Genet. Res., Camb. (1972), **20**, *pp.* 201–212
With 8 text-figures
Printed in Great Britain

Evidence of premeiotic control of chromosome pairing in *Triticum aestivum*

By M. W. BAYLISS* and RALPH RILEY

(*Received* 19 *April* 1972)

SUMMARY

A genotype of wheat deficient for a pair of chromosomes stabilizing chiasma frequency against extremes of temperature was investigated to determine the position and duration of temperature sensitivity with respect to first metaphase of meiosis. Temperature changes over a critical range, followed by sequential sampling and measurement of chiasma frequency, showed a relatively short temperature-sensitive stage, the position and duration of which were dependent on the final temperature used. Comparison with meiotic timings made independently showed that the temperature-sensitive stage occurred in the premeiotic interphase. Euploid wheat was shown to have a stage in chiasma formation sensitive to high-temperature treatments at a similar time.

Comparison with the work of others showed that the sensitive stage lay between the last premeiotic mitosis and the start of DNA synthesis. This modification of chromosome pairing at a much earlier stage than has been previously demonstrated is further evidence that the processes of chromosome pairing and crossing-over are probably more complex than formerly envisaged.

1. INTRODUCTION

The pairing of chromosomes at meiosis is the necessary precursor of genetic recombination in higher organisms. This is emphasized by the good cytogenetic evidence now available that the chiasmata associating meiotic chromosomes represent structures that arise as a result of the events that also lead to genetic crossing-over (Lewis & John, 1965). However, there is little understanding of the nature, causes or control of meiotic pairing. One approach to a description of the causal basis of pairing is the definition of the precise stage or stages at which pairing may be experimentally altered. The purpose of this paper is to show that events critical to the establishment of meiotic chromosome synapsis occur in the interphase prior to the visible initiation of meiosis.

The effects of environmental change on chiasma frequency and crossing-over have often been used in attempts to determine the time at which these events occur. Since both are dependent on chromosome pairing, the determination might also have indicated when processes take place that influence pairing. The results

* Work carried out during tenure of a Ministry of Agriculture, Fisheries and Food post-graduate Studentship. Present address: University of Leicester, Department of Botany, University Road, Leicester, LE1 7RH.

show that crossing over and chiasma frequency can be changed by events that take place at pachytene (Henderson, 1966; Lu, 1969; Peacock, 1970) at earlier stages of first prophase or during the premeiotic DNA synthesis (Grell & Chandley, 1965; Abel, 1965; Maguire, 1968) and during the premeiotic mitoses (McNelly-Ingle, Lamb & Frost, 1966; Lamb, 1971). Whether this diversity represents differences in the organisms or experimental methods used or whether distinct components in the sequence of an overall process were disturbed in separate experiments is not clear. In some instances difficulties were created by the need to use high temperatures or organisms with long meiotic durations. These were avoided in the present work by the use of *Triticum aestivum* and of a genetic variant with a marked temperature sensitivity.

T. aestivum provides a test organism with several features which can improve the accuracy of the timing of chiasma formation. First, there are reliable methods for measuring the meiotic duration, which is relatively short at normal temperatures in this organism. Secondly, an aneuploid genotype is available with a greatly increased sensitivity of zygotene chromosome pairing and chiasma formation to high and low temperatures (Bayliss & Riley, 1972). This genotype, nullisomic for chromosome 5D and tetrasomic for chromosome 5B, is almost completely asynaptic at 15 °C and shows a linear response of chiasma frequency up to 20 °C, where complete, normal chromosome pairing occurs.

This paper describes the use of temperature-change experiments, with this wheat genotype, to locate the period at which chiasma frequency is sensitive to temperature. As the timing measurements were made at a constant temperature after the temperature change and the change of chiasma frequency was in one direction only in any given experiment, the temperature-sensitive period could be defined with a high degree of accuracy for subsequent comparison with meiotic timing data.

In order to compare the results with euploid material, use was also made of the reduction in chiasma frequency in this genotype at high temperatures (Bayliss & Riley, 1972). Use of only short exposure to high temperatures allowed the detection of the reduction in chiasma frequency in subsequent samples without the effects on chromosome structure and stickiness resulting from prolonged exposure to high temperatures.

2. MATERIALS AND METHODS

The *Triticum aestivum* ($2n = 6x = 42$) euploid* and nullisomic 5D tetrasomic 5B (N5DT5B) genotypes of the variety Chinese Spring used throughout were described by Bayliss & Riley (1972).

For temperature-change experiments populations of 20–30 plants of one or both genotypes were grown initially in a lighted greenhouse at 20 °C until the leading tillers began to elongate prior to flowering. The plants were then transferred to controlled-environment chambers, with continuous lighting, at either 15 or

* This genotype will be referred to as 'Euploid'.

20 °C. Equilibration to these temperatures was checked by examination of temporary aceto-carmine squashes of anthers. When these showed chromosome pairing at first metaphase equivalent to that predicted by previous work (Bayliss & Riley, 1972), temperature-change experiments were performed.

The temperature changes were achieved by moving the populations of plants between similar growth rooms running at 15 or 20 °C. Tillers were then sampled at various time intervals after the temperature change. Samples consisted of anthers at first metaphase of meiosis from single tillers taken from each of two or three different plants in the populations. The anthers were fixed in acetic-alcohol (1:3) and subsequently permanent Feulgen-orcein stained squashes were prepared. Chiasma frequencies were scored in 20 metaphase plates per tiller and the results expressed as the mean number of chiasmata per chromosome – that is, mean number of chiasmata per cell/42.

Meiotic timing experiments were performed on both genotypes at both temperatures, using the sampling technique devised by Bennett, Chapman & Riley (1971). The rationale behind the method utilizes the synchrony of the three anthers in each floret of the wheat spike to compare the difference in meiotic stage between one of the three anthers fixed initially and the remaining two fixed at a measured time afterwards. Timing information of this nature was derived from 10–15 florets from each of eight spikes for each genotype at 15 and 20 °C. The intervals between a wide range of different stages were then collated to obtain the best fit of sequential time intervals throughout the course of the meiotic process.

3. RESULTS

(i) *Temperature transfer from 15 to 20 °C*

The initial experiment performed used samples taken at 24 h intervals after the temperature change to follow the change in chiasma frequency in N5DT5B. Euploid plants were similarly sampled as control. As shown in Fig. 1, the N5DT5B plants initially had a very low chiasma frequency which rose rapidly between 24 and 48 h after the temperature change and was quite stable from 72 h onwards. The Euploid genotype showed no significant response to the temperature change, ruling out the possibility that temperature-shock effects were influencing chiasma frequency.

To investigate more closely the period between 24 and 48 h after the temperature change, 3 and 6 h samples were taken during this period in a second experiment. Fig. 2 shows that the results of this experiment localized the response to between 36 and 42 h after the temperature change. Those samples having intermediate mean chiasma frequencies showed a large degree of variability. This was to be expected since when chiasma frequency was changing very rapidly with time, small differences in time of fixation or the stage of metaphase at which counts were made would show large differences in chiasma frequency.

In order to eliminate these sources of error a linear regression was fitted to the points lying in the period of maximum response over time and the regression was

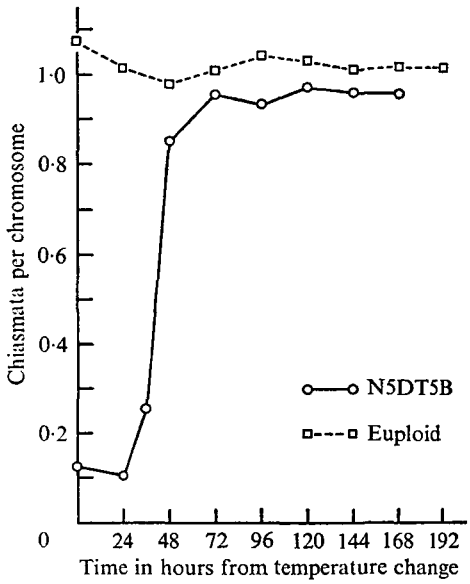

Fig. 1. The general relationship of chiasma frequency to time after a temperature change from 15 to 20 °C in Euploid and N5DT5B.

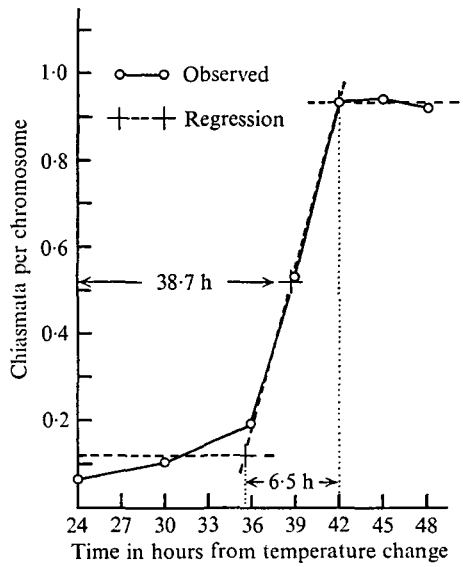

Fig. 2. The accurate relationship of chiasma frequency to time after a temperature change from 15 to 20 °C in N5DT5B.

extrapolated to intercept the mean initial and mean final chiasma frequencies. These initial and final means were calculated from the chiasma frequencies of plants sampled at those times at the beginning and end of the experiments when chiasma frequency did not change significantly with time. Though the true response is probably sigmoid there are insufficient data to permit this curve to be fitted.

The regression analysis used allowed the response to be defined in terms of two parameters – the time taken for the chiasma frequency to change from the initial to the final mean value and the time taken from the temperature switch to the mid-point of the shift in chiasma frequency. The fitted lines are shown in Fig. 2. The period of change was 6·5 h and the period from the imposition of the higher temperature to the mid-point of response in chiasma frequency was 38·7 h.

(ii) *Temperature transfer from 20 to 15 °C*

An initial experiment was conducted with samples taken every 24 h after the temperature change from 20 to 15 °C. The results (Fig. 3) show the fall in chiasma frequency to occur later and more gradually than the rise in chiasma frequency in the previous experiments. This is emphasized by the figures extrapolated from the fitted regression line.

To define more accurately the flex points of this downward response, a second experiment was performed with more frequent samples around these times. Unfortunately, as shown in Fig. 4, a fluctuation in the temperature of the 20 °C

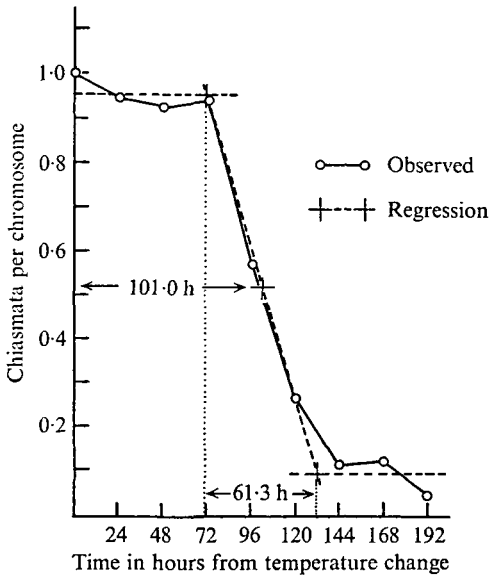

Fig. 3. The relationship of chiasma frequency to time after a temperature change from 20 to 15 °C in N5DT5B.

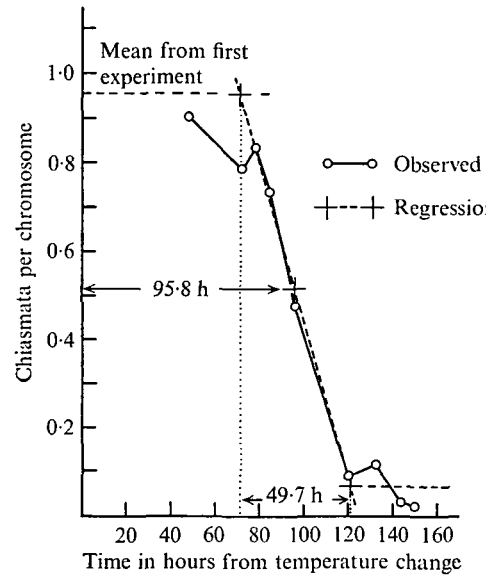

Fig. 4. The relationship of chiasma frequency to time after a temperature change from 20 to 15 °C in N5DT5B.

growth room prevented valid calculation of an initial high mean chiasma frequency for this experiment. However, regression analysis showed no significant difference between the regression coefficients of this and the first experiment. Extrapolation of the regression line from the second experiment to the high mean value obtained in the first experiment (shown as a dashed line in Fig. 4) showed the drop in chiasma frequency to begin at the same time, i.e. 72 h after the temperature change, in both experiments. As the second experiment included more frequent sampling at the lower end of the response, the regression line and low mean chiasma frequency for this experiment were used to calculate the position and duration of the temperature-sensitive stage. These calculations were performed in a similar way to those for the upward temperature change and showed the duration of the change in chiasma frequency to be 49·7 h and the mid-point of this response to be 95·8 h after the change in temperature.

(iii) *High-temperature treatment of Euploid*

To test whether or not the results obtained with N5DT5B were peculiar to this genotype, use was made of the reduction in chiasma frequency in Euploid material following high-temperature treatment. Euploid plants were exposed to a temperature of 35 °C for 12 h and then returned to 20 °C. This pulse treatment was used because continuous exposure to high temperatures caused chromosome stickiness and prevented scoring.

Chiasma frequency was checked immediately before and after the high-temperature treatment and thereafter at 12 h intervals from the end of the

https://doi.org/10.1017/S0016672300013719 Published online by Cambridge University Press

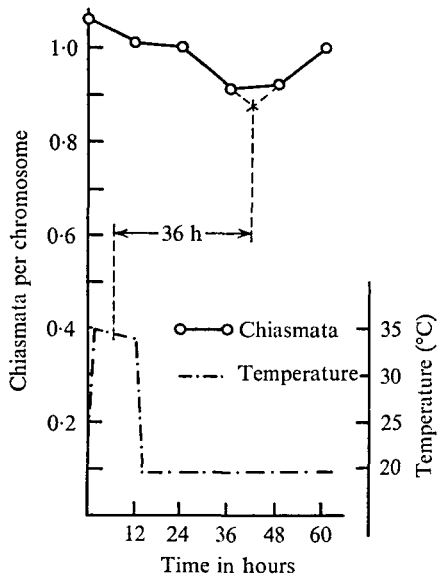

Fig. 5. The response of chiasma frequency in euploid after a 12 h high-temperature treatment.

treatment. The results, shown in Fig. 5, indicate that a reduction in chiasma frequency occurred at 36 and 48 h after the start of the high-temperature treatment, though pairing had returned to its original value by 60 h.

Assuming the mid-point of the chiasma frequency response to be at 42 h and the mid-point of the high temperature treatment to be at 6 h, the effect of the high-temperature treatment was detected 36 h after application.

(iv) *Meiotic timing experiments*

The results of timing the duration of meiosis in Euploid and N5DT5B at 15 and 20 °C are shown in Figs. 6 and 7. The start of leptotene was taken as simultaneous with the synchronous tapetal division. The dashed lines indicate borderlines between stages which could not be accurately placed. Similarly, very short stages such as diplotene–diakinesis and anaphase–telophase have not been separated, since accurate estimation of their individual durations was not possible.

Both genotypes show a very marked temperature response amounting to almost a doubling in duration for the 5 °C drop in temperature. The actual temperature coefficients are 1·78 for Euploid and 1·84 for N5DT5B. At 20 °C there are no significant differences between the genotypes either in terms of durations of individual stages or total duration. At 15 °C there is again probably not a significant difference in the overall durations. The main component of the increase in N5DT5B relative to Euploid is the extended duration of zygotene, possibly a consequence of the asynapsis at this temperature. The degree of chromosome contraction and the presence of characteristic loops of chromatin at the periphery of the nucleus (Bayliss & Riley, 1972) were used to distinguish the period equivalent to zygotene in the absence of chromosome pairing.

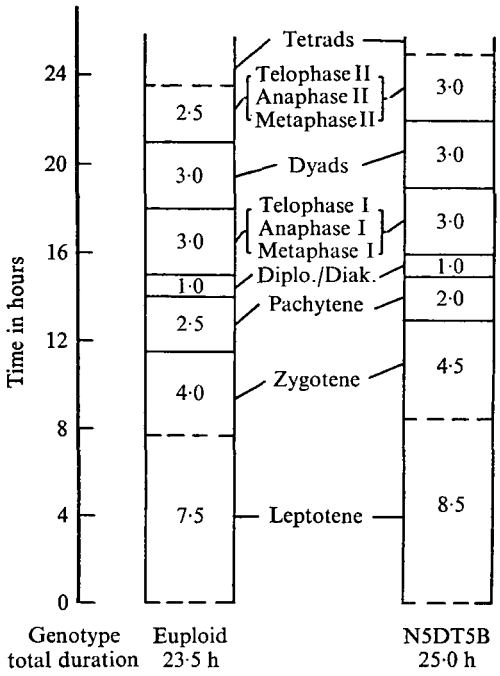

Fig. 6. The duration of meiosis and its constituent stages in Euploid and N5DT5B at 20 °C.

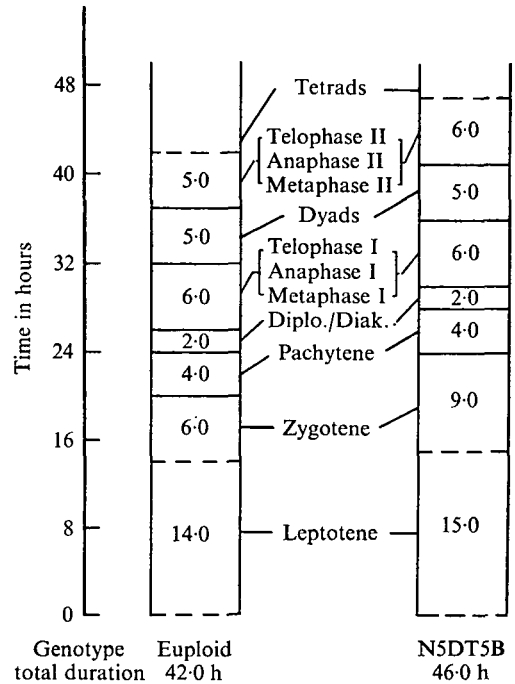

Fig. 7. The duration of meiosis and its constituent stages in Euploid and N5DT5B at 15 °C.

4. CONCLUSIONS AND DISCUSSION

Interpretation of the timing relationships measured in the temperature transfer experiments first requires consideration of the temperature regimes involved. Measurement, by means of inserted sensors, of the temperature within the spikes of plants subject to changes in environmental temperature have shown that the plants equilibrate to the new air temperature within 12 min of transfer (Bayliss, 1972). Thus the temperature change can be considered as occurring virtually instantaneously in terms of the subsequent time intervals measured. Additionally, after transfer the plants can be considered as growing at a constant temperature, and timing measurements thus refer to developmental processes occurring at that constant temperature.

Measurements of chiasma frequency at first metaphase of meiosis are made at a fixed point in the time scale defined by the developmental sequence preceding and including meiosis in the pollen mother cells. Thus, a sequence of measurements made at intervals in real time will refer to the condition of the pollen mother cells at these intervals prior to first metaphase.

The localization of changes in chiasma frequency to certain time periods after temperature change, and the presence of intermediate chiasma frequency values, in spite of the fast rate of change in plant temperature, indicate that the

Table 1. *The duration and relationships of the stages measured in defining the time of the sensitivity to temperature of chromosome pairing in euploid and nullisomic 5D tetrasomic 5B plants of wheat*

Parameter	Temperature	Euploid	N5DT5B
Meiotic duration	15 °C	42·0 h	46·0 h
	20 °C	23·5 h	25·0 h
Q_5 meiotic duration	—	1·78	1·84
Start of leptotene to M1	15 °C	26·0 h	30·0 h
	20 °C	15·0 h	16·0 h
Temperature sensitive stage to M1	15 °C	—	96·0 h
	20 °C	36·0 h	39·0 h
Q_5 of period between temperature sensitive stage and M1	—	—	2·47
Duration of temperature-sensitive stage	15 °C	—	49·7 h
	20 °C	—	6·5 h
Q_5 of duration of temperature-sensitive stage	—	—	7·65

pollen mother cells are sensitive to temperature for a specific period of time in their developmental sequence. Thus the time taken for the change in chiasma frequency from the initial to the final mean value is a measure of the duration of this *temperature-sensitive stage* at the final temperature of the experiment. Similarly the time between the temperature change and the mid-point of the chiasma frequency change represents the time before first metaphase that the temperature-sensitive stage occurs.

Table 1 summarizes these timing data on the temperature-sensitive stage and duration of meiosis. Comparison shows that at both 15 and 20 °C the temperature-sensitive stage is in the premeiotic interphase, 66 and 23 h respectively before the start of leptotene. As N5DT5B is asynaptic, showing failure of zygotene chromosome pairing at low temperatures (Bayliss & Riley, 1972), it must be concluded that events affecting chromosome pairing take place in the premeiotic interphase, a considerable time before the start of leptotene.

That this result is not a peculiarity of the genotype is demonstrated by the effect of high-temperature treatment on the Euploid. Again the period of sensitivity occurred 36 h before first metaphase – that is, at the same time as the sensitive stage in N5DT5B grown at 20 °C. This confirms the earlier conclusion (Bayliss & Riley, 1972) that the Euploid and N5DT5B differ only in the sensitivity of chromosome pairing to temperature and not in the nature of the pairing failure.

As the work of Grell & Chandley (1965) has shown crossing-over to be sensitive to temperature during premeiotic DNA synthesis, it is of interest to relate the sensitive stage in wheat to the time of DNA synthesis. Unfortunately critical experiments to test the relationship in N5DT5B were unsuccessful for technical reasons. However, the work of Bennett *et al.* (1971), Bennett & Smith (1972) and

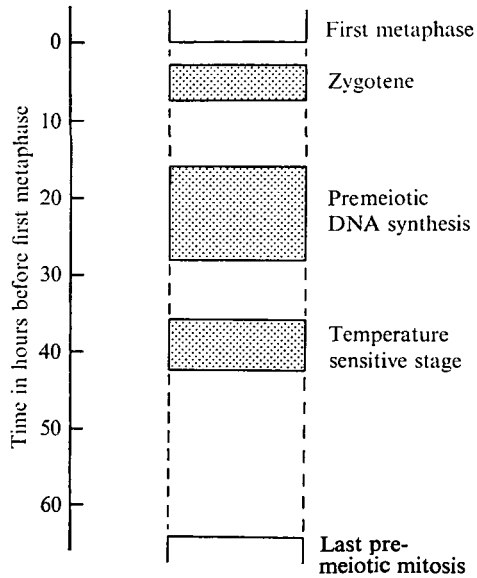

Fig. 8. The position of the temperature-sensitive stage in N5DT5B at 20 °C with relation to zygotene and premeiotic DNA synthesis.

Bennett (personal communication) has demonstrated that in euploid wheat, at 20 °C, premeiotic DNA synthesis occurs during the 10 or 12 h immediately prior to the start of leptotene and that the latest time at which the asynchronous premeiotic mitoses can occur is approximately 48 h before the start of leptotene. These data for euploid wheat are assumed to be applicable to N5DT5B at 20 °C as other meiotic timing relationships measured in both genotypes at 20 °C have been very similar.

Assembling this information (Fig. 8) shows that at 20 °C the temperature-sensitive stage occurs in the premeiotic interphase at least 25 h after the last premeiotic mitosis and 9 h before the start of DNA synthesis. This is the first time that the occurrence of a premeiotic stage determining meiotic pairing has been demonstrated in a higher plant. The only instance of a possibly similar situation is the demonstration of a premeiotic sensitivity to a bivalent interlocking shown in *Locusta migratoria* (Buss & Henderson, 1971).

There is no direct evidence as to the mechanism by which chromosome pairing is influenced during the temperature-sensitive stage though two observations are suggestive. First the temperature coefficients of the interval between the temperature-sensitive stage and first metaphase are reasonably similar to those for the duration of meiosis but the coefficient for the duration of the stage is markedly different. Thus at low temperatures, when there is a failure of chromosome pairing, the temperature-sensitive stage is of much greater length than predicted by the temperature coefficients of the other meiotic components. This suggests sensitivity to temperature of a specific event or sequence of events which limit the rate of progress through this premeiotic stage. Intermediate chiasma frequencies in the

transfer experiments can then be explained as arising in pollen mother cells which spent part of their sensitive stage at one temperature and part at another, the proportion spent at the higher temperature determining the proportion of the maximum chiasma frequency attained.

The second relevant observation is that of Bayliss & Riley (1972) on the behaviour of an isochromosome in a nullisomic 5D genotype. As the arms of the isochromosome fail to pair with each other at low temperatures in the absence of 5D, though induction of asynapsis in euploid wheat by colchicine does not disrupt isochromosome pairing (Driscoll & Darvey, 1970), it could be argued that the failure of pairing in N5DT5B resulted from disruption of detailed synapsis rather than from the disturbance of the relative positions of chromosomes which has also been shown to interrupt pairing.

Thus it is possible to speculate that the increased sensitivity of chromosome pairing to extremes of temperature, in the absence of chromosome 5D, is initiated by the modification of events associated with the mechanism of chromosome pairing.

Previous use of temperature treatments to locate the time of crossing-over or chiasma formation has produced widely different results in the variety of organisms studied. Assuming that the apparent similarities of meiosis in all organisms precludes the use of species differences to explain the diversity of results, it is necessary to reconcile the variation to fit a common sequence of events producing chromosome pairing, chiasmata and recombination.

One interesting relation apparent between previous reports is that high-temperature treatments invariably increase the amount of genetic recombination while decreasing the chiasma frequency (compare Grell & Chandley, 1955; Maguire, 1968; Lamb, 1971; and Abel, 1965, with Henderson, 1962; and Peacock, 1970). An apparently similar contradiction exists in the asynaptic mutant of maize originally described by Beadle (1933). Here an asynaptic type of pairing failure and consequent low chiasma frequency appears to be associated with an increase in recombination (Grell, 1969).

The timing work mentioned in the introduction has generally shown the sensitive stage to occur before zygotene when measurements of crossing-over were made, but during zygotene or pachytene when chiasma frequency measurements were used. As a first approximation, this suggests there are at least two components, independently affected by temperature, in the sequence of events producing chiasmata and recombination.

The absolute prerequisite for both chiasma formation and recombination is the pairing of homologous chromosomes. Though this is seen to occur at zygotene, effective pairing might also occur during the premeiotic interphase (Grell, 1969). Thus there is no absolute test of whether the temperature effects on chiasma frequency and recombination, described above, result from direct action on chromosome pairing or in some way predetermine a subsequent pairing pattern. However, it is important to recognize that chromosome pairing, whether affected directly or indirectly, must be the first process susceptible to temperature treatments.

The results presented in this paper, involving sensitivity to low temperatures, may thus be interpreted as the first instance of a change in chromosome pairing produced at an early, premeiotic stage. An example of a temperature-induced change in cross-over frequency occurring at zygotene is provided by the study of Lu (1969) in *Coprinus lagopus*.

The concept of a multi-stage process influencing crossing-over and chiasma frequency was also suggested by the work of Lawrence (1961*a*, *b*) on *Lilium longiflorum* and *Tradescantia paludosa*. In both these species, radiation treatments during premeiotic interphase reduced chiasma frequency whereas treatments at pachytene or diplotene increased chiasma frequency.

A two-stage process had previously been invoked by Faberge (1942) to explain the mechanism of chromosome pairing. He envisaged a rough initial alignment of homologous chromosomes followed by a detailed synaptic stage. In present terms the rough alignment would occur during premeiotic interphase and might well be related to the somatic association effects also suggested as controlling chromosome pairing (Feldman, 1968; Stack & Brown, 1969). The precise pairing dependent upon molecular recognition would then occur at a later stage, possibly zygotene, in association with the synaptonemal complex (Moses, 1969). Obviously the ultimate extent of effective pairing may be altered during the early alignment stage or during detailed recognition, but the later events are dependent upon premeiotic pairing determination.

Present information on the time of events influencing chromosome pairing and recombination are too fragmentary to allow more than speculation on the nature and number of processes involved. However, there would now seem to be sufficient evidence (see also reviews by Grell, 1969; Stern & Hotta, 1969) strongly to suggest a complex sequence of events controlling chromosome pairing and recombination. What is now certain is that the processes producing meiotic chromosome pairing, though they may terminate in zygotene or pachytene, are initiated early in the premeiotic interphase, before DNA replication takes place.

REFERENCES

Abel, W. O. (1965). Über den Zeitpunkt des Crossing-over und der Chromosomeuperdoppling bei *Sphaerocarpis*. *Zeitchrift fur Vererbungslehre* **96**, 228–233.

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

Bayliss, M. W. & Riley, R. (1972). An analysis of temperature-dependent asynapsis in *Triticum aestivum*. *Genetical Research*. (In the Press.)

Bennett, M. D., Chapman, V. & Riley, R. (1971). The duration of meiosis in pollen mother cells of wheat, rye and *Triticale*. *Proceedings of the Royal Society of London* B **178**, 259–275.

Bennett, M. D. & Smith, J. M. (1972). The effect of polyploidy on meiotic duration and pollen development in cereal anthers. *Proceedings of the Royal Society of London* B **181**, 81–107.

Buss, M. & Henderson, S. A. (1971). Induced bivalent interlocking and the course of meiotic chromosome synapsis. *Nature* **234**, 243–246.

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

Faberge, A. C. (1942). Homologous chromosome pairing: the physical problem. *Journal of Genetics* **43**, 121–144.

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

Grell, R. (1969). Meiotic and somatic pairing. In *Genetic Organisation* vol. I (ed. E. W. Caspari and A. W. Ravin), pp. 361–492. New York: Academic Press.

Grell, R. & Chandley, A. C. (1965). Evidence bearing on the coincidence of exchange and DNA replication in the oöcyte of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the U.S.A.* **53**, 1340–1346.

Henderson, S. A. (1966). Time of chiasma formation in relation to the time of desoxyribonucleic acid synthesis. *Nature* **211**, 1043–1047.

Henderson, S. A. (1962). Temperature and chiasma formation in *Schistocerea gregaria*. II. Cytological effects and the mechanism of heat induced univalence. *Chromosoma* **13**, 437–463.

John, B. & Lewis, K. R. (1965). The meiotic system. *Protoplasmatologia*, Band VI. Springer-Verlag.

Lawrence, C. W. (1962*a*). The effect of irradiation of different stages on microsporogenesis and chiasma frequency. *Heredity* **16**, 83–89.

Lawrence, C. W. (1961*b*). The effect of radiation on chiasma frequency in *Tradescantia*. *Radiation Botany* **1**, 92–96.

Lamb, B. C. (1971). Some details and effects of the premeiotic control of recombination frequencies in *Neurospora crassa*. *Genetical Research* **18**, 255–264.

Lu, B. C. (1969). Genetical recombination in Coprinus. I. The precise timing as revealed by temperature treatment experiments. *Canadian Journal of Genetics and Cytology* **11**, 834–847.

Maguire, M. (1968). Evidence of the stage of heat induced cross-over effect in maize. *Genetics* **60**, 353–362.

McNelly-Ingle, C. A., Lamb, B. C. & Frost, L. C. (1966). The effect of temperature on recombination frequency in *Neurospora crassa*. *Genetical Research* **7**, 169–183.

Moses, M. J. (1969). Structure and function of the synaptinemal complex. *Genetics* **61**, 41–51.

Peacock, W. J. (1970). Replication, recombination and chiasmata in *Goniaea australasiae* (Orthoptera: Acrididae). *Genetics* **65**, 593–617.

Stack, S. M. & Brown, W. V. (1969). Somatic pairing, reduction and recombination: an evolutionary hypothesis of meiosis. *Nature* **222**, 1275–1276.

Stern, H. & Hotta, Y. (1969). DNA synthesis in relation to chromosome pairing and chiasma formation. *Genetics* **61**, 27–39.