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An analysis of temperature-dependent asynapsis in *Triticum aestivum*

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SUMMARY

Measurements of chiasma frequency over a range of temperatures from 9 to 35 °C in euploid wheat and lines nullisomic for chromosome 5D showed the absence of chromosome 5D greatly to increase the sensitivity of chiasma frequency to temperature, resulting in complete univalence at the lowest temperatures. Careful examination of meiotic prophase showed the lowering of chiasma frequency to be correlated with failure of zygotene chromosome pairing, justifying the use of the term asynaptic. Observation of the pairing behaviour of an isochromosome in the nullisomic 5D background suggested that the asynapsis was due to a failure in the mechanism of chromosome pairing rather than of the pre-alignment of homologues. The observed effects in the absence of chromosome 5D could be due to the presence of a recessive allele at the locus of chromosome 5A, also known to control stability of chiasma frequency to temperature. In conclusion it is pointed out that in euploid wheat the sensitivity of chiasma frequency to temperatures within the normal meteorological range could influence the cytological stability of the wheat crop.

1. INTRODUCTION

Sensitivity of chiasma frequency to low temperature has already been shown to be under the control of loci on chromosomes 5A and 5D of wheat (Riley, 1966). In the variety studied, Chinese Spring, the presence of chromosome 5D was shown to stabilize chiasma frequency against the effect of temperatures below 20 °C. This was detected by the drop in chiasma frequency in plants grown below that temperature in the absence of chromosome 5D.

In some hexaploid wheat varieties a locus, probably on chromosome 5A, has a recessive allele, *ltp*, which, in the absence of chromosome 5D, is unable to stabilize chiasma frequency to low temperature. This allele exhibits a weak stabilizing activity, revealed by plants tetrasomic 5A and nullisomic 5D, which do not show reduction in chiasma frequency at temperatures below 20 °C (Riley *et al.* 1966). The dominant allele at the 5A locus, *Ltp*, is present in the AABB tetraploid wheats, maintaining chiasma frequency at low temperatures in the absence of the D genome (Riley & Hayter, 1967).

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The work presented in this paper is a detailed analysis of the response of chiasma frequency to temperature in normal euploid wheat and in genotypes deficient for chromosome 5D. A further paper (Bayliss & Riley, 1972) will show, by the use of temperature changes followed by sequential sampling, that the sensitivity to temperature is localized at a specific time in the meiotic sequence.

2. MATERIALS AND METHODS

The wheat (*Triticum aestivum*) genotypes used were all derived from stocks of the variety Chinese Spring ($2n = 6x = 42$). The nullisomic 5D tetrasomic 5B (N5DT5B) genotype was produced originally as outlined by Riley (1966). The response of N5DT5B was compared with that of normal 42-chromosome euploid plants, referred to for convenience as 'Euploid'. A 40-chromosome plant nullisomic for chromosome 5D was isolated from a population of plants mono-telosomic for 5D. The line containing an isochromosome of the long arm of chromosome 5B was produced from N5DT5B and plants mono-isosomic for 5B in three generations as illustrated below.

N5DT5B × mono-iso-5B

$2n = 42 \downarrow 2n = 41_{iso}$

monosomic-5D, disomic-5B, monoisi-5B

$2n = 42_{iso}$

↓ selfed

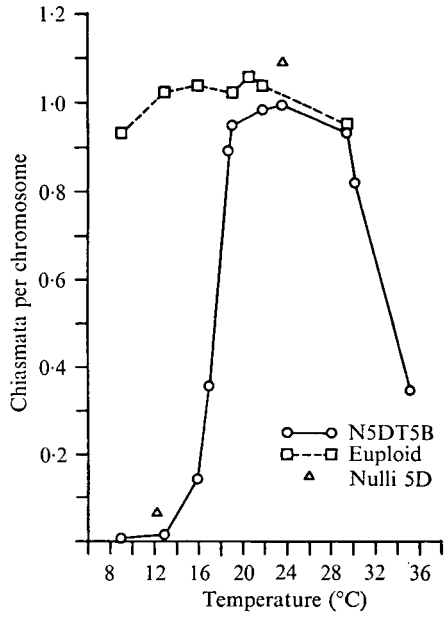
nullisomic-5D, disomic-5B, monoisosomic-5B

$2n = 41_{iso}$

The final genotype was selected cytologically from the segregating progeny of the F_1 plants. Chromosome numbers in this and all other material were checked in the root-tips of germinating seedlings after pre-treatment with 1-bromonaphthalene and fixation in glacial-acetic acid. Temporary preparations were made using the Feulgen-squash technique of Hillary (1939).

The populations of plants used for different temperature treatments were grown initially in a lighted greenhouse at 20 °C and transferred to continuously lighted controlled environment chambers just before meiosis. Temperature was continuously monitored within the plant population using a multi-channel recorder and thermistor-type probes. Before sampling for meiotic stages the plants were allowed to equilibrate to the controlled temperatures. The periods of equilibration used ranged from 2 weeks at the lowest temperature to 3 days at the highest temperature. This ensured that sampled anthers had undergone their complete meiotic development at the controlled temperature. After sampling the temperature was raised and the procedure repeated. Because of the need for equilibration, three different populations of plants had to be used to cover the entire temperature range.

For measurement of chiasma frequency, anthers at first metaphase of meiosis were fixed in acetic-alcohol (1:3) and permanent Feulgen-orcein squash prepara-



Text-fig. 1. The relationship of chiasma frequency to temperature in euploid, N5DT5B and nullisomic 5D.

tions made subsequently. For examination of earlier meiotic stages, whole spikes of approximately the correct age were fixed and Feulgen stained. Anthers were then dissected out and squash preparations made on slides numbered to correspond to the position of the florets within the spike. The graduation of meiotic stages between spikelets and florets then ensured correct interpretation of the sequence of meiotic stages.

3. RESULTS

(i) *The relation of chiasma frequency to temperature*

The results for Euploid showed that, between 12·9 °C (Plate 3*a*) and 21·75 °C, temperature had no significant effect on chiasma frequency. At temperatures above or below this range, chiasma frequency was reduced (Table 1, Text-fig. 1). Euploid plants were also examined at 35 °C, and though the chromosomes were too sticky to allow accurate scoring the numbers of rod and ring bivalents indicated a chiasma frequency similar to that at 29 °C.

By contrast, the results for N5DT5B showed an almost linear relationship of chiasma frequency to temperature between 15·9 °C and 19 °C. Below 15·9 °C the response tailed off slowly to 9·1 °C. At 12·9 and 9·1 °C the majority of cells were completely asynaptic (Table 2, Text-fig. 1, Plate 3*b*). Between 19·0 and 23·5 °C the chiasma frequency was relatively constant and slightly lower than euploid at the same temperature (Plate 3*c*). At 29·25 °C and above, the chiasma frequency falls progressively. It was possible to analyse only a few cells in plants examined at 35 °C and as these showed mainly rod bivalents and univalents, it would seem

Table 1. *Relationship to temperature of the number of chiasmata and types of chromosome configuration at first metaphase of meiosis in euploid plants*

Mean temp (°C)	No. plants		Mean per cell: Univ.	Bivalents: Rod 1Xa	Rod 2Xta	Ring 2Xta	Ring > 2Xta	Triv. and quad.	Mean Xta per chromosome
9·1	4	1st expt.	0·40	3·2	0·09	16·7	0·8	0	0·934
12·9	4		0·05	1·1	0·09	17·5	2·2	0	1·028
15·9	4		0·01	1·4	0·12	16·3	3·1	0	1·045
19·0	4	2nd expt.	0·07	1·7	0·32	16·1	2·9	0	1·028
21·75	2		0·05	1·3	0·17	16·5	2·9	0	1·042
20·5	3	3rd expt.	0·03	1·2	0·22	15·9	3·7	0	1·063
29·25	2		0·30	3·4	0·37	15·1	1·9	0	0·955

Xa = chiasma. Xta = chiasmata.

Table 2. *Relationship to temperature of the number of chiasmata and types of chromosome configuration at first metaphase of meiosis in plants nullisomic 5D, tetrasomic 5B*

Mean temperature (°C)	No. plants		Mean per cell: Univ.	Bivalents: Rod 1Xa	Rod 2Xta	Ring 2Xta	Ring > 2Xta	Triv. and quad.	Mean Xta per chromosome
9·1	4	1st expt.	41·5	0·24	0	0	0	0	0·006
12·9	4		40·6	0·67	0	0	0	0	0·016
15·9	4		30·8	5·26	0	0·32	0	0	0·141
18·7	2		0·77	4·30	0·25	13·98	0·82	0·52	0·896
19·0	4	2nd expt.	0·69	3·64	0·45	13·70	2·25	0·34	0·952
21·75	4		0·26	2·87	0·51	14·46	2·61	0·21	0·989
23·5	4		0·35	2·65	0·50	14·24	2·86	0·30	0·999
17·0	2	3rd expt.	17·45	9·45	0·12	2·7	0	0	0·359
29·25	4		0·74	4·24	0·25	12·91	2·50	0·40	0·937
30·0	2		2·30	6·75	0·72	10·42	1·72	0·15	0·821
35·0	2		19·0	8·29	0	3·12	0	0	0·349

Xa = chiasma. Xta = chiasmata.

that the chiasma frequency of N5DT5B was lower than that of Euploid at this temperature.

The two additional points shown in Text-fig. 1 are for the plant nullisomic for chromosome 5D examined at 12·25 and 23·5 °C (Table 3). Though the general response of this genotype was similar to N5DT5B, analyses showed that on a per chromosome basis (to compensate for the differing chromosome numbers) nullisomic 5D had a significantly ($P < 0·05$) higher chiasma frequency when compared with the N5DT5B plants analysed at 12·9 and 23·5 °C.

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Table 3. *Relationship to temperature of the number of chiasmata and types of chromosome configuration at first metaphase of meiosis in plants nullisomic 5D*

		Mean per cell						
			Bivalents					
Mean temperature (°C)	No. plants	Univ.	Rod 1Xa	Rod 2Xa	Ring 2Xa	Ring >2Xa	Triv. and quad.	Mean Xta per chromosome
12·25	1	35·1	2·45	0	0·05	0	0	0·064
23·5	1	0·1	0·90	0·35	13·9	4·8	0	1·097

The effect of variation in chiasma frequency on the distribution of chiasmata between chromosomes was examined by scoring the frequencies of different bivalent configurations. In Euploid (Table 1) the lowering of chiasma frequency at high and low temperatures was accompanied by an increase in frequency of rod bivalents and univalents and a corresponding decrease in frequency of ring bivalents with more than two chiasmata.

This shift towards a predominance of rod bivalents and univalents occurred to a much greater extent in N5DT5B (Table 2), owing to the greater reduction in chiasma frequency. It was apparent, however, that not all paired chromosomes proceeded through this sequence at the same rate, as the rod bivalent frequency rose to a maximum at 17 °C and then fell again, although some ring bivalents were still present at 15·9 °C. The multivalents occurring in N5DT5B between 19·0 and 23·5 °C resulted from pairing between the four 5B chromosomes present.

Analysis of the pairing behaviour of nullisomic 5D showed similar trends to those in N5DT5B. At 12·25 °C rod bivalents and univalents predominated whereas at 23·5 °C the increase in chiasma frequency above N5DT5B was accompanied by a lower frequency of rod bivalents and an increased frequency of ring bivalents with more than two chiasmata.

(ii) *Pairing of an isochromosome in a nullisomic 5D background*

When two homologous chromosome arms are attached to a single centromere in an isochromosome, they can pair at meiosis and chiasma formation leads to the production of a ring univalent. Moreover, the probability of this occurring should be independent of the requirement, in normal bivalent formation, of initial juxtaposition of the two homologous partner chromosomes.

As shown in Plate 3(*d*) the isochromosome ring formed normally in the nullisomic 5D background at 20 °C. At 13 °C, when most cells were completely asynaptic, there was no evidence of the isochromosome ring.

(iii) *Comparison of stages of meiotic prophase at low temperatures*

To test whether the reduced chiasma frequency at low temperatures was due to failure of chromosome pairing at zygotene (asynapsis) or precocious separation of paired homologues at diplotene (desynapsis), preparations of meiotic prophase stages were examined in Euploid and N5DT5B plants grown at 13 °C.

In Euploid, zygotene pairing could clearly be seen occurring quite normally at 13 °C (Plate 1*a*), while little or no zygotene pairing occurred in N5DT5B (Plate 1*b*). Indeed, as a sequence of florets of slightly different ages was examined in chronological order it could be concluded unequivocally that no pairing occurred. Plate 1(*b*) was typical and was at a stage when synapsis would have taken place in Euploid. Verification was obtained by examination of pachytene and diplotene. At diplotene the paired homologues were clearly held together at exchange points in Euploid (Plate 2*a*), while in N5DT5B unpaired chromosomes lay at random in the cell (Plate 2*b*). It was therefore concluded that N5DT5B showed failure of zygotene pairing at extremes of temperature and could be designated asynaptic.

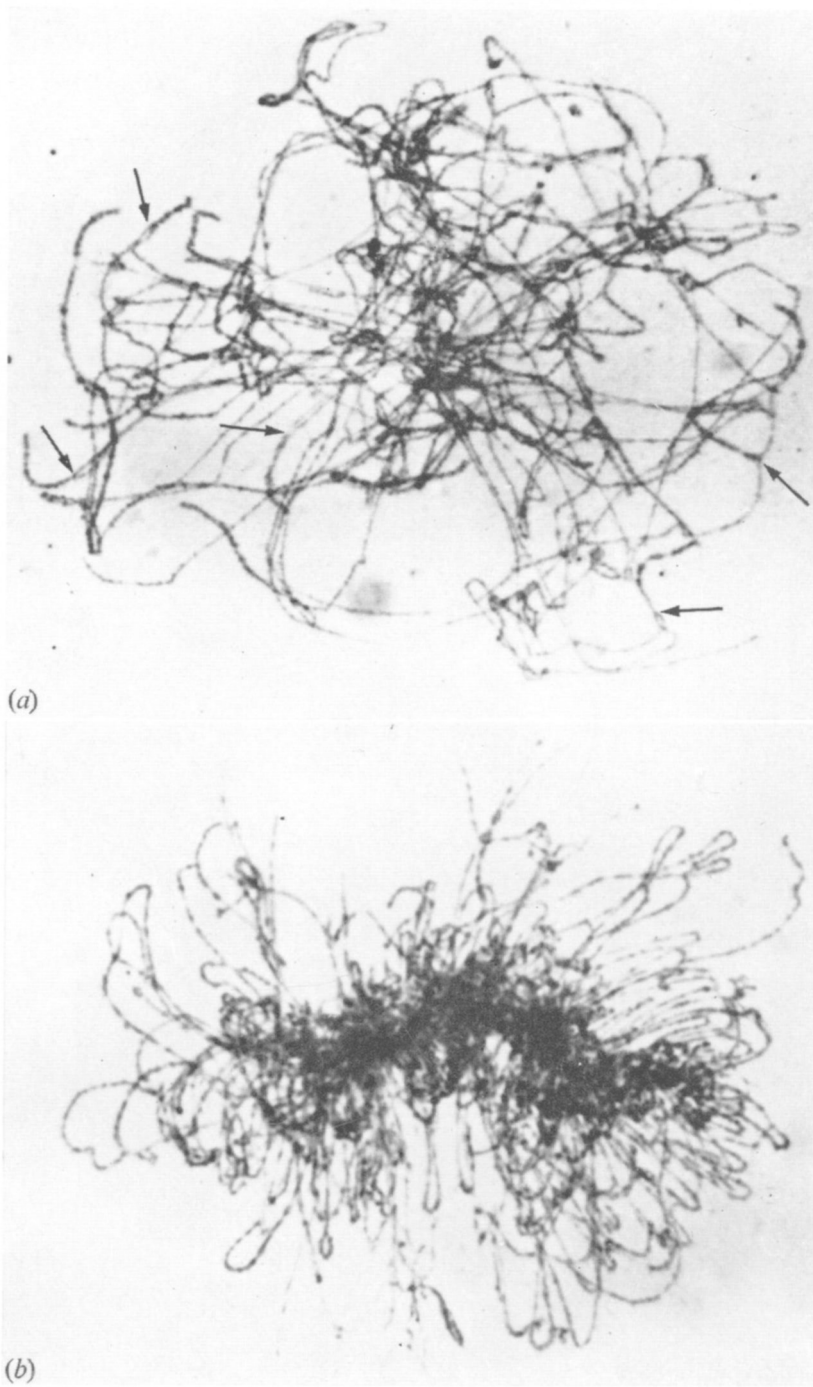
4. CONCLUSIONS AND DISCUSSION

The reduction in chiasma frequency found in Euploid plants at high and low temperatures is similar to the responses found in several other plant species. Dowrick (1965) showed similar relations of chiasma frequency to temperature in *Uvularia perfoliata* and *Tradescantia bracteata*, though the exact temperature co-ordinates varied between these species and differ from the present results. The review by Wilson (1959) classified the responses of chiasma frequency to temperature by earlier workers into several categories, but concluded that inconsistencies in experimental design precluded exact comparisons.

The effect of the deficiency of chromosome 5D in wheat is greatly to increase the sensitivity of chiasma formation to temperature rather than to cause unique pairing failure at extremes of temperature. The failure in 5D-deficient plants may be ascribed to the failure of the *ltp* alleles on chromosome 5A to stabilize chiasma frequency to temperature (Riley & Hayter, 1967). The effect is then analogous to that of mutants affecting the relation of chiasma frequency to temperature described in *Oryza sativa* (Chao & Hu, 1961), *Lycopersicum esculentum* (Soost, 1951) and *Triticum aestivum* (Li, Pao & Li, 1945). In this last example the description of the mutant as desynaptic suggests that it may have been a mutant in a system other than that described in the present work.

The higher chiasma frequency of nullisomic 5D in comparison with N5DT5B, independently of temperature, must be ascribed to the different dosages of chromosome 5B in these two genotypes. This increase in chiasma frequency accompanying a reduced 5B dosage is similar to that shown by Riley & Chapman (1967) and by Bayliss (1972).

Though it is not possible to show precisely how the distribution of chiasmata is controlled in wheat, comparison of the frequencies of various bivalent configurations with data on chromosome arm lengths (Sears, 1954) allows tentative conclusions to be drawn. The increase in numbers of rod bivalents occurring at intermediate temperatures may occur because the majority of wheat chromosomes have submedian centromeres – that is, one chromosome arm is longer than the other. The one or two ring bivalents present even at low chiasma frequencies correspond to the presence of chromosome pairs with short arms longer than the



(*a*) Zygotene of meiosis in euploid wheat at 13 °C, showing pairing of homologous chromosomes (arrowed).

(*b*) Zygotene of meiosis in nullisomic 5D tetrasomic 5B, showing absence of chromosome pairing.

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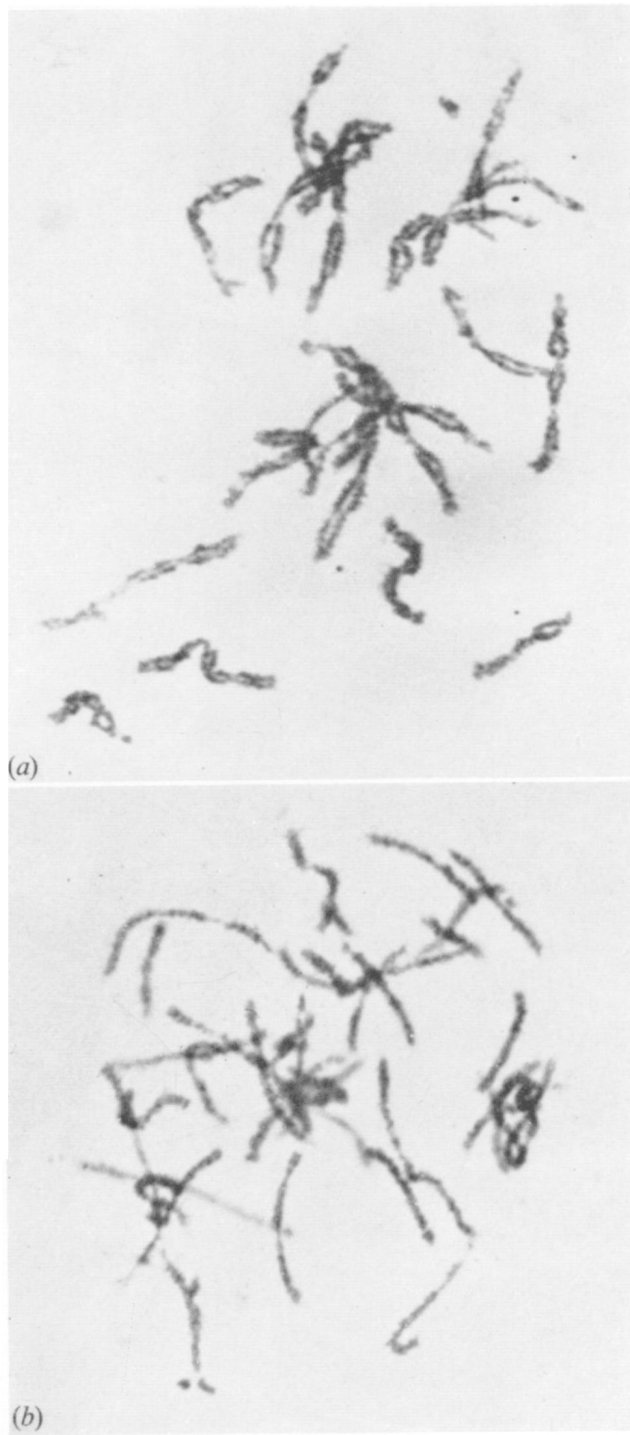

(*a*) Diplotene of meiosis in euploid wheat at 13 °C, showing separating homologues held at exchange points.

(*b*) Diplotene of meiosis in N 5 DT 5 B at 13 °C, showing only unpaired univalents.

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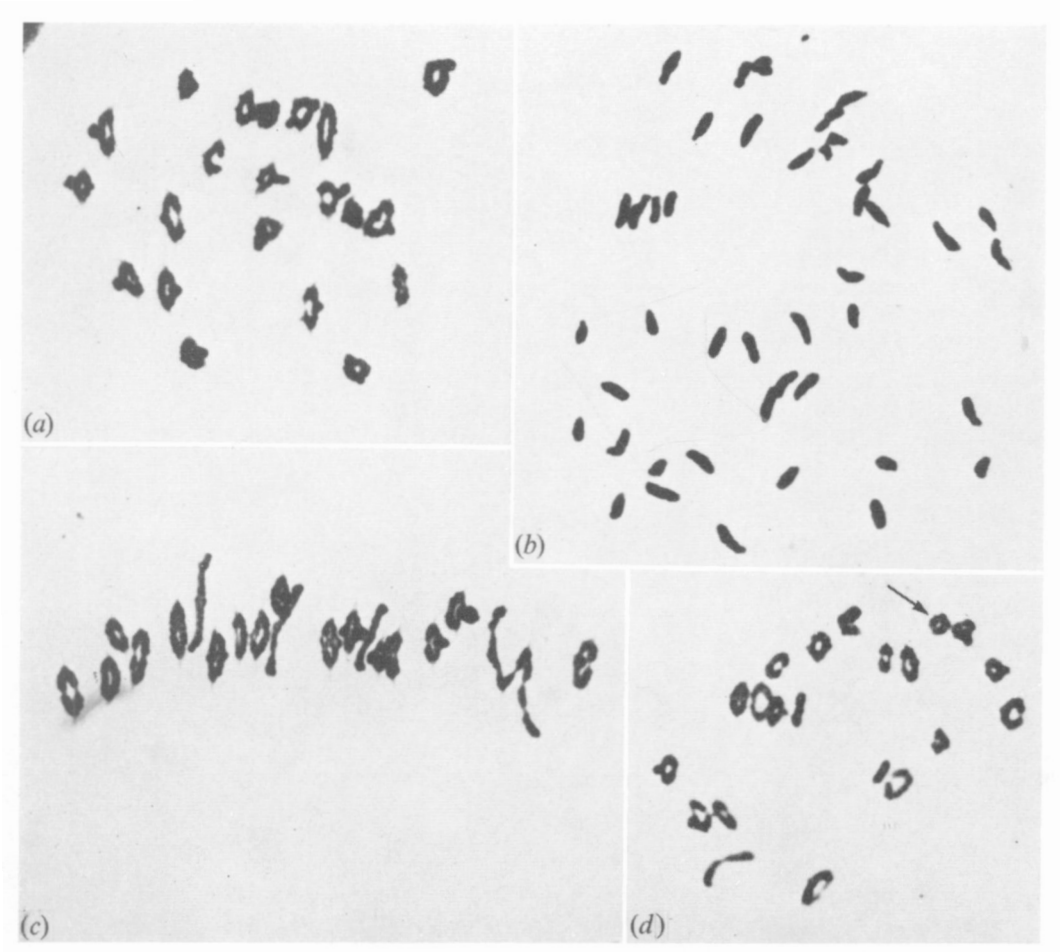

First metaphase of meiosis, showing (*a*) 21 ring bivalents in euploid at 13 °C, (*b*) 42 univalents in N 5DT 5B at 13 °C, (*c*) 19 bivalents and one quadrivalent in N 5DT 5B at 21·75 °C, (*d*) 20 bivalents and one isochromosome ring in nulli 5D, disomic 5B, di-iso $5B^{L}$ at 20 °C.

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long arms of the remaining chromosomes. It thus appears that in wheat the distribution of chiasmata is related to the distribution of chromosome arm lengths. This type of relationship was interpreted by Mather (1938) as a response of the 'differential distance' of the first chiasma from the centromere. This principle may be expected to apply in wheat where maximal chiasma frequencies rarely exceed one chiasma per chromosome arm.

Examination of zygotene and subsequent meiotic stages at low temperatures shows that the low chiasma frequencies are due to failure of normal zygotene pairing (asynapsis) rather than to precocious disolution of pairing at diplotene (desynapsis), using the definitions of Rieger, Michaelis & Green (1968). Though this can only be determined at the gross level of complete asynapsis at low temperatures in N5DT5B, the similarity in shape of response of Euploid suggests some asynapsis occurs in this case also. The behaviour of the isochromosome may be interpreted in relation to the work of Driscoll & Darvey (1970). They found that if meiotic cells containing an isochromosome were treated with colchicine, pairing failure was general except between the arms of the isochromosome. Using the arguments of Feldman (1968) and Avivi, Feldman & Bushuk (1969) they concluded that colchicine affected somatic association prior to chromosome pairing rather than the mechanism of chromosome pairing. In the present material the failure of the isochromosome to pair at low temperatures suggests a failure in the mechanism of chromosome pairing rather than merely a failure in somatic association.

It may thus be generally concluded that in euploid wheat the presence of a gene or genes on chromosome 5D largely stabilizes chromosome pairing against extremes of temperature. When chromosome 5D is absent, the recessive *ltp* allele present in chromosome 5A is unable to counteract the affects of temperature extremes, resulting in a partial or complete failure of the mechanism of zygotene chromosome pairing.

In practical terms, the demonstration that low temperatures to which the developing ear of a euploid wheat crop might normally be exposed can cause a significant degree of asynapsis, suggests this may be an important source of the naturally observed rates of aneuploidy (Riley & Kimber, 1961) and the cytological instability that sometimes occurs in wheat varieties. It might be possible to counteract this by incorporation of the dominant *Ltp* allele from the A genome of tetraploid wheats into the hexaploid background. This should then give enhanced stability to extremes of temperature.

REFERENCES

Avivi, L., Feldman, M. & Bushuk, W. (1969). The mechanism of somatic association in common wheat, *Triticum aestivum* (L.). I. Suppression of somatic association by colchicine. *Genetics* **62**, 745–752.

Bayliss, M. W. (1972). Ph.D. Thesis, University of Cambridge.

Chao, C. Y. & Hu, W. L. (1961). The effect of temperature on a desynaptic gene in rice. *Botanical Bulletin of Academia Sinica* (Taipei) **2** (2), 87–100.

Dowrick, G. J. (1956). The influence of temperature on meiosis. *Heredity* **11**, 37–49.

Driscoll, C. J. & Darvey, N. L. (1970). Chromosome pairing: effect of colchicine on an isochromosome. *Science* **169**, 290–291.

Feldman, M. (1968). Regulation of somatic association and meiotic pairing in common wheat. *Proceedings Third International Wheat Genetics Symposium*, pp. 169–178.

Hillary, B. B. (1939). Improvements to the permanent root-tip squash technique. *Stain Technology* **14**, 97–99.

Li, H. W., Pao, W. K. & Li, C. H. (1945). Desynapsis in common wheat. *American Journal of Botany* **32**, 92–100.

Mather, K. (1938). Crossing-over. *Biological Reviews* **13**, 252–289.

Rieger, R., Michaelis, A. & Green, M. M. (1968). *Glossary of Genetics and Cytogenetics*. Berlin: Springer-Verlag.

Riley, R. (1966). Genotype–environmental interaction affecting chiasma frequency in *T. aestivum*. In *Chromosomes Today*, vol. 1 (ed. C. D. Darlington and K. R. Lewis), pp. 57–65. Edinburgh: Oliver and Boyd.

Riley, R. & Chapman, V. (1967). Effect of $5B^S$ in suppressing the altered dosage of $5B^L$ on meiotic chromosome pairing in *T. aestivum*. *Nature* **216**, 60–62.

Riley, R., Chapman, V., Young, R. M. & Belfield, A. (1966). Control of meiotic chromosome pairing by the chromosomes of homoeologous group 5 of *T. aestivum*. *Nature* **212**, 1475–1477.

Riley, R. & Hayter, A. M. (1967). Duplicate genetic activities affecting meiotic chromosome pairing at low temperatures in *Triticum*. *Nature* **216**, 1028–1029.

Riley, R. & Kimber, G. (1961). Aneuploids and the cytogenetic structure of wheat varietal populations. *Heredity* **16**, 275–290.

Sears, E. R. (1954). The aneuploids of common wheat. *Bulletin of the University of Missouri Agricultural Experiment Station*, no. 572.

Soost, R. K. (1951). Comparative cytology and genetics of asynaptic mutants in *Lycopersicum esculentum*. *Genetics* **36**, 410–434.

Wilson, J. Y. (1959). Chiasma frequency in relation to temperature. *Genetica* **29**, 290–303.