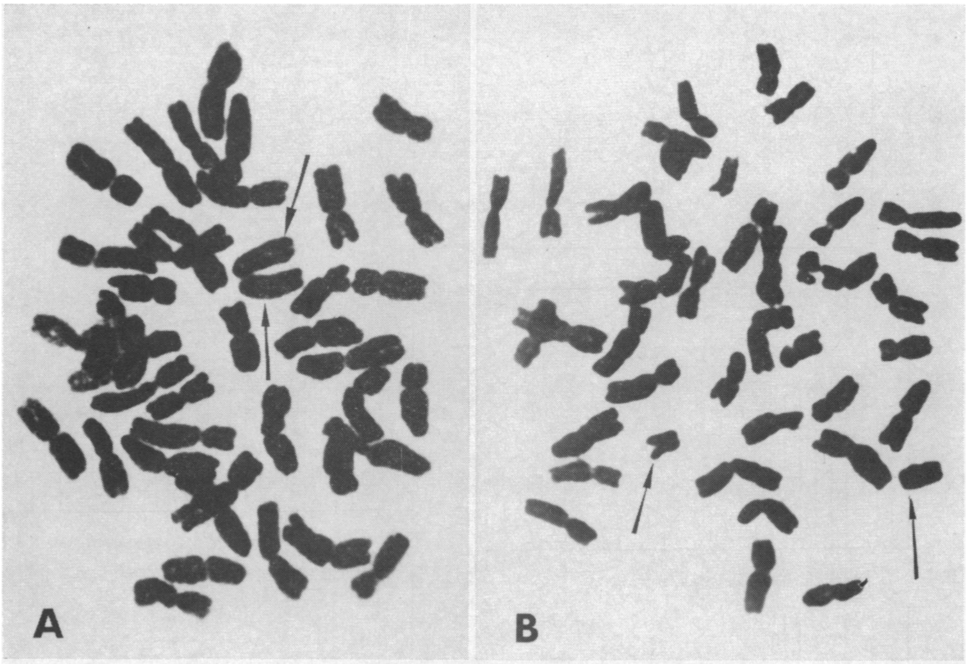

FIG. 1.—Root-tip mitosis in plants of *T. aestivum* with two telocentric chromosomes (indicated by arrows). (*A*) Homologues ($3B^L - 3B^L$). (*B*) Nonhomologues ($6B^L - 1D^L$). Normal 6B and 1D chromosomes also present but not identified.

significantly (at the 0.05 level) from the calculated random distribution (Figs. 2*F*, 2*G*, and 3), nor did their distributions differ from each other (Fig. 3). The excellent correspondence with the theoretical curve tends to verify the essential validity of the assumption that the chromosomes of the squashed cell are uniformly distributed in a nearly circular area. If there were a strong tendency for many of the chromosomes to be clumped, for example, this would result in a lower mean distance between the two chromosomes being measured and a different shape of distribution curve.

Homologous telocentrics: A quite different type of distribution was obtained with the two pairs of homologous telocentrics, $3B^L$-$3B^L$ and $5A^L$-$5A^L$ (Figs. 2*B* and *C*). The mean distances between homologues were only 0.308 and 0.333, respectively, compared with the 0.45 expected if they were randomly distributed; and in each case the deviation from the random curve was significant at the 0.005 point (Fig. 3). The two distributions of homologues showed no significant difference from each other at the 0.05 point.

Two arms of the same chromosome: If the attractive forces which bring about somatic association depend solely upon the presence of homologous loci, telocentrics for opposite arms of the same chromosome ought not to show association. If, however, homology of centromeres is partly or wholly responsible for somatic association, opposite-arm telocentrics might be associated. Opposite halves of the same centromere are fully homologous, if Lima-de-Faria[10] is right in believing that the centromere is a reversed repeat. Even if this assumption is invalid, it is possible that each stable telocentric has a complete or nearly complete centromere, for

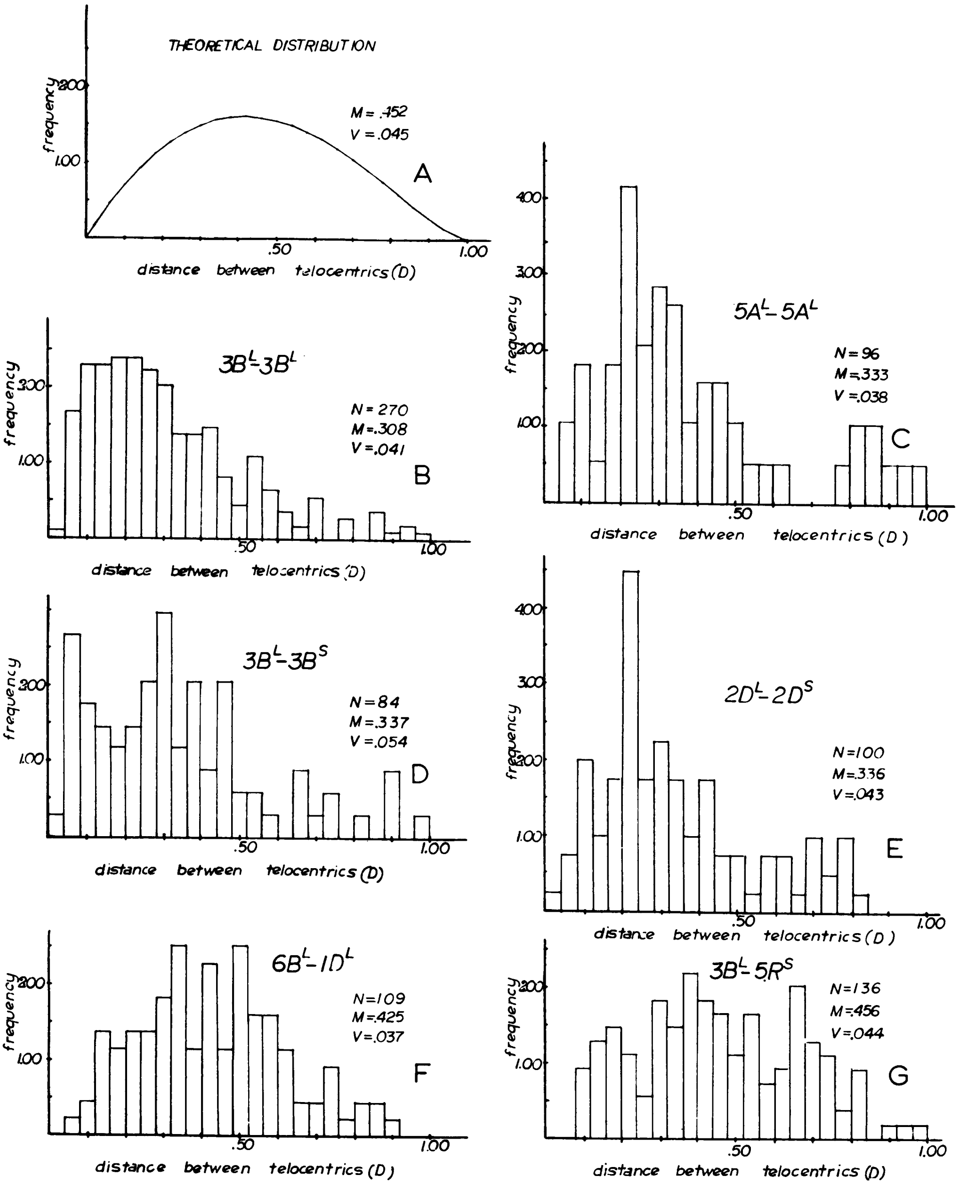

FIG. 2.—(*A*) Theoretical distribution of distances between two randomly located chromosomes in squashes of root-tip cells. (*B–G*) Observed distribution of distances between two telocentric chromosomes which were (*B*, *C*) homologous, (*D*, *E*) opposite arms of the same chromosome, or (*F*, *G*) nonhomologous. N = number of observations; M = mean; V = variance.

there is some evidence that telocentrics with only half a centromere are highly unstable and are quickly lost.[11]

Two different pairs of relatively stable opposite-arm telocentrics were studied, $3B^L$-$3B^S$ and $2D^L$-$2D^S$, in each case with no other representative present of the chromosome concerned. In both cases the two opposite arms of the same chromo-

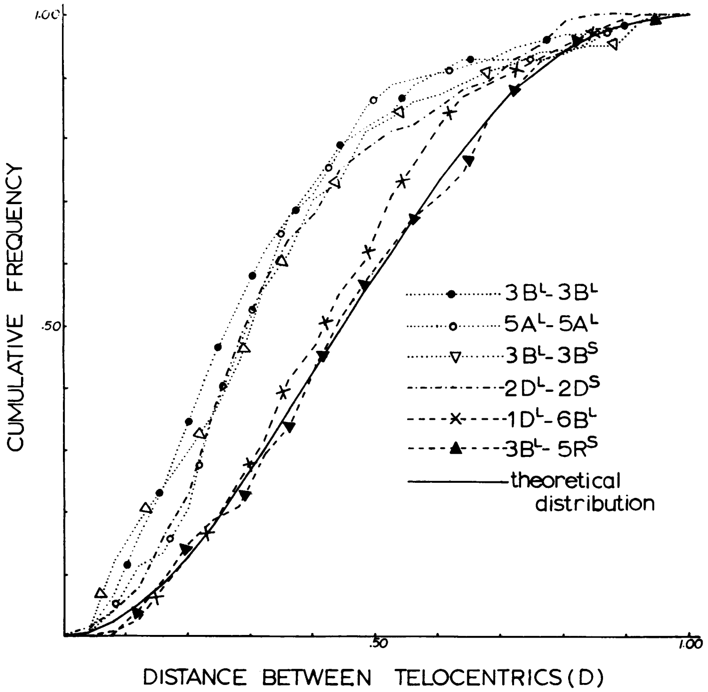

FIG. 3.—Expected and observed cumulative frequencies of distances between two telocentric chromosomes.

some had the same distributional pattern as homologous telocentrics (Figs. 2*D*, 2*E*, and 3). There were no significant differences between the cumulative curves obtained for 3B^L-3B^S or 2D^L-2D^S and those for 3B^L-3B^L and 5A^L-5A^L.

Two homologues and a nonhomologue in the same cell: For a comparison of somatic association free of many of the variables involved in comparisons of distribution curves, material was available with two homologous telocentrics, 3B^L-3B^L, and one nonhomologue, 5R^S. In 31 of the 43 cells measured, the homologues were closer to each other than to the nonhomologous telocentric (Table 1). When the distances in each cell were taken into account, the homologues were significantly closer together (at the 0.001 level). Plotting the distances as in the previous experiments yielded curves for nonhomologues and homologues which closely followed, respectively, the random curve and the one previously obtained for homologues.

Discussion.—From the evidence presented, it seems clear that homologous chromosomes of wheat are associated in root-tip cells. Homologous telocentric chromosomes were found to be located near to each other in squashed cells oftener than was at all likely on the basis of chance alone. Since the cold treatment which the cells had been given suppressed the mitotic spindle, the proximity of the telocentrics cannot be attributed to a tendency for chromosomes of a particular size to lie in a particular portion of the spindle. In any case, the telocentrics used were substantially the same length as many of the short, normal chromosomes of the wheat complement. A similar tendency for somatic association of homologues has been found for human chromosomes.[12]

TABLE 1

Comparison of Distances between Homologous ($3B^L$-$3B^L$) and Nonhomologous ($3B^L$-$5R^S$) Telocentrics within the Same Cells of Plants with Two $3B^L$ + One $5R^S$

Cell	Distance between homologous telocentrics	Average distance between nonhomologous telocentrics	*d*
1	0.04	0.75	0.71
2	0.06	0.73	0.67
3	0.08	0.70	0.62
4	0.25	0.86	0.61
5	0.13	0.71	0.58
6	0.15	0.64	0.49
7	0.10	0.54	0.44
8	0.25	0.67	0.42
9	0.14	0.54	0.40
10	0.11	0.48	0.37
11	0.10	0.47	0.37
12	0.40	0.75	0.35
13	0.34	0.67	0.33
14	0.11	0.40	0.29
15	0.14	0.42	0.28
16	0.07	0.34	0.27
17	0.11	0.38	0.27
18	0.08	0.35	0.27
19	0.30	0.56	0.26
20	0.41	0.61	0.20
21	0.31	0.51	0.20
22	0.31	0.48	0.17
23	0.04	0.18	0.14
24	0.46	0.56	0.10
25	0.28	0.37	0.09
26	0.29	0.37	0.08
27	0.10	0.18	0.08
28	0.05	0.10	0.05
29	0.52	0.56	0.04
30	0.05	0.09	0.04
31	0.28	0.31	0.03
32	0.41	0.39	−0.02
33	0.21	0.19	−0.02
34	0.64	0.55	−0.09
35	0.26	0.16	−0.10
36	0.34	0.19	−0.15
37	0.55	0.38	−0.17
38	0.37	0.19	−0.18
39	0.55	0.36	−0.19
40	0.48	0.28	−0.20
41	0.59	0.38	−0.21
42	0.94	0.49	−0.45
43	0.87	0.31	−0.56

P is significant above the 0.001 level.

It is reasonable to assume that homologues lie even nearer together before the cells have been squashed. The unsquashed chromosome group is approximately spherical. Any ordered arrangement the chromosomes may have at that time is likely to be reduced in the process of flattening the sphere into one plane. It is also likely that the onset of cell division tends to disturb whatever association there may be between homologues. Therefore, it is probable that the association observed is only a vestige of a much closer and more regular association which exists during interphase. This assumption is supported by the observations of Metz,[2] who found that somatic association in the *Diptera* was most intimate in the beginning of prophase or in late anaphase, and who concluded that a close association of homologues is maintained during interphase. Similarly, Kitani[4] found that homologous chromosomes of *Crepis capillaris* became more closely associated at mitotic telophase.

Feldman[1] presented evidence that homologues are already associated at the time meiotic synapsis begins. This and the present finding of association in root-tip cells strongly suggest that association of homologues occurs throughout the life of the plant.

The discovery that telocentrics representing the two different arms of the same chromosome are as closely associated as homologous telocentrics suggests that it is the centromere which is primarily responsible for positioning the homologues near to each other. Although it is conceivable that the opposite arms of chromosomes 3B and 2D have one or more segments in common, it is most unlikely that either chromosome has the amount of interarm duplication that may be assumed necessary to make the opposite arms associate as if they were homologous. Although 2D when univalent at meiosis sometimes tends to fold back on itself, as though there were some attraction of the two arms for each other, no such tendency has been observed for 3B. It should be emphasized that these telocentrics, having been derived through misdivision of univalents, had genuinely terminal centromeres.

The tendency for opposite-arm telocentrics to associate somatically is strong support for the assumption of Steinitz-Sears[11] that the centromere of each chromosome is unique. Since stable telocentrics, such as those used in the present study, are believed to have substantially more than half-centromeres,[11] the results may be explained without assuming, as Lima-de-Faria[10] does, that the centromere is a reversed repeat. With such an assumption, however, the results are all the more credible.

Although the present results can be explained solely on the basis of somatic association of homologous centromeres, mutual attraction of other regions is not ruled out. Hinton[13] found in *Diptera* that translocated segments paired normally; and Sved[14] suggested that chromosomes attach in pairs to the nuclear membrane by their telomeres. Kitani[4] showed that in prophase of *Crepis capillaris* all the centromeric regions lie near one end of the nucleus, and that the distal ends lie near the other end. Finally, Kasha and Burnham[15] showed that in barley effective meiotic pairing begins at the ends rather than at the centromere, suggesting that at least in the stage immediately preceding meiosis, ends are as close or closer together than centromeres.

Whether wheat homologues are closely associated throughout their length in interphase somatic nuclei is at present a matter for conjecture. Homologous segments are known to attract each other in meiosis, but there is little reason to assume that this is also true in somatic stages. Whereas meiotic pairing is always limited in any one region to two homologues, somatic association may involve more than two.[2] This suggests that somatic association is brought about in a different way than is meiotic synapsis. Also, the effect of deficiency for chromosome 5B indicates that somatic association and meiotic pairing are controlled by different genes. Somatic association, as reflected in the occurrence of homoeologous pairing at meiosis, is greater in the absence of $5B^L$, but there is no increase in chiasma frequency[16] to indicate a corresponding rise in meiotic attraction.

Meiotic pairing is very intimate and precise, making possible the frequent exchange of homologous segments. Somatic association is evidently relatively loose, only rarely leading to interchange. However, somatic exchanges between homologues, which sometimes occur spontaneously or may be induced by irradiation, are often nearly enough at the same level to simulate crossing over. This suggests that the homologues do lie fairly close together throughout their length.

The existence of special, long-range attractive forces operating during prophase of meiosis to bring homologues together is called into question by the finding that homologues are normally already associated when meiosis begins. There is little need to assume such forces, at least in the wheat material.

There is some evidence that somatic interphase chromosomes are regularly attached to the nuclear membrane by their centromeres[17] and also by their ends.[14] If each centromere and/or telomere were attached to a specific site on the membrane, this would explain the observed somatic association. However, the maintenance of such specificity of sites by the membrane, which breaks down during each mitosis, is at present difficult to understand. Furthermore, the existence of specific sites would result in apparent association of nonhomologues which happened to occupy adjacent or nearby sites, and possibly in negative association of nonhomologues with sites on opposite sides of the nucleus. No departures from randomness were observed in the present study in the distribution of nonhomologues. The data are adequately explained by an assumption of attraction between centromeres and possibly other regions, with subsequent attachment of centromeres and possibly telomeres to the membrane.

Summary.—Whereas the distances between nonhomologous telocentric chromosomes in squashes of mitotic cells of root tips of wheat conformed to a theoretical model based on the distance between points taken at random from a uniform distribution, homologues tended to lie near each other. The two telocentrics for opposite arms of the same chromosome were associated to approximately the same degree as homologous telocentrics, suggesting that the centromere is at least partly responsible for somatic association.

The authors are much indebted to Dr. Lotti M. Steinitz-Sears for several of the stocks used, and to Dr. Gary F. Krause for invaluable assistance with the statistical treatment of the results. Dr. Krause called their attention to the applicability of the Deltheim formula to their data and also pointed out the advantages of the Kolmogorov-Smirnov and Wilcoxon Matched-Pairs Signed-Ranks tests of significance.

---

* Cooperative investigations of the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the Field Crops Department, Missouri Agricultural Experiment Station. Supported in part by grant GB-1185 from the National Science Foundation. Journal Series no. 4046 of the Missouri Station.

† Fellow of the Fundacao Calouste Gulbenkian, Centro de Biologia, Oeiras, Portugal.

‡ Research geneticist, Agricultural Research Service, U.S. Department of Agriculture.

[1] Feldman, M., these PROCEEDINGS, **55**, 1447 (1966).

[2] Metz, C. W., *J. Exptl. Zool.*, **21**, 213 (1916).

[3] Boss, J. M. N., *Texas Rept. Biol. Med.*, **13**, 213 (1955).

[4] Kitani, Y., *Japan. J. Genetics*, **38**, 244 (1963).

[5] Barber, H. N., and H. G. Callan, *Proc. Roy. Soc.* (*London*), **B131**, 258 (1943).

[6] Hammersley, J. M., *Ann. Math. Statistics*, **21**, 447 (1950).

[7] Lord, R. D., *Ann. Math. Statistics*, **25**, 794 (1954).

[8] Deltheil, R., *Probabilités Géometriques* (Paris: Gauthier-Villars, 1926), vol. 2, section 2.

[9] Siegel, S., *Nonparametric Statistics* (New York: McGraw-Hill Book Company, Inc., 1956).

[10] Lima-de-Faria, A., *Hereditas*, **35**, 422 (1949).

[11] Steinitz-Sears, L. M., *Genetics*, **51**, 241 (1966).

[12] Schneiderman, L. J., and C. A. B. Smith, *Nature,* **195,** 1229 (1962).
[13] Hinton, T., *J. Exptl. Zool.,* **102,** 232 (1946).
[14] Sved, J. A., *Genetics,* **53,** 747 (1966).
[15] Kasha, K. J., and C. R. Burnham, *Can. J. Genet. Cytol.,* **7,** 620 (1965).
[16] Riley, R., *Heredity,* **15,** 407 (1960).
[17] Vanderlyn, L., *Botan. Rev.,* **14,** 270 (1948).