PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Inferences from Genetical Evidence on the Course of Meiotic Chromosome Pairing in Plants

G. A. Dover and R. Riley

Phil. Trans. R. Soc. Lond. B 1977 **277**, 313-326
doi: 10.1098/rstb.1977.0020

Phil. Trans. R. Soc. Lond. B. **277**, 313–326 (1977) [313]
Printed in Great Britain

Inferences from genetical evidence on the course of meiotic chromosome pairing in plants

By G. A. Dover
Department of Genetics, University of Cambridge, Downing Street, Cambridge
and R. Riley, F.R.S.
Plant Breeding Institute, Maris Lane, Trumpington, Cambridge

[Plates 1–3]

Meiotic chromosome pairing is a process that is amenable to genetic and experimental analysis. The combined use of these two approaches allows for the process to be dissected into several finite periods of time in which the developmental stages of pairing can be precisely located. Evidence is now available, in particular in plants, that shows that the pairing of homologous chromosomes, as observed at metaphase I, is affected by events occurring as early as the last premeiotic mitosis; and that the maintenance of this early determined state is subsequently maintained by constituents (presumably proteins) that are sensitive to either colchicine, temperature or gene control.

A critical assessment of this evidence in wheat and a comparison of the process of pairing in wheat with the course of meiotic pairing in other plants and animals is presented.

1. Introduction

Several recent studies in plants have shown that the precision of synapsis of homologous chromosomes at zygotene is the final outcome of a series of sequential steps in the process of pairing during the period of development from the last mitotic division to the end of leptotene. The dissection of this critical developmental interval is possible with the combined use of genetic mutants that block one or other of the steps in the process, and of environmental agencies that cause such blocks to be phenocopied. The concept of an association of homologous chromosomes occurring during the last premeiotic mitosis and the subsequent G1 of the meiotic cycle is not new. It is based on observations in plants and animals that go back to the early part of the century (admirably reviewed by Grell 1969) and on contemporary observations using more refined optical techniques (Maguire 1974, and this volume). The early cytologists were careful to note that in a diverse range of species the chromosomes at the last mitotic anaphase and telophase were associated in pairs and that the number of prochromosomes, observed during the premeiotic interphase, was haploid in number (Montgomery 1901, Dublin 1905; Overton 1909; Guyénot & Naville 1933). More recent observations on the haploid number of prochromosomes have been made in the liverwort, *Sphaerocarpus* (Abel 1965), and in the flowering plants *Haplopappus* and *Rhoeo* (Stack & Brown 1968). Identification of chromosomes by shape in *Brachystola magna* (Sutton 1902) showed that the associations at the last anaphase were indeed homologous. Similarly, in maize, Maguire (1974) has substantiated the early findings and has found that the homologous associations extend back as far as the last premeiotic metaphase.

There are observations that have failed to reveal associations of any sort before the intimate synapsis of homologues at zygotene. For example, Walters (1970) found that, during the pre-leptotene contraction period in *Lilium longiflorum*, the chromosomes were distributed at random and that there was no evidence of alignment or association of homologues. Furthermore the analysis of fine-structure by serial sections of early first meiotic prophase in *Locusta migratoria* (Moens 1973) has shown that associations can be both homologous and non-homologous in nature. Variation in the course of meiotic pairing in different groups of organisms, presumably the result of selection for alternative adaptive forms of meiotic development, is to be expected. Whatever the precise course taken by the chromosomes in the pairing process, it is clear that homologous synapsis is a developmental process consisting of a series of sequential steps that are amenable to genetical and experimental analysis.

TABLE 1. CLASSIFICATION OF THE CHROMOSOMES OF *T. AESTIVUM*

homoeologous groups	genomes		
	A	B	D
1	1A	1B	1D
2	2A	2B	2D
3	3A	3B	3D
4	4A	4B	4D
5	5A	5B	5D
6	6A	6B	6D
7	7A	7B	7D

2. GENETICAL ANALYSIS OF THE PAIRING PROCESS

Mutants that affect the pairing process either before (asynaptic) or after (desynaptic) zygotene pairing have been reported extensively and reviewed (Rees 1961; Riley & Law 1965). Several recent findings, however, suggest that the pairing of chromosomes is a multistage process and that the genetic controls of these steps can be specific to chromosomes and sex. For example, analysis of X-ray induced meiotic aberrations in *Pisum* (Gottschalk 1973) has shown that there may be up to 50 genes controlling stages of microsporogenesis. One large group of 20 desynaptic mutants falls into several intergenic complementation groups, each presumably controlling a different developmental step. A second group of ten male-sterile mutants allows meiosis to proceed normally until a point of failure is reached that is specific to each mutant. Furthermore the same mutants have no effect on macrosporogenesis; a situation analogous to that in *Drosophila melanogaster* in which Baker & Hall (1976) found that ethylmethanesulphonate (EMS)-induced meiotic mutants were frequently sex specific (see also Lindsley, this volume).

Controls of pairing that are specific to single pairs of homologues have been reported in *Hypochoeris* and *Crepis* (Parker 1975). Preliminary results of breeding tests suggest that there may be at least two loci in *Hypochoeris* that effect this control (J. S. Parker, personal communication).

However, it is in polyploid plants that the most detailed information has been obtained on the extent and nature of the controls of meiotic pairing; and manipulation of these controls has allowed for the beginning of a picture of the process of pairing to be composed. Work in hexaploid wheat, *Triticum aestivum*, (Okamoto 1957; Riley & Chapman 1958) showed that a locus (*Ph*) on chromosome 5B restricts meiotic pairing to homologues despite the genetic

similarities of the three genomes (table 1) (see Sears 1969 and Riley 1974 for reviews). Similar gene-effected restrictions have been found subsequently in hexaploid oats, *Avena sativa* (Rajhathy & Thomas 1972) and possibly also in hexaploid, *Festuca arundinacea* and *F. rubra* (Jauhar 1975*a*).

3. Genetics of pairing in wheat

Genotypes of wheat that vary in the degree of meiotic pairing prove to be ideal for experiments aimed at elucidating the mechanisms through which control and the process of pairing are effected. Experimental manipulation of pairing is possible in several ways. First, through the exploitation of natural variation at loci in two related diploids, *Aegilops speltoides* and *Ae. mutica* that suppress the 5B effect (Dover & Riley 1972*a*). Secondly, with the introduction of supernumerary chromosomes of the diploids that affect the levels of pairing in certain genotypes. Thirdly, by the use of genotypes of wheat, nullisomic for chromosome 5D, in which meiotic chromosome pairing is temperature dependent (Bayliss & Riley 1972*a*, *b*), and fourthly, by the induction of asynapsis in different genotypes resulting from the application of colchicine to developing anthers (Dover & Riley 1973; Dover 1972).

The controls of meiotic pairing in wheat are complex (see Dover 1973, for review). There are at least two loci in both *Ae. speltoides* and *Ae. mutica* that suppress *Ph* in interspecific hybrids with wheat (figure 2*b*) (Dover & Riley 1972*a*; Kimber & Athwal 1972). Allelic variation at these two loci generates a series of hybrids in which the pattern of pairing ranges from almost complete asynapsis to full homoeologous pairing of all chromosomes in multivalents (Vardi & Dover 1972). The presence of supernumerary chromosomes of *Ae. speltoides* or *Ae. mutica* does not directly affect the levels of pairing determined by the pairing-control genes. However, hybrids normally having very high levels of pairing in the absence of chromosome 5B, are asynaptic in the presence of supernumerary chromosomes (Dover & Riley 1972*b*). The supernumerary chromosomes compensate for the absence of chromosome 5B in preventing the pairing of homoeologues. Similar restrictions of pairing are caused by the supernumeraries present in species of the *Lolium–Festuca* complex (Evans & Macefield 1972, 1974). Recent observations of Jauhar (1975*b*) that supernumerary chromosomes in this species group do not necessarily reduce levels of homoeologous pairing need to be treated with caution. A complete analysis is necessary of the degree of pairing affected by the normal complement in the between- and within-family variance of single crosses, before the supernumerary chromosome contribution to variation in pairing can be assessed.

Chromosome 5D of wheat carries a locus that normally buffers the meiotic pairing process from extremes of temperature (Riley 1966). In its absence plants held below 19 °C and above 29 °C have sharply reduced levels of pairing (Bayliss & Riley 1972*a*). Evidence on meiotic processes has been obtained from work on the temperature-dependent pairing pattern in 5D-deficient genotypes (see §5).

Additional evidence on the pairing process has been obtained with induction of asynapsis by colchicine (Driscoll, Darvey & Barber 1967; Dover 1972; Dover & Riley 1973; Shepard, Boothroyd & Stern 1974). Injection of 0.5 and 0.01 % colchicine into anthers, at different premeiotic stages of development, of genotypes of wheat and wheat/*Aegilops* hybrids having varying levels of pairing, has allowed the critical steps in the pairing process to be placed at an early stage of the meiotic G1.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

4. The premeiotic interphase in wheat

Stages in the development of pollen mother cells from the last premeiotic mitosis to the beginning of tetrad formation are shown in figure 1. The time and durations of premeiotic DNA synthesis, the 5D temperature-sensitive stage, the colchicine-sensitive stage and the occurrence of intranuclear fibrils are also indicated. The duration and diagnostic features of stages 1, 2 and 3 in figure 1 are listed in table 2, compiled from data of Bennett, Rao, Smith & Bayliss (1973). The precision of timing, in conjunction with optical and electron-microscopical identification of individual stages, has proved crucial for a hypothesis to be constructed about the process of pairing in relation to the timing of the temperature and colchicine sensitive stages. Consideration of all of the cytological features listed in table 2 makes identification of individual stages unambiguous.

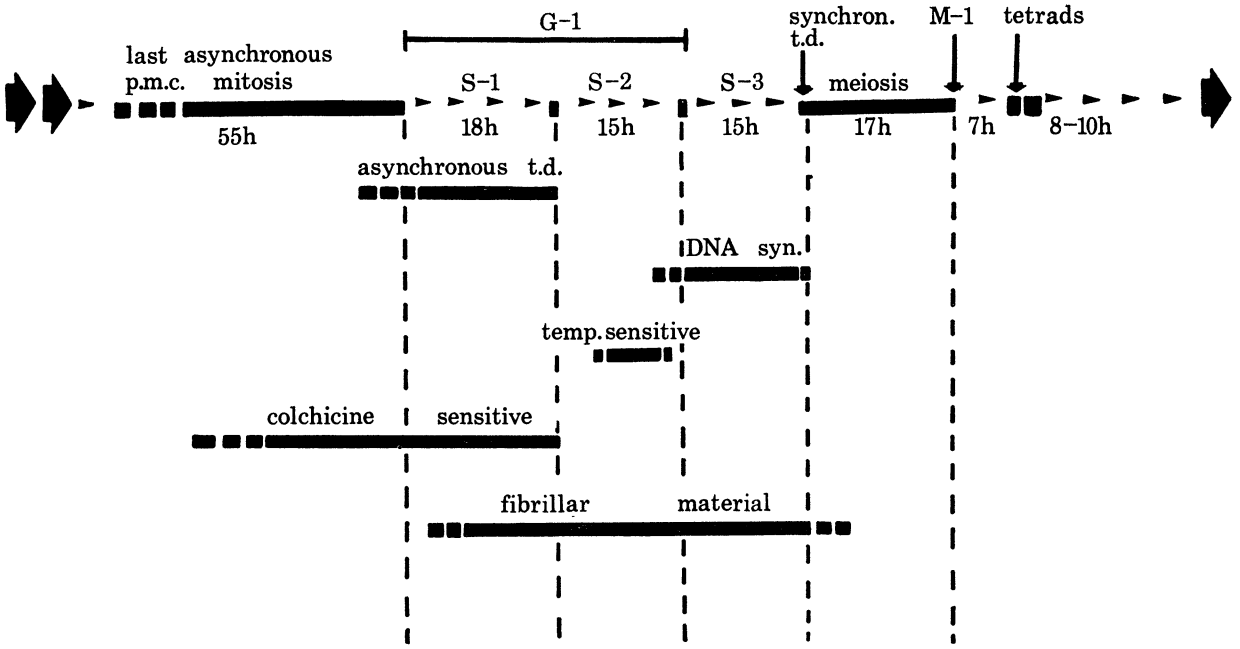


Figure 1. Stages of development of pollen mother cells of *Triticum aestivum* from the last premeiotic mitosis to tetrad formation. Durations of stages at 20 °C are indicated in hours. Horizontal bars represent the limits at which stages are sensitive to colchicine or low temperatures with respect to the degree of pairing observed at first meiotic metaphase. The times of DNA synthesis, tapetal cell divisions (t.d.) and the appearance of intranuclear fibrillar material are also indicated.

The times of the last three somatic cell cycles increase from 25 to 35 to 55 h (see Bennett, this volume), and during this period the archesporial cells are asynchronous. Cells completing the last mitotic division accumulate in G1 and all subsequent development of pollen mother cells is synchronous. The adjacent cells of the tapetum go through a penultimate asynchronous division terminating with stage 1 of the pollen mother cells; and a final synchronous division coincidental with the beginning of leptotene (figure 1).

Fine-structural analysis of nuclei of pollen mother cells from anthers at stages 1, 2 and 3 (Bennett, Stern & Woodward 1974) has shown the presence of bundles of fibrillar material, 1.2 μm × 0.2 μm in dimensions, lying between the chromatin and the nuclear membrane during stage 1, figure 6*b*. In stage 2 the chromatin is separated from the membrane by a 200 nm

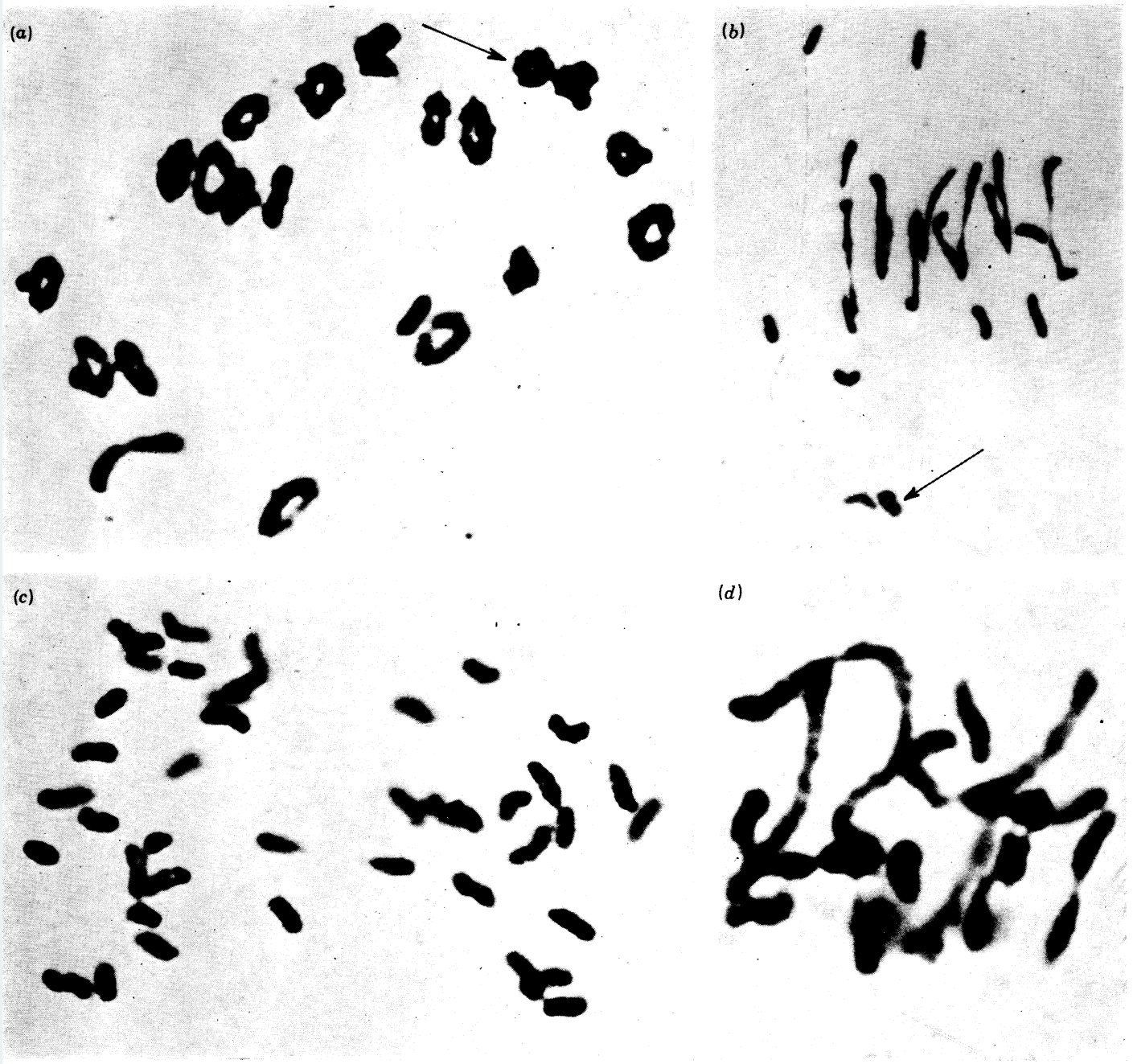


FIGURE 2. First metaphase in pollen mother cells of (*a*) *Triticum aestivum* nullisomic 5D, tetrasomic 5B, monoisosomic $5B^L$ at 20 °C. $2n = 42 +$ isosome $5B^L$ (arrow indicates isochromosome ring); (*b*) *Triticum aestivum*, diisosomic $5B^L \times$ *Aegilops mutica* (high pairing line); $2n = 27 +$ isosome $5B^L$ (arrow indicates isochromosome ring); (*c*) same genotype as (*a*) at 15 °C; (*d*) same genotype as (*b*) treated with 0.5 % colchicine at stage 3 and leptotene (see figure 1), showing pairing of chromosomes in multivalents.

(*Facing p.* 316)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

wide zone that is less electron dense and contains no chromatin. By stage 3, paracrystalline bodies, representing either a reorganization or degradation of the fibrillar material, become apparent. Lateral elements and the complete synaptonemal complex are fully apparent during leptotene and zygotene respectively.

Microdensitometry of Feulgen stained anther squashes shows that during stage 3 (figure 1) both tapetal and pollen mother cell nuclei undergo synchronous DNA synthesis and their DNA contents increase from 2C to 4C. Autoradiographic studies confirm the time and duration of premeiotic DNA synthesis (Bennett, Chapman & Riley 1971).

TABLE 2. DIAGNOSTIC FEATURES OF STAGES OF DEVELOPMENT OF POLLEN MOTHER CELLS OF *TRITICUM AESTIVUM* FROM THE LAST PREMEIOTIC MITOSIS TO THE BEGINNING OF LEPTOTENE

	stage 1	stage 2	stage 3
duration (h)	18	15	15
DNA amount	2C	2C	4C
relative appearance of nucleus	diffuse, reticulate, chromatin threads visible	contracted, denser, darker staining	large, densely staining
nucleoli	usually three within nucleus	usually three within nucleus	one; peripheral to nucleus
callose deposition	absent	beginning	complete
tapetal cell divisions	present	absent	absent
fine structure	chromatin adjacent to membrane; fibrillar material present.	chromatin separate from membrane; fibrillar material present.	chromatin separate from membrane; fibrillar material, paracrystalline bodies and some lateral elements present.

5. THE TIMING OF THE TEMPERATURE-SENSITIVE STAGE

Populations of plants used in different temperature treatments were initially grown at 20 °C and transferred before meiosis to controlled environment chambers which were continuously lighted. Below 15 °C, the majority of cells in wheat nullisomic 5D tetrasomic 5B are completely asynaptic, figure 2*c*, plate 1, in contrast to cells of euploid wheat that show little reduction in chiasma frequency at this temperature (Bayliss & Riley 1972*a*).

Temperature-switch experiments have been used to locate the period at which chiasma frequency is sensitive to temperature. As the timing measurements are made at a constant temperature after the temperature change and the change in chiasma frequency is 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 (Bayliss & Riley 1972*b*). Tillers are sampled at 3 h and 6 h intervals after the temperature change. Figure 3 depicts the response in chiasma frequency as scored at first metaphase of wheat nullisomic 5D tetrasomic 5B after a temperature switch from 15 to 20 °C. Linear regression lines, fitted to the curves, show that the mid-point of response in first metaphase chiasma frequency is 38.7 h after the temperature change, and the period of change is 6.5 h. Reciprocal experiments in which the temperature is switched from 20 to 15 °C, figure 4, show the mid-point to be 101 h after the change and 61.3 h in duration. Measurement, by means of inserted sensors, showed that the plants equilibrated to the new temperature within 12 min of transfer (Bayliss 1972).

The locating of changes in first metaphase chiasma frequency to certain time intervals after

the temperature change, indicates that the pollen mother cells are sensitive to temperature for a specific period of time in their developmental sequence. The duration of this period is the time taken for the change in chiasma frequency from the initial to the final mean value. Timing of the duration of meiosis at 20 °C (Bennett & Smith 1972) and at 15 °C (Bayliss & Riley 1972*b*) clearly indicates that at either temperature the temperature-sensitive stage lies in stage 2 of the premeiotic interphase (figure 1) and before premeiotic DNA synthesis that occurs in stage 3.

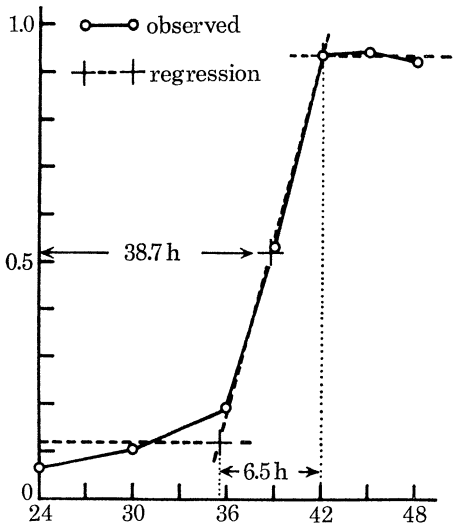


FIGURE 3. The relation of chiasma frequency to time after a temperature change from 15 to 20 °C in *T. aestivum* nullisomic 5D tetrasomic 5B (from Bayliss & Riley 1972*b*).

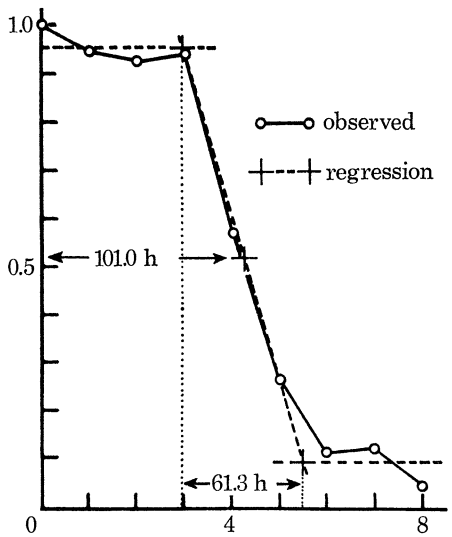


FIGURE 4. The relation of chiasma frequency to time after a temperature change from 20 to 15 °C in *T. aestivum* nullisomic 5D tetrasomic 5B (from Bayliss & Riley 1972*b*).

One important clue to the nature of the cellular events, occurring in stage 2, that relate to temperature sensitivity of chiasma frequency, comes from studies on the pairing of arms of an isochromosome in 5D-deficient genotypes (Bayliss & Riley 1972*a*). At 20 °C, interarm pairing of an isochromosome is apparent (figure 2*a*). At 15 °C there is no evidence of an isochromosome

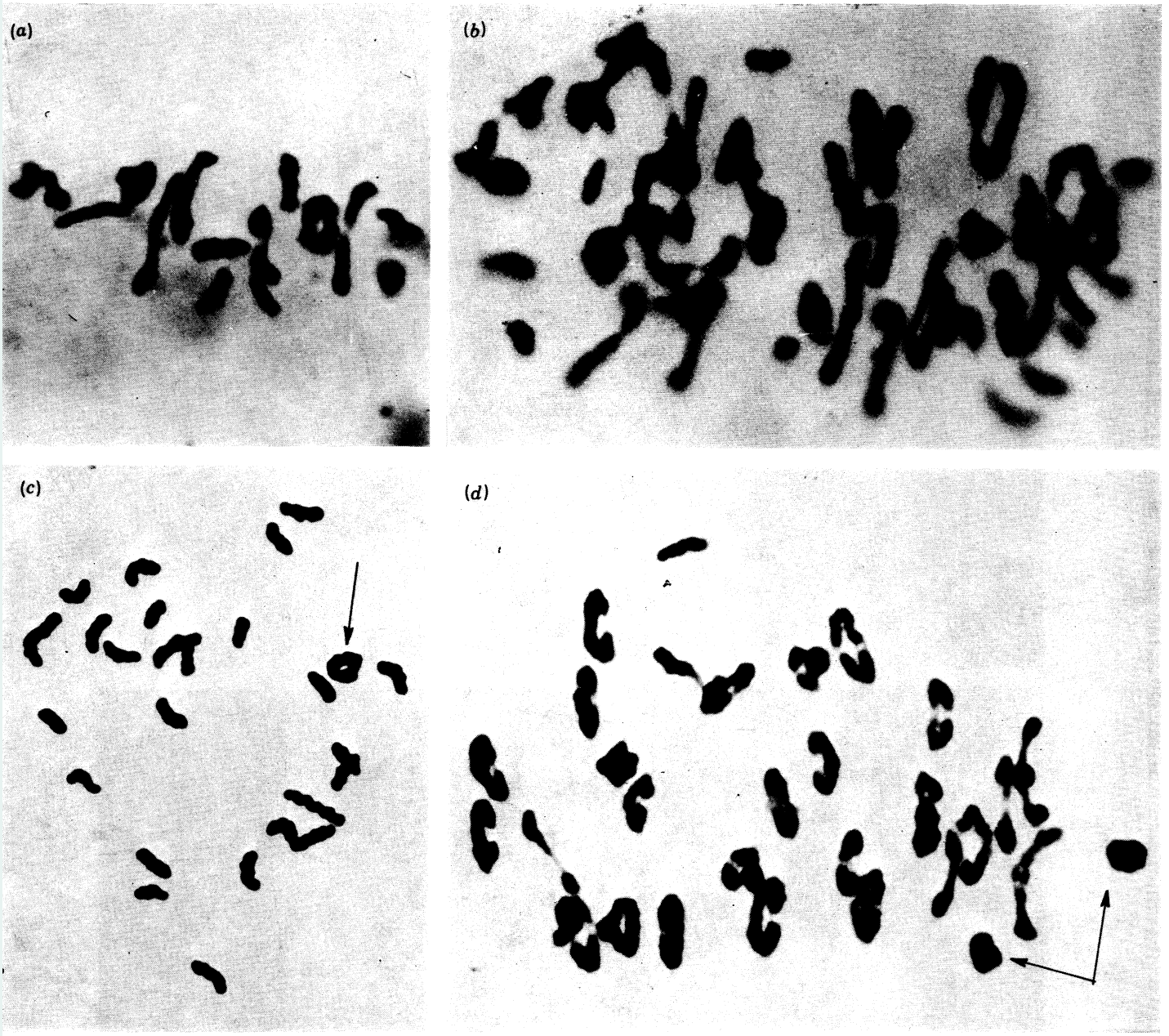


FIGURE 5. First metaphase in pollen mother cells of:

(*a*) Same genotype as figure 2*b*, treated with 0.01% colchicine at the last premeiotic mitosis. Cell from anther mosaic with low number of chromosomes, showing pairing of chromosomes in multivalents.

(*b*) Same genotype as figure 2*b*, treated with 0.01% colchicine at the last premeiotic mitosis. Cell from anther mosaic with high number of chromosomes, showing pairing of chromosomes in multivalents.

(*c*) Same genotype as figure 2*b*, treated with 0.5% colchicine at stage 1, (see figure 1). Arrow indicates isochromosome ring.

(*d*) Same genotype as figure 2*b*, treated with 0.5% colchicine at the last premeiotic mitosis. Tetraploid pollen mother cell showing bivalent pairing of all chromosomes and interarm pairing of isochromosomes (arrowed). (From Dover & Riley 1972*b*.)

(*Facing p.* 318)

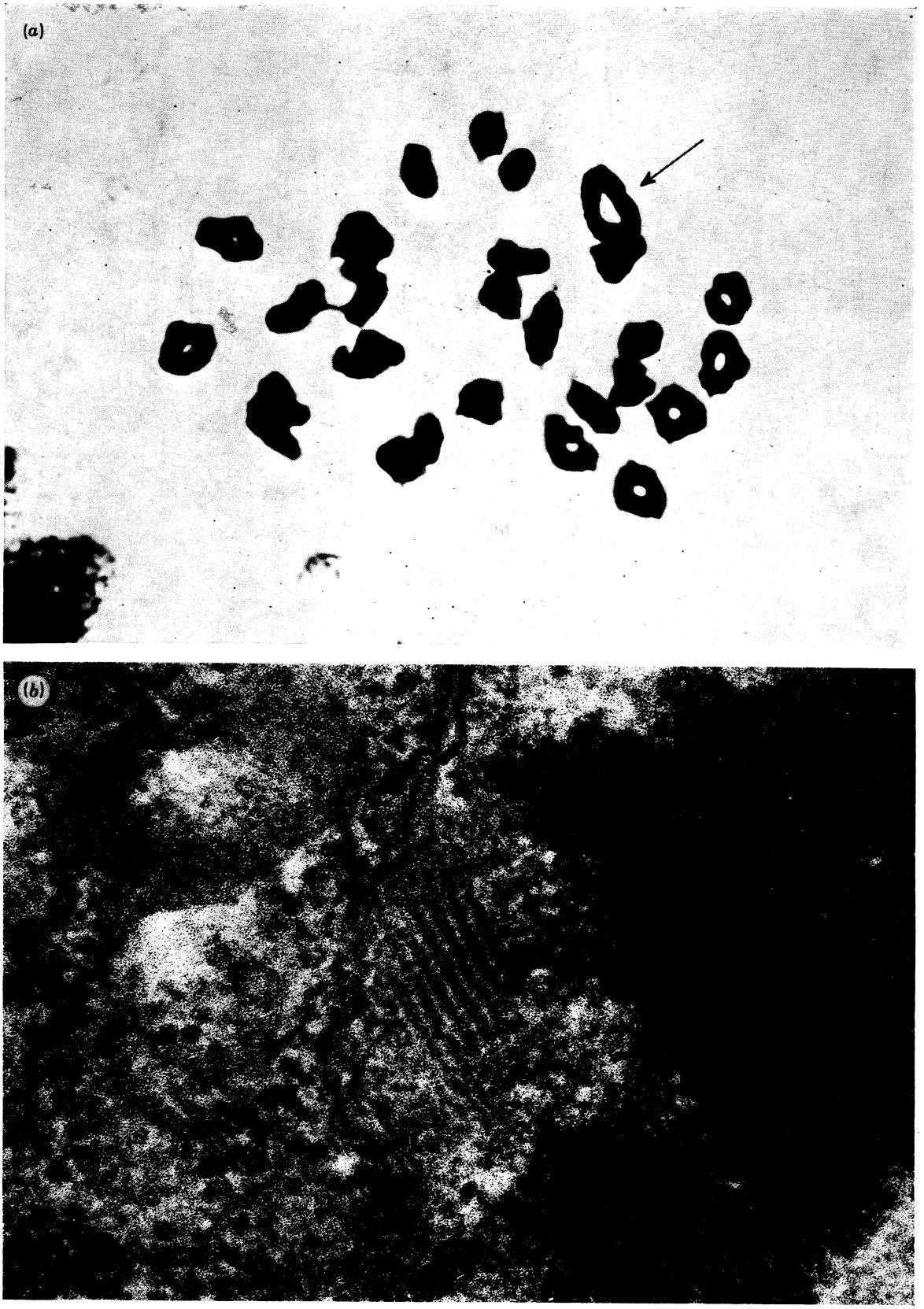


FIGURE 6. (*a*) Pollen mother cell with double the number of chromosomes from F1 hybrid of *Triticum aestivum* × *Aegilops mutica* having a wheat chromosome in the disomic condition and two supernumeraries; 26 bivalents of regular chromosomes, 2 small ring bivalents of supernumerary chromosomes and 1 ring quadrivalent of wheat chromosomes in the tetrasomic condition (arrowed) (from Vardi & Dover 1972).

(*b*) Electron micrograph of pollen mother cell of *Triticum aestivum*, showing fibrillar material located between the nuclear membrane and chromatin masses at stage 1 (see figure 1). (Magn. × 28000; from Bennett *et al.* 1974.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ring (figure 2*c*). Chiasma failure is brought about at low temperatures despite the proximity of the homologous arms of the isochromosome; and it is unlikely therefore that chromosome proximity is the factor affected by temperature to cause reduced chiasma frequency. This is in marked contrast to the pairing behaviour of an isochromosome under the influence of colchicine at stage 1 (figure 1) (see §6).

6. The timing of the colchicine-sensitive stage

Induction of asynapsis by colchicine depends on the time of application of the chemical to developing anthers (Driscoll *et al.* 1967; Dover 1972; Dover & Riley 1973; Shepard *et al.* 1974). In wheat, colchicine in aqueous solution is injected into the space formed by the leaf sheaths surrounding the immature spikes, and anthers are sampled and scored at first metaphase at varying time-intervals after injection. The time at which colchicine first takes effect is determined by the chromosome ploidy levels of the pollen mother cells and the adjacent tapetal cells; this being related to the numbers of failures of nuclear division after inhibition of spindle formation (Dover & Riley 1973). For example, tetraploid pollen mother cells and 16 ploid tapetal cells are observed after application of 0.5% colchicine during the last premeiotic mitosis of the pollen mother cells.

Injection of 0.5% colchicine at stages 2, 3 and leptotene (figure 1) has no observable effects on the levels of pairing characteristic of the plant genotype. Figure 2*b* depicts high levels of homoeologous pairing in wheat/*Ae. mutica* hybrids, due to the suppression of the 5B-effect by alleles at the *mutica* pairing-control loci (Vardi & Dover 1972; Dover & Riley 1972*a*). No reduction in pairing occurs in this genotype after application of the colchicine at stages 2, 3 and leptotene (figure 2*d*) despite the obvious disruption of the first metaphase spindle induced by colchicine leading to the formation of tetraploid monads. Anthers at stage 3 and leptotene that are cultured in modified White's medium with colchicine show no effect of colchicine on synapsis. This is in contrast to the asynapsis induced during leptotene by colchicine in cultured anthers of *Lilium* (Shepard *et al.* 1974).

Injection of 0.5% colchicine at stage 1 causes two meiotic irregularities that may be causally related: the induction of asynapsis as observed at first metaphase (figure 5*c*, plate 2) (Dover & Riley 1973) and the induction of poreless pollen (Dover 1972, 1974). It is known that the position of pores is related to the polarities of the spindles at second meiotic division. The coincidence of failure of pairing and pore formation could be related to the absence, under colchicine, of pole determinants operating at stage 1 (discussed in §8).

Application of 0.5% colchicine at the last premeiotic mitosis induces tetraploid pollen mother cells in which all chromosomes are paired as bivalents despite the tetrasomic condition of each homologue or the presence of high-pairing alleles of *Aegilops* spp. (figure 5*d*). It is possible that such 'ski-pairing' (Barber 1942) is due to limited movement of chromosomes that were formerly sister chromatids which maintain their proximity until synapsis.

To test for this possibility, 0.1% colchicine has been applied during the last mitosis. At this dilution, colchicine induces multipolar spindles that result in the eventual formation of pollen mother cell mosaics at first metaphase in which cells have widely different numbers of chromosomes (figure 5*a*, *b*). If the pairing of chromosomes at meiosis is a reflection of their relative movements during the last mitotic anaphase, then the irregular movement of chromosomes to multiple poles after random fragmentation of the nucleus would induce high levels of

multivalent pairing in genotypes normally having bivalents or univalents only. Observation of multivalent pairing in anther mosaics after such treatment (figure 5*a*, *b*) in wheat, wheat/*Aegilops* and wheat/rye hybrids (Dover & Riley 1973; Bennett, Dover & Riley 1974) strongly supports the hypothesis.

The occurrence of interarm pairing in an isochromosome indicates that the colchicine-sensitive events affecting the pairing process are earlier and separate from the temperature-sensitive events. Unlike the low-temperature effect in 5D-deficient genotypes (see §5) colchicine applied at stage 1, does not prevent the interarm chiasma formation to give an isochromosome ring at first metaphase (figure 5*c*) (see also Driscoll & Darvey 1970).

7. The timing of the supernumerary chromosome effects on pairing

F_1 hybrids of wheat/*Ae. mutica* and wheat/*Ae. speltoides* fall into four classes with regards the extent of pairing as measured by chiasma formation (Dover & Riley 1972*a*). The presence of supernumerary chromosomes from either diploid *Ae. speltoides* or *Ae. mutica* does not alter the mean frequency of chiasma of each class of hybrid (Vardi & Dover 1972). However, in the presence of supernumerary chromosomes there are reduced levels of pairing in two instances: first, in high-pairing hybrids of wheat/*Aegilops* spp. subjected to low temperatures (Vardi & Dover 1972; Dover 1973), and secondly, in wheat/*Aegilops* spp. hybrids normally having very high levels of pairing in the absence of chromosome 5B (Dover & Riley 1972*b*). Temperature-switch experiments, similar to those described in §5 in which wheat/*Ae. mutica* hybrids of the high-pairing class were taken from 20 °C to 15 °C to 20 °C over a period of days, have shown that chiasma frequencies are substantially reduced at the lower temperature, and rise again to their previous mean value on return to the higher temperature (see table 7; Vardi & Dover 1972). Successive sampling of tillers after transfer from 20 to 15 °C or 15 to 20 °C clearly shows that the final mean chiasma frequency is reached only after a lapse of time equivalent to the duration of time required for development from the 5D-deficient temperature-sensitive period to first metaphase in each of the temperature regimes. It is apparent that the supernumerary chromosome mediated reduction in chiasma frequency is the consequence of low temperatures affecting early stages of the premeiotic interphase.

In addition, the frequent observation of tetraploid pollen mother cells in wheat/*Aegilops* spp. hybrids with supernumerary chromosomes, figure 6*c* (Vardi & Dover 1972) lends support to the suggestion that the supernumerary chromosome effects on pairing are through their effects on events during the last premeiotic mitosis and subsequent development. Pollen mother cells with doubled chromosome numbers occur singly and not in clusters, indicating that only the spindle of the last mitosis is affected. It may be that the ability of supernumerary chromosomes to compensate for the absence of chromosome 5B operates through their effects on the preliminaries to pairing that occur during or soon after the last premeiotic mitosis (Dover 1973, 1974; Riley 1974). This would suggest that the 5B activity also affects these preliminaries.

It has been suggested that one possible role of highly repetitious (satellite) DNA, in particular that located in contiguous blocks around the centromeres, might be in chromosome pairing or chromosome movement during division (Walker 1971; Yunis & Yasmineh 1971; Flamm 1972). The 5B-like diploidizing effect of supernumerary chromosomes of *Aegilops* spp. and of *Lolium* spp. (Evans & Macefield 1972, 1974) and the supernumerary chromosome effect on spindles might be the result of supernumerary chromosome satellite DNA interfering with chromosome

behaviour controlled by satellite DNA of the regular chromosomes. The original finding of a satellite DNA specific to supernumerary chromosomes in *Myrmeleotettix maculatus* lent support to this suggestion (Gibson & Hewitt 1972). However, recent extensive analysis of DNA of individuals of *Myrmeleotettix maculatus* with zero, one or two supernumeraries has failed to confirm the presence of supernumerary satellite (Dover & Henderson 1976).

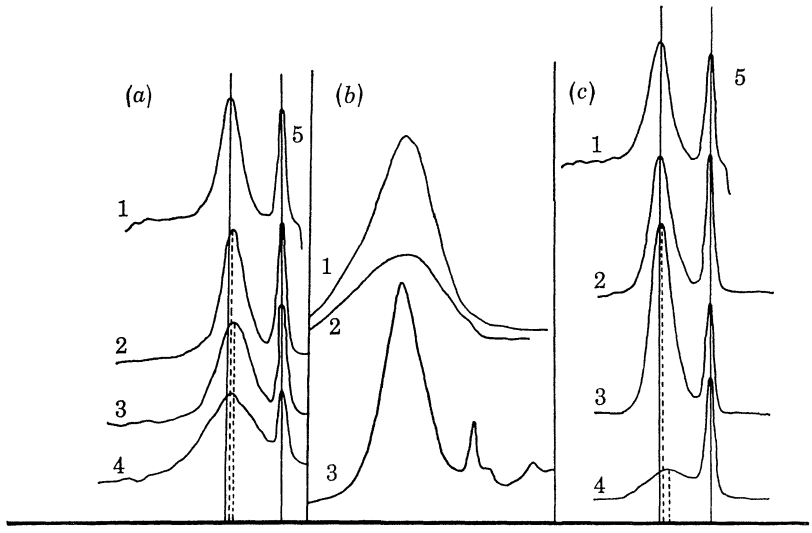


FIGURE 7. (*a*) and (*c*) Buoyant density patterns after analytical centrifugation in neutral CsCl of native DNA of *T. aestivum*, *Aegilops speltoides* ± supernumeraries and *Aegilops mutica* ± supernumeraries. Ten μg DNA in 10 mM tris-HCl, pH 7.5, plus 0.5 μg of marker DNA, *Pseudomonas aeruginosa* (buoyant density 1.726 g cm^{-3}) were loaded and spun in CsCl (initial density 1.700 g cm^{-3}) at 44000 rev./min for 20 h at 25 °C. Buoyant densities calculated relative to the marker according to Mandel, Schildkraut & Marmur (1969).

(*a*) 1, *T. aestivum*; 2, *Ae. speltoides*/+6 supernumeraries; 3, *Ae. speltoides*/+3 supernumeraries; 4, *Ae. speltoides*/zero supernumeraries; 5, *Pseudomonas aeruginosa*.

(*b*) Buoyant density patterns in neutral CsCl of native DNA of *Ae. speltoides*/+6 supernumeraries and *Ae. mutica*/+4 supernumeraries after binding to actinomycin-D. Native unsheared main-band and satellite DNA of *Drosophila melanogaster* were similarly bound to actinomycin-D as a check on the success of the method. Ten μg DNA were bound to 20 μg actinomycin-D in 10 mM tris-HCl, pH 7.5 and 1 mM EDTA and spun in CsCl (initial density 1.66 g cm^{-3}) at 44000 rev./min for 24 h at 25 °C. 1, *Ae. speltoides*/+6 supernumeraries; 2, *Ae. mutica*/+4 supernumeraries; 3, *Drosophila melanogaster* (main-band DNA and satellites).

(*c*) 1, *T. aestivum*, 2, *Ae. mutica*/zero supernumeraries, 3, *Ae. mutica*/+3 supernumeraries, 4, *Ae. mutica*/+4 supernumeraries, 5, *Pseudomonas aeruginosa*, (from Dover 1975*b*).

Results of comparative experiments testing for DNA compositional similarities and differences between regular and supernumerary chromosomes of wheat and *Aegilops* spp. make it clear that the disruption of spindle formation and meiotic pairing in the presence of supernumerary chromosomes is not due to a repetitive DNA which is exclusive to supernumeraries. The extent of heteroduplex formation between regular and supernumerary chromosome DNA under conditions allowing for highly and moderately repetitive DNA to reanneal; and the high thermal stabilities of the duplexes show that the supernumerary chromosomes contain representatives of the same spectrum of molecular families found on regular chromosomes (Dover 1975*a*, *b*).

Buoyant density patterns in neutral CsCl have not revealed satellites additional to main-band of supernumerary chromosome DNA (figure 7*a*, *c*). Density patterns in actinomycin D-CsCl gradients have similarly not revealed any additional cryptic main-band satellites (figure 7*b*: see legend for details); although the large spread of main-band DNA suggests considerable

heterogeneity in base composition of families of DNA binding differentially to actinomycin-D (Dover 1975*b*).

The lack of a resolvable difference, underpinning a DNA exclusive to supernumeraries, does not support the suggestion that satellite DNAs located around the centromeres might be responsible for chromosome behaviour; although it is clear that the relative changes in centromere orientation and form during meiosis are closely related to meiotic chromosome behaviour (see Nicklas, this volume; Stack 1975). At the least, in the wheat group of species, the complex set of interactions of regular and supernumerary chromosomes affecting meiotic pairing and spindles cannot be explained so simply.

The molecular similarity between regular and supernumerary chromosome DNA in several species indicates the origin of supernumeraries from the regular complement (Chilton & McCarthy 1973; Dover 1975*b*; Dover & Henderson 1976; Rimpau & Flavell 1975). It is possible, therefore, that the successful survival of supernumeraries in a population in which they arise would depend on an immediate ban on pairing of supernumeraries and regulars. Such a restrictive control 'locus' arising on the supernumeraries might explain the ability of these chromosomes to compensate for the absence of chromosome 5B. Translocation of the putative control region to regular chromosomes of *Aegilops* would form a basis for the varying ability of genotypes of *Aegilops* to control the level of pairing in hybrids with wheat. It might be more than coincidence that the only two species of *Aegilops* that have pairing-control loci interacting with the 5B-system of wheat also carry supernumerary chromosomes.

8. The process of pairing in wheat and other species

Identification of the developmental stages of the premeiotic interphase during which meiotic synapsis may be affected by temperature, colchicine or supernumerary chromosomes leads to proposals about the construction of the events affecting pairing from the last mitosis to zygotene, in wheat (figure 8).

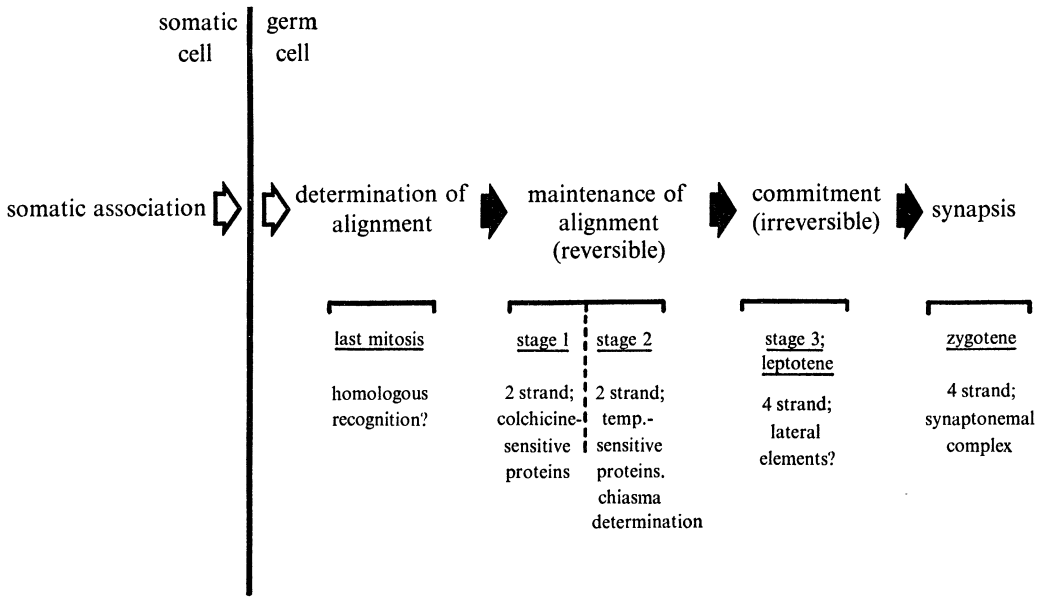


Figure 8. Sequential steps in the process of pairing of homologous chromosomes from the last premeiotic mitosis to zygotene (see text for discussion).

The patterns of pairing observed after the effects of colchicine on the chromosomes or spindles of the last mitotic division suggests that a prealignment of homologous chromosomes occurs at this time : and this is in part the result of the relative movements of chromosomes during anaphase. Observations of close homologous associations that are specific to the last mitotic metaphase in maize, but not to other somatic divisions (Maguire 1974, and this volume), support the suggestion that homologues become juxtaposed following division in a unique preliminary to meiosis.

Feldman (1968) has argued that the somatic associations of homologous chromosomes in root-tip cells of wheat, also under the control of loci on chromosome 5B, are of sufficient precision to account for strictly homologous synapsis at meiosis. However, neither colchicine, low-temperature nor supernumerary chromosomes exert their effects on pairing during stages of germ cell development other than the premeiotic interphase. It is arguable that a more precise alignment, probably exploiting pre-existing somatic associations, is fixed during the last mitosis, making this developmental period both sensitive to the above experimental stimuli and the point at which genetic control of pairing is effected.

The lack of evidence for generalized somatic association of homologues or nucleolus organizers at metaphase in neuroblast cells of *Locusta migratoria* (Fox, Mello-Sampayo & Carter 1975) indicates that in this species the prealignment of chromosomes during the last premeiotic mitosis (Buss & Henderson 1971) is unique to this division. Heat-shock induction of bivalent interlocking of only the longest chromosomes in *Locusta migratoria* (Buss & Henderson 1971 ; Henderson 1970) and the interval of time between treatment and observation has led to the suggestion that an initial pre-alignment of chromosomes takes place during the last mitotic division when chromosome size differences are maximized on contraction. Alignment of homologues after contraction alleviates the teasing problem of unravelling the 10 m of chromosome thread (referred to by Stern, this volume) that is found in nuclei of *Lilium* at leptotene.

Recent experimental analysis of segregation patterns of compound autosomes in *Drosophila melanogaster* (Novitsky 1975) has led to the proposition of the 'chromocentral' single-phase pairing theory of meiosis. To quote Novitsky: 'the initial "pairing" process occurs prior to interphase of meiosis when the chromosomes, having completed the last premeiotic mitosis, have their centromere regions opposed, with a subsequent formation of the chromocenter. This, then brings homologues into proximity so that they may exchange segments, after which the remaining chromosomes find themselves in a configuration which will determine the subsequent segregation pattern'. An alignment of homologues during the final mitosis, possibly mediated by the close apposition of like centromeres, as is the case in *Drosophila*, may be of more widespread occurrence.

After the establishment of alignment, there may be a necessity for either a continuous monitoring or maintenance, within the nucleus, of this state. Differences in sensitivity of stages 1 and 2 to colchicine and low temperature suggest that the manner of maintenance is different, although both are reversible (figure 8). The bundles of fibrillar material, lying between the chromatin and the nuclear membrane may be the means by which alignment is maintained, or by which polarity is established, in stage one. Colchicine-sensitive proteins, not necessarily microtubulin, have been located both in membranes and chromatin in nuclei of rat liver and brain (Stadler & Franke 1972) and in nuclear membranes of *Lilium* meiocytes (Hotta & Shepard 1973).

At stage 2 the chromatin is separate from the membrane and the nuclei are no longer sensitive

to colchicine. Maintenance of alignment, possibly of greater precision during stage 2, might well be mediated by temperature-sensitive proteins. Furthermore, the differences in sensitivity of interarm chiasmate pairing of an isochromosome during stages 1 and 2 supports the idea that the greater precision of alignment is related to the events that pre-determine chiasma formation (figure 8).

It is apparent that the maintenance of the aligned state in stages 1 and 2 is reversible; however, the insensitivity of stage 3 to experimental stimulation suggests that the chromosomes are now committed to synapse and that this commitment is irreversible (figure 8). An analogous situation of premeiotic readiness and commitment to sporulation has been described in *Saccharomyces cerevisiae* (Simchen, Pinon & Salts 1972).

The synthesis of DNA and replication of chromosomes in stage 3 might not be unrelated to the process of commitment. Grell & Day (1974) have shown that premeiotic DNA synthesis, sensitivity of recombination to heat shock, and the formation of the synaptonemal complex are coincidental in time in *Drosophila melanogaster*. However, no such strict coincidence is observed in wheat, although the replication of chromosomes during stage 3 could play a significant part in the final stages of the pairing process.

Observation of synaptonemal complexes complete with lateral elements and cores between homologous chromosomes at zygotene clearly indicates that synapsis, in the classical sense, occurs at this stage.

The process of pairing outlined above and depicted in figure 8, involving alignment, maintenance of alignment and commitment, during early stages of meiotic development may be unique to plant polyploids in which there is the additional problem of prevention of homoeologous pairing. There are clear indications however that similar processes are operating in animals (Montgomery 1901; Sutton 1902; Dublin 1905; Guyénot & Naville 1933; Illert 1956; Buss & Henderson 1971; Novitsky 1975) and the picture of pairing taking shape in wheat may prove indicative of the large canvas.

We are grateful for helpful discussions with, and assistance from, our colleagues M. W. Bayliss, M. D. Bennett, V. Chapman, R. B. Flavell and C. N. Law.

References (Dover & Riley)

Abel, W. O. 1965 Über den Zeitpunkt des Crossing over und der Chromosomenverdopplung bei *Sphaerocarpus*. *Z. Vererb Lehre* **96**, 228–233.

Baker, B. S. & Hall, J. C. 1976 Meiotic mutants: genic control of meiotic recombination and chromosome segregation. In *The genetics and biology of Drosophila*, vol. 1*a* (eds. M. Ashburner & E. Novitski), pp. 352–434. London: Academic Press.

Barber, H. N. 1942 The experimental control of chromosome pairing in *Fritillaria*. *J. Genet.* **43**, 359–374.

Bayliss, M. W. 1972 Ph.D. thesis, University of Cambridge.

Bayliss, M. W. & Riley, R. 1972*a* An analysis of temperature-dependent asynapsis in *Triticum aestivum*. *Genet. Res., Camb.* **20**, 193–200.

Bayliss, M. W. & Riley, R. 1972*b* Evidence of premeiotic control of chromosome pairing in *Triticum aestivum*. *Genet. Res., Camb.* **20**, 201–212.

Bennett, M. D., Chapman, V. C. & Riley, R. 1971 The duration of meiosis in pollen mother cells of wheat, rye and *Triticale*. *Proc. R. Soc. Lond.* B **178**, 259–275.

Bennett, M. D., Dover, G. A. & Riley, R. 1974 Meiotic duration in wheat genotypes with or without homoeologous meiotic chromosome pairing. *Proc. R. Soc. Lond.* B **187**, 191–207.

Bennett, M. D., Rao, M. K., Smith, J. B. & Bayliss, M. W. 1973 Cell development in the anther, the ovule, and the young seed of *Triticum aestivum* L. var. Chinese Spring. *Phil. Trans. R. Soc. Lond.* B **266**, 39–81.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Bennett, M. D. & Smith, J. B. 1972 The effect of polyploidy on meiotic duration and pollen development in cereal anthers. *Proc. R. Soc. Lond.* B **181**, 81–107.
Bennett, M. D., Stern, H. & Woodward, M. 1974 Chromatin attachment to nuclear membrane of wheat pollen mother cells. *Nature, Lond.* **252**, 395–396.
Brown, W. V. & Stack, S. M. 1968 Somatic pairing as a regular preliminary to meiosis. *Bull. Torrey bot. Club* **95**, 369–378.
Buss, E. M. & Henderson, A. S. 1971 Induced bivalent interlocking and the course of meiotic chromosome synapsis. *Nature New Biol.* **234**, 243–246.
Chilton, M. D. & McCarthy, B. J. 1973 DNA from maize with and without B chromosomes: a comparative study. *Genetics* **74**, 605–614.
Dover, G. A. 1972 Organisation and polarity of pollen mother cells of *Triticum aestivum*. *J. Cell Sci.* **11**, 699–713.
Dover, G. A. 1973 The genetics and interactions of A and B chromosomes controlling meiotic chromosome pairing in the Triticinae. *Proc. 4th Int. Wheat Genet. Symp.*, pp. 653–667.
Dover, G. A. 1974 On pores and pairing. In *Chromosomes today*, vol. 4 (eds. J. Wahrman & K. R. Lewis), pp. 197–204. Israel Universities Press.
Dover, G. A. 1975*a* Observations on the repeated sequence DNA of A and B chromosomes of genotypes in the Triticinae having contrasting patterns of meiotic chromosome pairing. In *Chromosomes today* (eds. J. Wahrman & K. R. Lewis), vol. 5. Israel Universities Press.
Dover, G. A. 1975*b* The heterogeneity of B-chromosome DNA: No evidence for a B-chromosome specific repetitive DNA correlated with B-chromosome effects on meiotic pairing in the Triticinae. *Chromosoma* **53**, 153–173.
Dover, G. A. & Henderson, S. A. 1976 No detectable satellite DNA in supernumerary chromosomes of the grasshopper *Myrmeleotettix maculatus*. *Nature, Lond.* **259**, 57–58.
Dover, G. A. & Riley, R. 1972*a* Variation at two loci affecting homoeologous meiotic chromosome pairing in *Triticum aestivum* × *Aegilops mutica* hybrids. *Nature New Biol.* **235**, 61–62.
Dover, G. A. & Riley, R. 1972*b* The prevention of pairing of homoeologous meiotic chromosomes of wheat by a genetic activity of supernumerary chromosomes of *Aegilops*. *Nature, Lond.* **240**, 159–161.
Dover, G. A. & Riley, R. 1973 The effect of spindle inhibitors applied before meiosis on meiotic chromosome pairing. *J. Cell Sci.* **12**, 143–161.
Driscoll, C. J. & Darvey, N. L. 1970 Chromosome pairing: effect of colchicine on an isochromosome. *Science N.Y.* **169**, 290–291.
Driscoll, C. J., Darvey, N. L. & Barber, H. N. 1967 Effect of colchicine on meiosis of hexaploid wheat. *Nature, Lond.* **216**, 687–688.
Dublin, L. I. 1905 The history of the germ cells in *Pedicellina americana* (Leidy). *Ann. N.Y. Acad. Sci.* **16**, 1–64.
Evans, G. M. & Macefield, A. J. 1972 The suppression of homoeologous pairing by B chromosomes in a *Lolium* species hybrid. *Nature, Lond.* **236**, 110–111.
Evans, G. M. & Macefield, A. J. 1974 The effect of B chromosomes on homoeologous pairing in species hybrids. II. *Lolium multiflorum* × *L. perenne*. *Chromosoma* **45**, 369–378.
Feldman, M. 1968 Regulation of somatic association and meiotic pairing in common wheat. *Proc. 3rd Int. Wheat Genet. Symp.* (Australian Acad, Sci., Canberra), pp. 31–40.
Flamm, W. G. 1972 Highly repetitive sequences of DNA in chromosomes. *Int. Rev. Cytol.* **32**, 1–43.
Fox, D. P., Mello-Sampayo, T. & Carter, K. C. 1975 Chromosome distribution in neuroblast metaphase cells of *Locusta migratoria* L. *Chromosoma* **53**, 321–333.
Gibson, I. & Hewitt, G. M. 1972 Interpopulation variation in the satellite DNA from grasshoppers with B-chromosomes. *Chromosoma* **38**, 121–138.
Gottschalk, W. 1973 The genetic control of meiosis. *Proc. XIIIrd Int. Cong. Genet.* **74**, 99.
Grell, R. F. 1969 Meiotic and somatic pairing. In *Genetic organisation I* (eds. E. W. Caspari & A. W. Ravin), vol. 1, pp. 361–492. New York: Academic Press.
Grell, R. F. & Day, J. W. 1974 Intergenic recombination, DNA replication and synaptonemal complex formation in the *Drosophila* oocyte. In *Mechanisms in recombination* (ed. R. F. Grell), pp. 327–349. Plenum Publishing Corporation.
Guyénot, E. & Naville, A. 1933 Les bases cytologiques de la théorie du 'crossing-over'. Les premiéres phases de l'ovogenése de *Drosophila melanogaster*. *Cellule* **42**, 213–230.
Henderson, S. A. 1970 The time and place of meiotic crossing-over. *A. Rev. Genet.* **4**, 295–324.
Hotta, Y. & Shepard, J. 1973 Biochemical aspects of colchicine action on meiotic cells. *Mol. gen. Genet.* **122**, 243–260.
Illert, G. 1956 Die meiose in der spermatogenese von *Aphcophora salicina* (Goeze). *Chromosoma* **7**, 608–619.
Jauhar, P. 1975*a* Genetic regulation of diploid-like chromosome pairing in the hexaploid *Festuca arundinacea* and *F. rubra*. *Chromosoma* **52**, 363–382.
Jauhar, P. 1975*b* Chromosome relationships between *Lolium* × *Festuca* (Gramineae). *Chromosoma* **52**, 103–121.
Kimber, G. & Athwal, R. S. 1972 A reassessment of the course of evolution of wheat. *Proc. natn. Acad. Sci. U.S.A.* **69**, 912–915.
Maguire, M. P. 1974 A new model of homologous chromosome pairing. *Carylogia* **27**, 349–357.

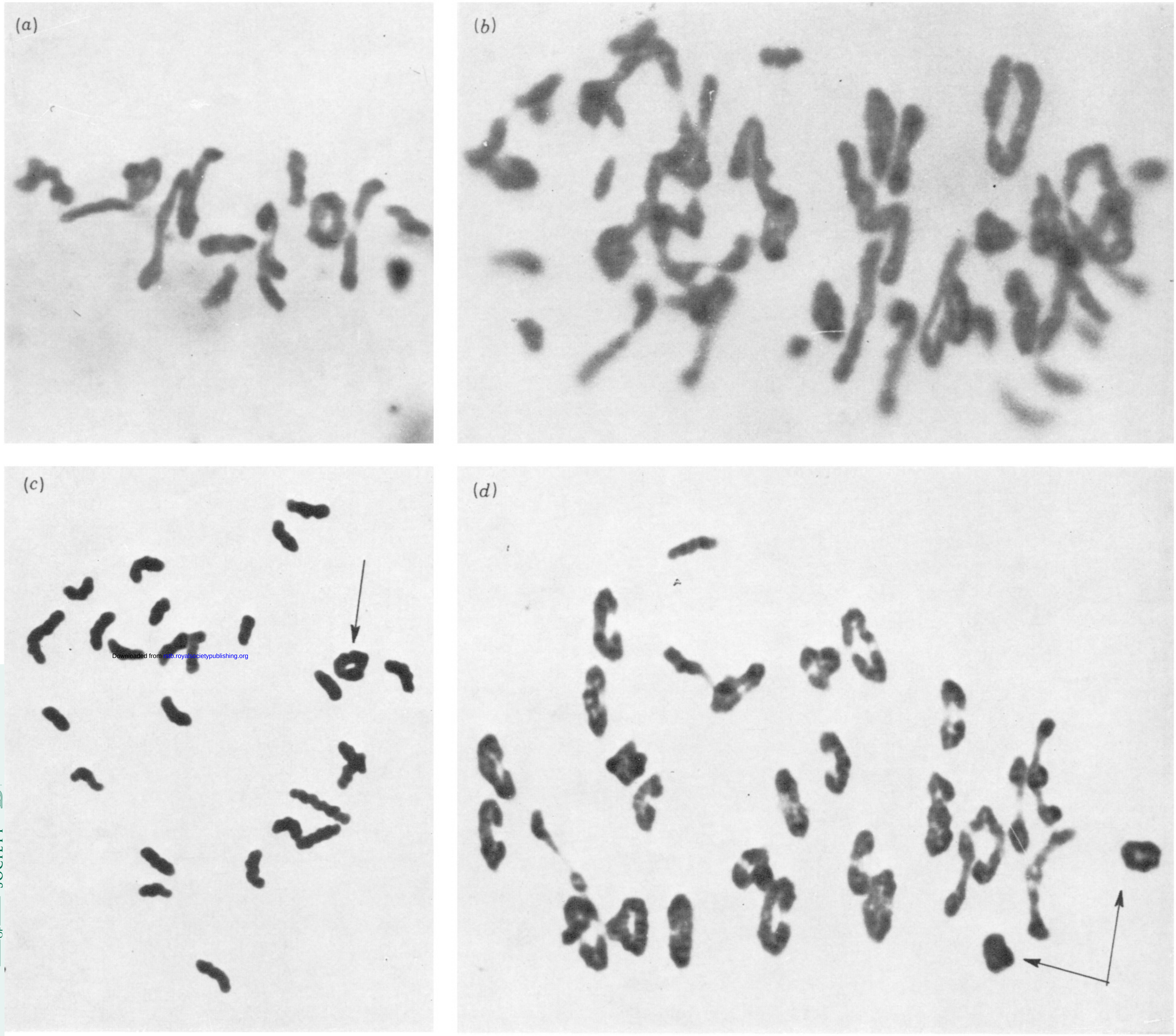
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Mandel, M., Schildkraut, C. & Marmur, J. 1968 Use of CsCl density gradient analysis for determining the guanine plus cytosine content of DNA. In *Meth. Enzym.* **12**B, 184–185.
Moens, P. 1973 Quantitative electronmicroscopy of chromatin organisation at meiotic prophase. *Cold Spring Harb. Symp. quant. Biol.* **38**, 99–107.
Montgomery, T. H. 1901 A study of the chromosomes of the germ cells of Metazoa. *Trans. Am. Phil. Soc.* **20**, 154–235.
Novitsky, E. 1975 Evidence for the single phase pairing theory of meiosis. *Genetics* **79**, 63–71.
Okamoto, M. 1957 Asynaptic effect of chromosome V. *Wheat Inf. Serv. Kyoto Univ.* **5**, 6.
Overton, J. B. 1909 On the organisation of the nuclei in the pollen mother cells of certain plants, with special reference to the permanence of the chromosomes. *Ann. Bot.* **23**, 19–57.
Parker, J. S. 1975 Chromosome-specific control of chiasma formation. *Chromosoma* **49**, 391–406.
Rajhathy, T. & Thomas, H. 1972 Genetic control of chromosome pairing in hexaploid oats. *Nature, Lond.* **239**, 217–219.
Rees, H. 1961 Genotypic control of chromosome form and behaviour. *Bot. Rev.* **27**, 288–318.
Riley, R. 1966 Genotype–environmental interaction affecting chiasma frequency in *T. aestivum*. In *Chromosomes today* (eds. C. D. Darlington & K. R. Lewis), vol. 1, pp. 57–65. Edinburgh : Oliver and Boyd.
Riley, R. 1974 Cytogenetics of chromosome pairing in wheat. *Genetics* **78**, 193–203.
Riley, R. & Chapman, V. 1958 Genetic control of the cytological diploid behaviour of hexaploid wheat. *Nature, Lond.* **182**, 713–715.
Riley, R. & Law, C. N. 1965 Genetic variation in chromosome pairing. *Adv. Genet.* **13**, 57–114.
Rimpau, J. & Flavell, R. B. 1975 Characterisation of rye B chromosome DNA by DNA/DNA hybridisation. *Chromosoma* **52**, 207–217.
Sears, E. 1969 Wheat cytogenetics. *A. Rev. Genet.* **3**, 451–467.
Shepard, J., Boothroyd, E. R. & Stern, H. 1974 The effect of colchicine on synapsis and chiasma formation in microsporocytes of *Lilium*. *Chromosoma* **44**, 423–437.
Simchen, G., Piñon, R. & Salts, Y. 1972 Sporolation in *Saccharomyces cerevisiae :* premeiotic DNA synthesis, readiness and commitment. *Expl Cell Res.* **75**, 207–218.
Stack, S. M. 1975 Differential Giemsa staining of kinetochores in meiotic chromosomes of two higher plants. *Chromosoma* **51**, 357–363.
Stack, S. M. & Brown, W. V. 1968 Somatic pairing as a regular preliminary to meiosis. *Bull. Torr. bot. Club* **95**, 369–378.
Stadler, J. & Franke, W. W. 1972 Colchicine-binding proteins in chromatin and membranes. *Nature New Biol.* **237**, 237–238.
Sutton, W. S. 1902 On the morphology of the chromosome group in *Brachystola magna*. *Biol. Bull. mar. biol. Lab., Woods Hole* **4**, 24–39.
Vardi, A. & Dover, G. A. 1972 The effects of B chromosome on meiotic and premeiotic spindles and chromosome pairing in *Triticum/Aegilops* hybrids. *Chromosoma* **38**, 367–385.
Walker, P. M. B. 1971 Origin of satellite DNA. *Nature, Lond.* **228**, 306–308.
Walters, M. S. 1970 Evidence on the time of chromosome pairing from the preleptotene spiral stage in *Lilium longiflorum* 'Croft'. *Chromosoma* **29**, 375–418.
Yunis, J. J. & Yasmineh, W. G. 1971 Heterochromatin, satellite DNA and cell function. *Science, N.Y.* **174**, 1200–1209.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 2. First metaphase in pollen mother cells of (*a*) *Triticum aestivum* nullisomic 5D, tetrasomic 5B, monoisosomic $5B^L$ at 20 °C. $2n = 42 +$ isosome $5B^L$ (arrow indicates isochromosome ring); (*b*) *Triticum aestivum*, diisosomic $5B^L \times$ *Aegilops mutica* (high pairing line); $2n = 27 +$ isosome $5B^L$ (arrow indicates isochromosome ring); (*c*) same genotype as (*a*) at 15 °C; (*d*) same genotype as (*b*) treated with 0.5 % colchicine at stage 3 and leptotene (see figure 1), showing pairing of chromosomes in multivalents.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

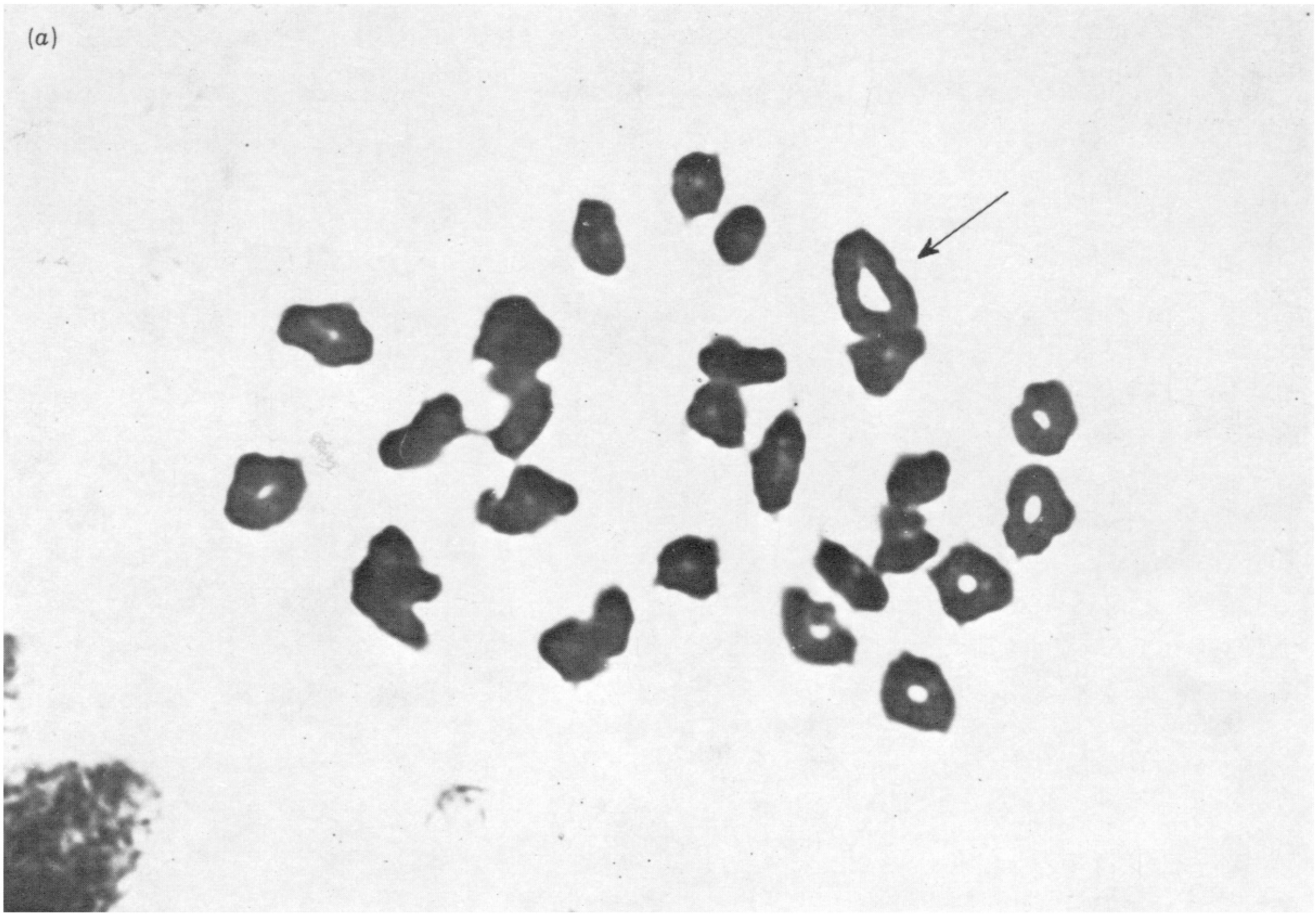
FIGURE 5. First metaphase in pollen mother cells of:

(*a*) Same genotype as figure 2*b*, treated with 0.01% colchicine at the last premeiotic mitosis. Cell from anther mosaic with low number of chromosomes, showing pairing of chromosomes in multivalents.

(*b*) Same genotype as figure 2*b*, treated with 0.01% colchicine at the last premeiotic mitosis. Cell from anther mosaic with high number of chromosomes, showing pairing of chromosomes in multivalents.

(*c*) Same genotype as figure 2*b*, treated with 0.5% colchicine at stage 1, (see figure 1). Arrow indicates isochromosome ring.

(*d*) Same genotype as figure 2*b*, treated with 0.5% colchicine at the last premeiotic mitosis. Tetraploid pollen mother cell showing bivalent pairing of all chromosomes and interarm pairing of isochromosomes (arrowed). (From Dover & Riley 1972*b*.)



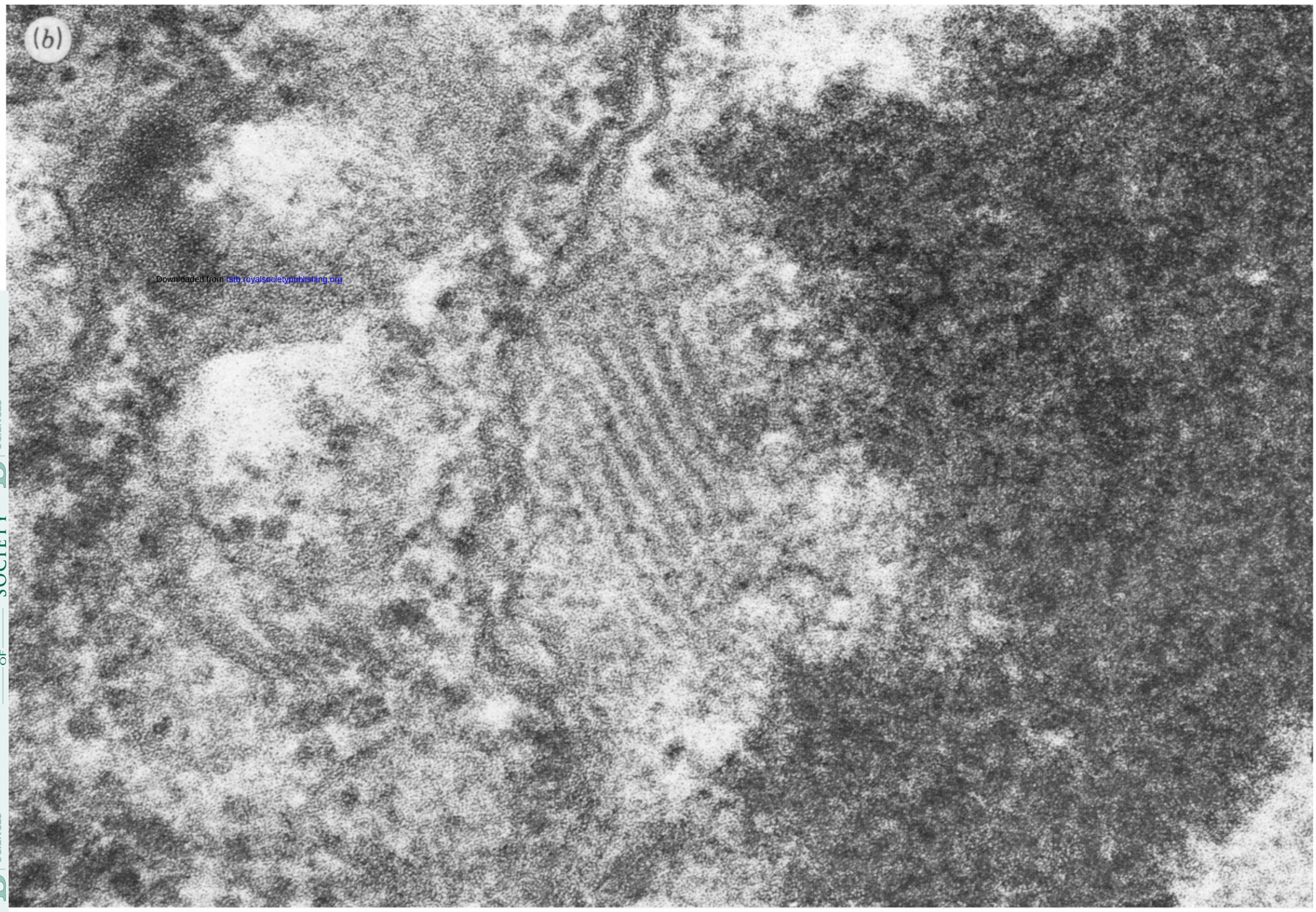


FIGURE 6. (*a*) Pollen mother cell with double the number of chromosomes from F1 hybrid of *Triticum aestivum* × *Aegilops mutica* having a wheat chromosome in the disomic condition and two supernumeraries ; 26 bivalents of regular chromosomes, 2 small ring bivalents of supernumerary chromosomes and 1 ring quadrivalent of wheat chromosomes in the tetrasomic condition (arrowed) (from Vardi & Dover 1972).

(*b*) Electron micrograph of pollen mother cell of *Triticum aestivum*, showing fibrillar material located between the nuclear membrane and chromatin masses at stage 1 (see figure 1). (Magn. × 28000 ; from Bennett *et al.* 1974.)