CHAPTER 4

Meiosis in normal and interchange heterozygotes of rye

1. Normal rye — introduction

Meiosis is a unique cell division connected with sexual reproduction in eukaryotes. Unlike mitosis, it halves the zygotic chromosome number (2n) to the gametic number (2n) and thereby introduces a haploid phase into the life cycle of an organism. It compensates for the increase in chromosome number upon fertilisation of gametes, a number which would otherwise double at each generation. Meiosis is a highly conserved process, which facilitates the recombination of genes through the crossing over of linked genes on homologous chromosomes, and the independent assortment of genes carried on maternal and paternal chromosomes (Fig. 4.1). Meiosis is, therefore, the fundamental mechanism upon which the laws of Mendelian genetics and heredity are based.

Meiosis is preceded by an interphase, comprising G_1, S and G_2, during which the chromosomes are replicated. There is biochemical and genetic evidence that premeiotic interphase differs in several ways from regular mitotic interphase. Meiosis II is not preceded by a DNA replication S-phase. The substages of meiosis are listed below and are shown on Fig. 4.2, although it should be realised that these phases constitute part of a continuum, and have been defined simply for our convenience.

Meiosis I (Reductional Division)

Prophase I	leptotene (chromosomes visible as thin threads) zygotene (chromosomes begin and complete synapsis) pachytene (thick threads and crossing over) diplotene (chiasmata visible) diakinesis (further contraction)
Metaphase I	(bivalents at equilibrium on metaphase plate)
Anaphase I	(segregation of half-bivalents)
Telophase I	(nuclear envelope reforms around each haploid daughter nucleus)

Interkinesis (interkinesis lacks DNA synthesis!)

Meiosis II (Equational Division)

Prophase II	(chromosomes condense)
Metaphase II	(chromosomes at equilibrium on metaphase plate)
Anaphase II	(segregation of sister chromatids)
Telophase II	(nuclear envelope reforms around 4 haploid recombinant products — spores)

Normal rye (*Secale cereale* L.) of the Poaceae is a diploid with 14 chromosomes ($2n = 2x = 14$), which can be represented diagrammatically as an idiogram comprising seven pairs of homologues (Fig. 4.3). Homologous chromosomes form seven bivalents during meiosis, and can be visualised by light (Fig. 4.4) and electron microscopy.

2. Scientific problem

The scientific problems of this experiment are to:

- observe and identify the sub-phases of meiosis using light microscopy of squash preparations
- relate meiotic chromosome behaviour to the recombination and segregation of genes.

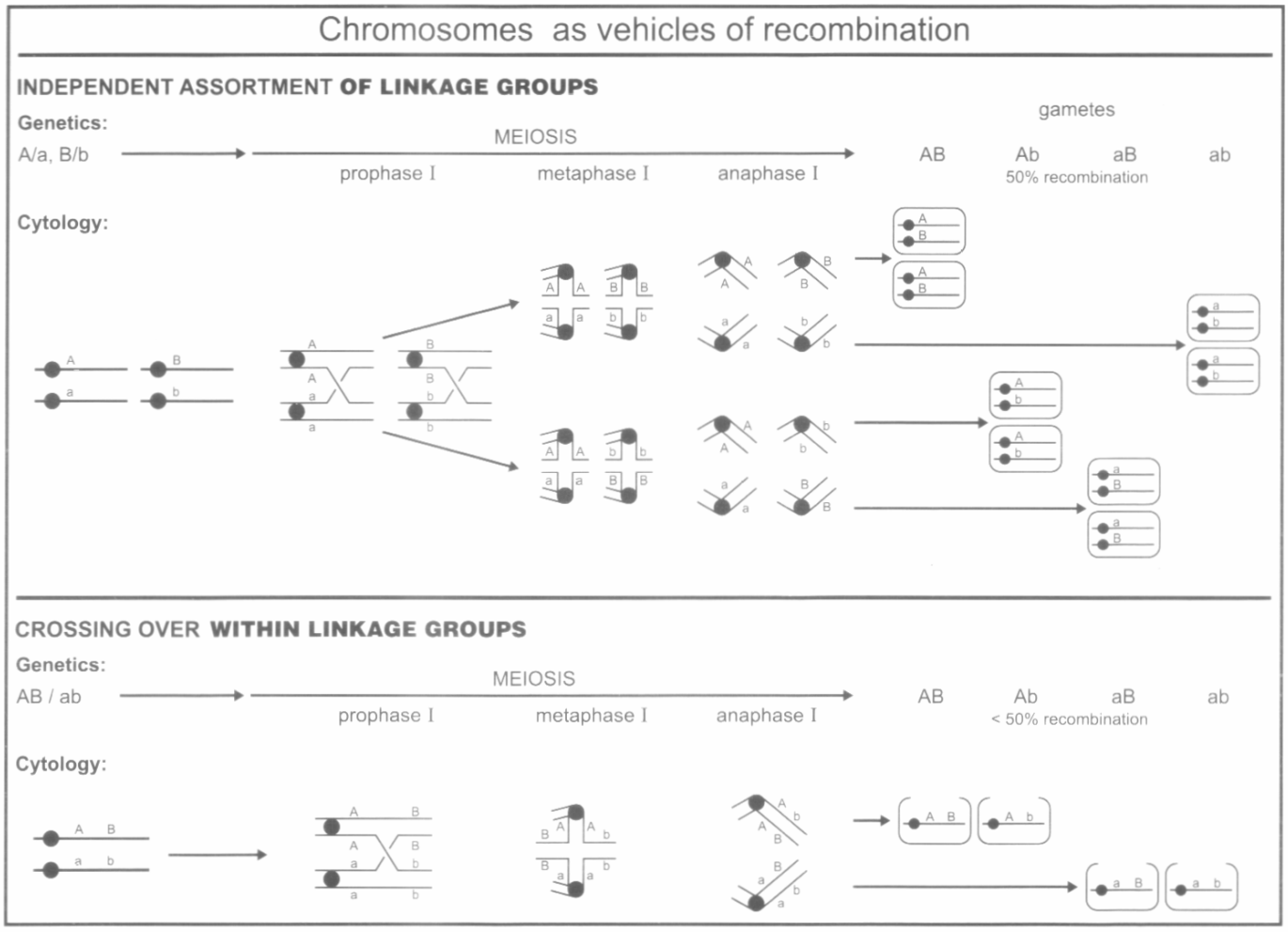


Fig. 4.1. Chromosomes as vehicles of genetic recombination

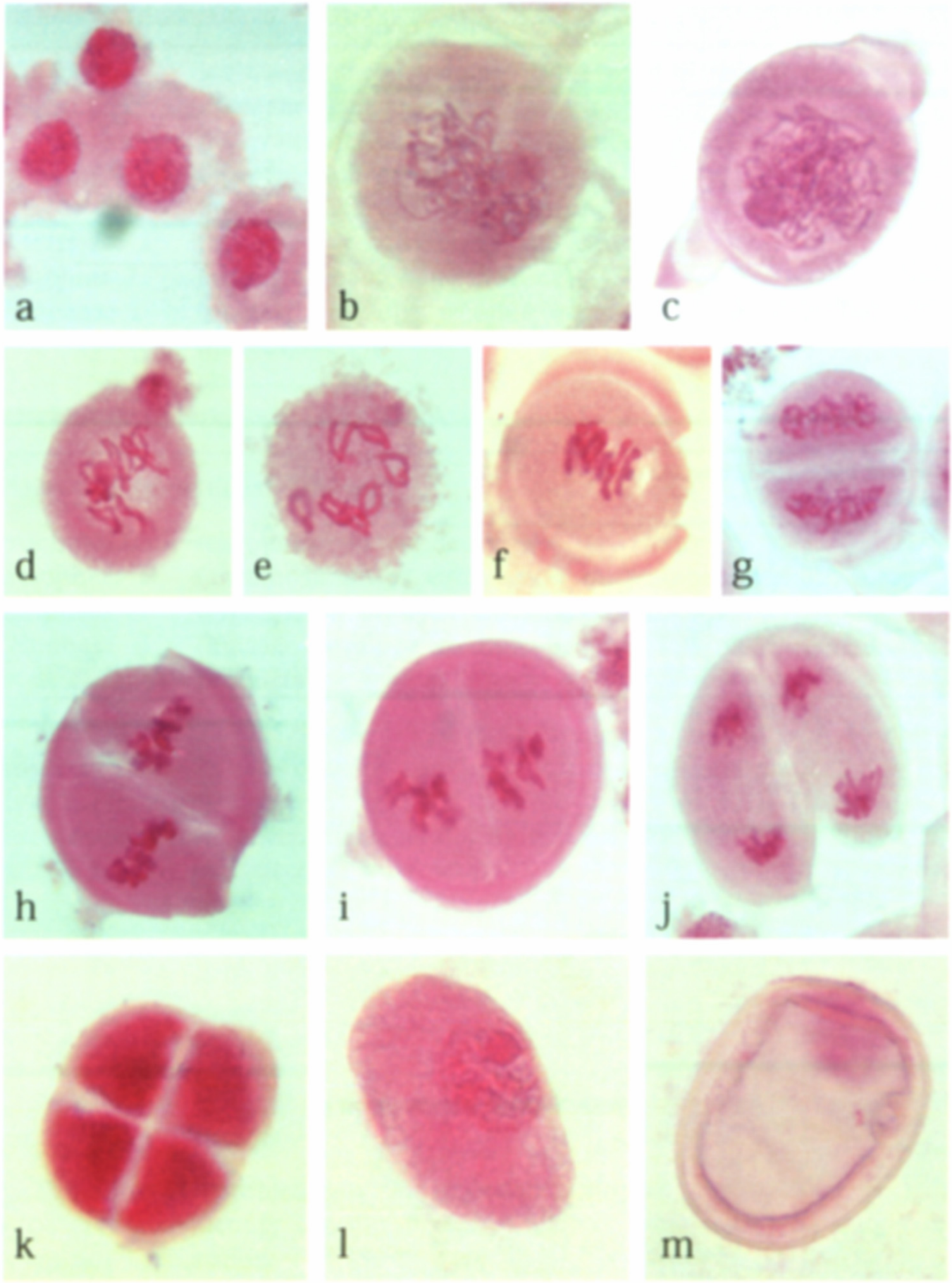


Fig. 4.2. Meiosis in rye (*Secale cereale*; $2n = 2x = 14$). Stained with acetocarmine. (a) premeiosis/leptotene, (b) zygotene, (c) pachytene, (d) diplotene, (e) diakinesis, (f) metaphase I, (g) anaphase I, (h) metaphase II, (i) anaphase II, (j) telophase II, (k) tetrad, (l) young pollen, (m) matured pollen

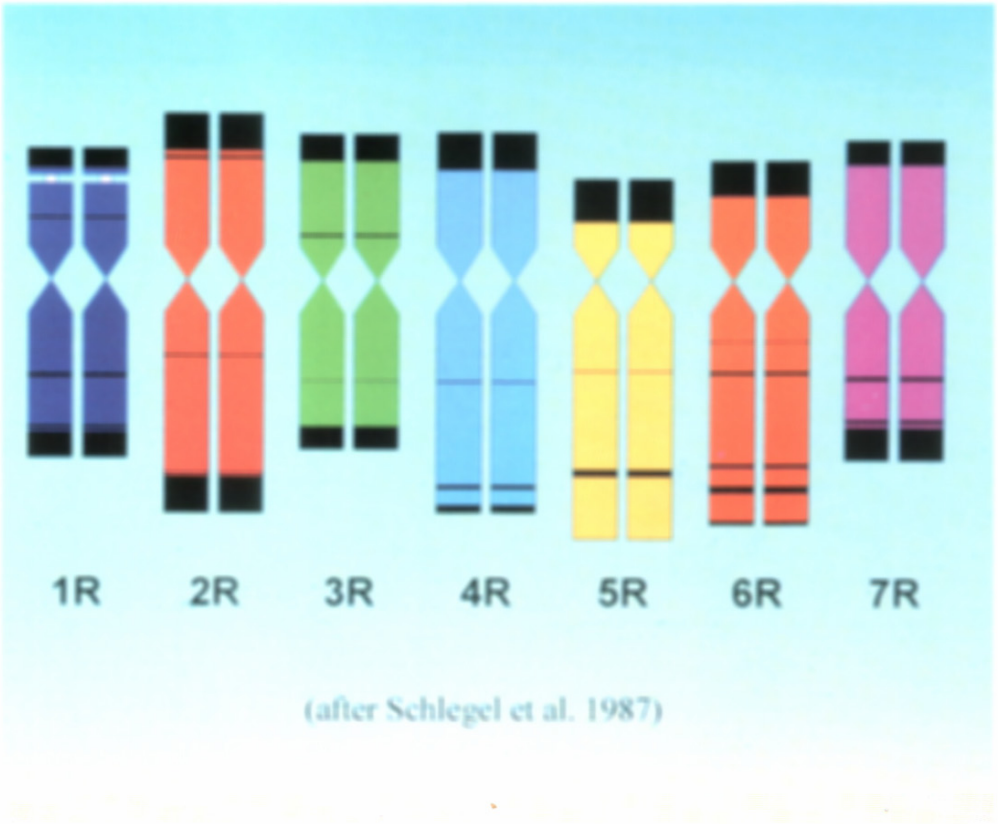


Fig. 4.3. Idiogram of the mitotic chromosomes of normal rye

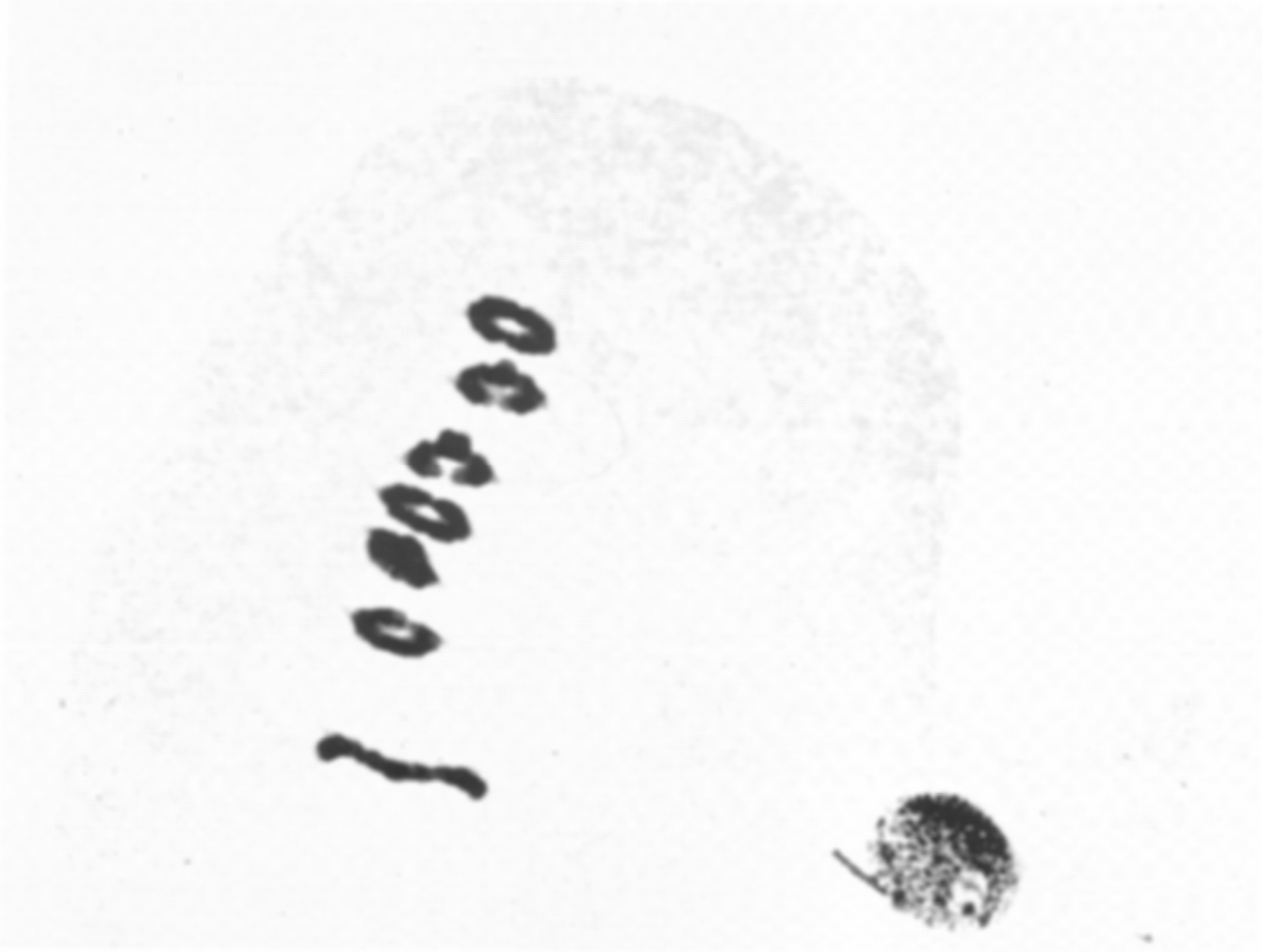


Fig. 4.4. Metaphase I showing six ring bivalents and one rod bivalent

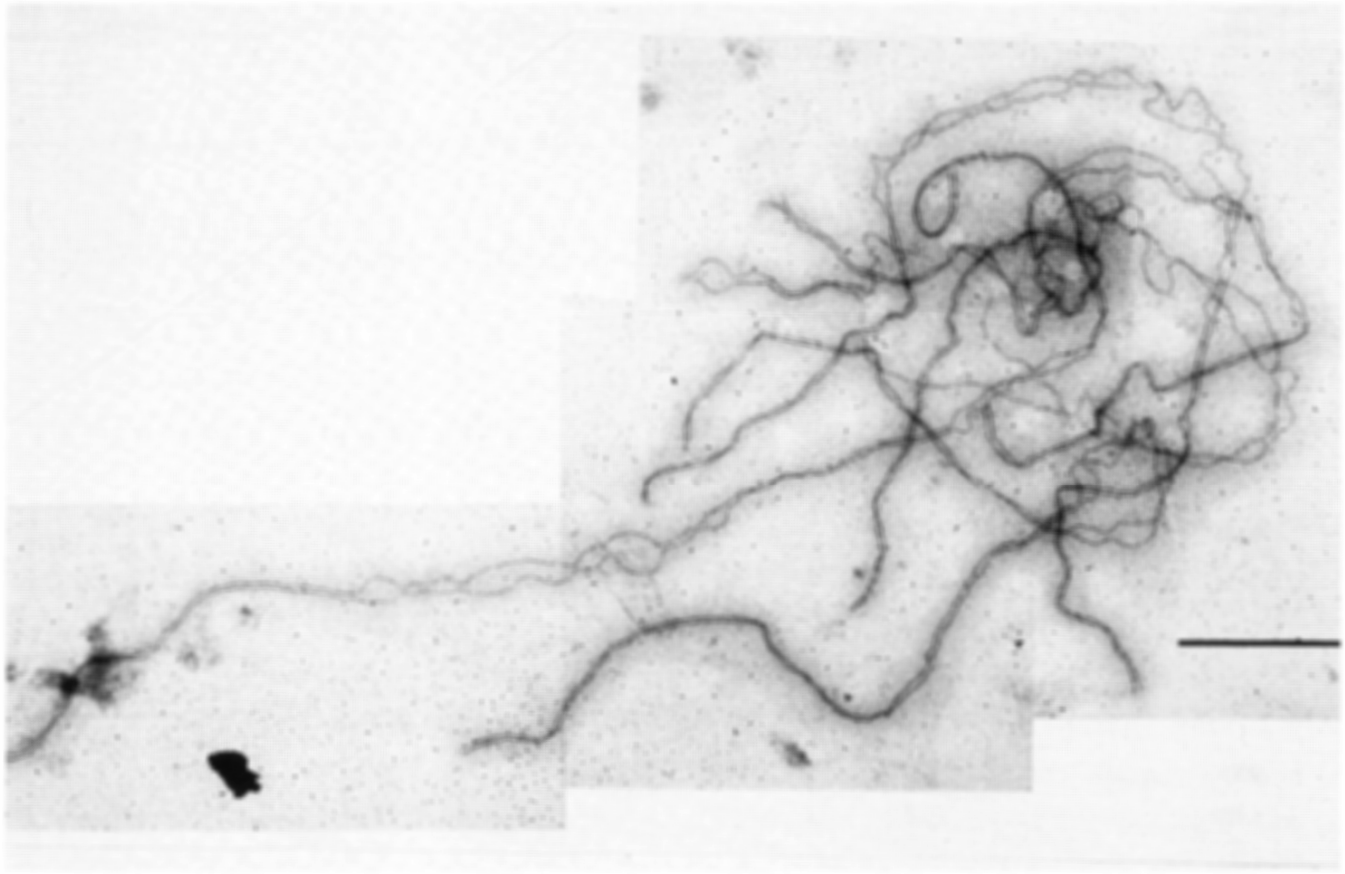


Fig. 4.5. Electron micrograph of the synaptonemal complex complement of rye

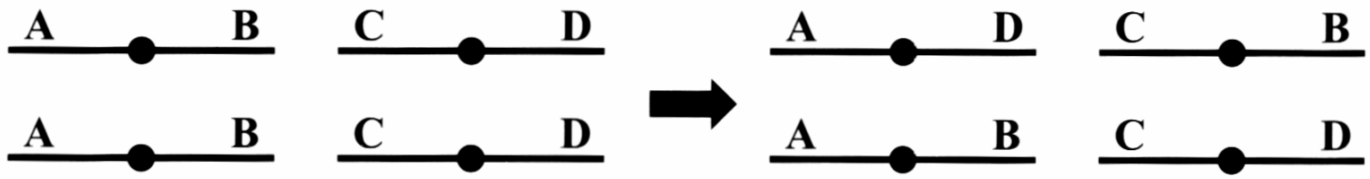
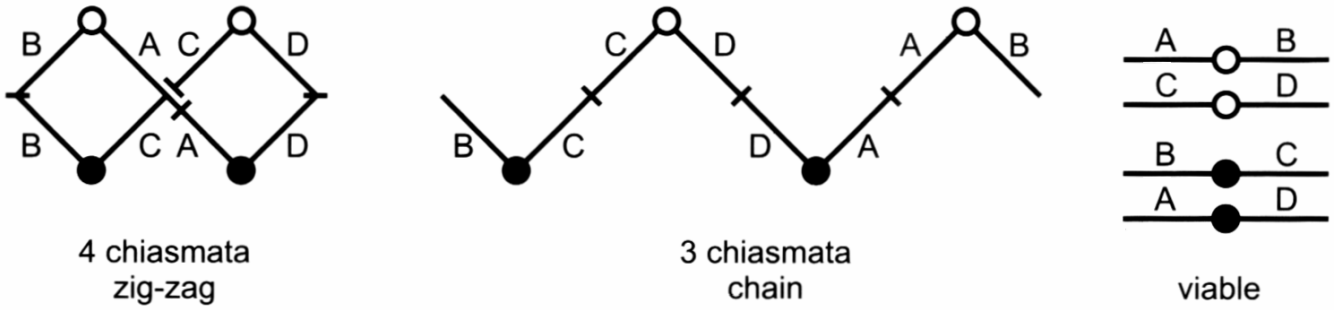


Fig. 4.6. The interchange of B and D of two nonhomologous chromosomes creates homology between four chromosomes in the heterozygote

(1) Alternate segregation

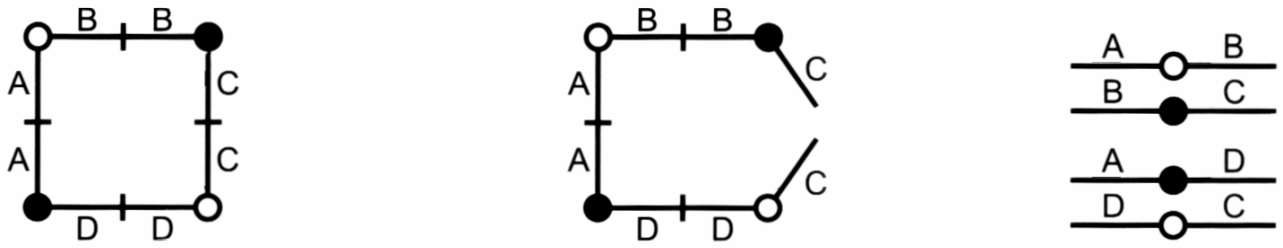


4 chiasmata zig-zag

3 chiasmata chain

viable

(2) Adjacent-1 segregation

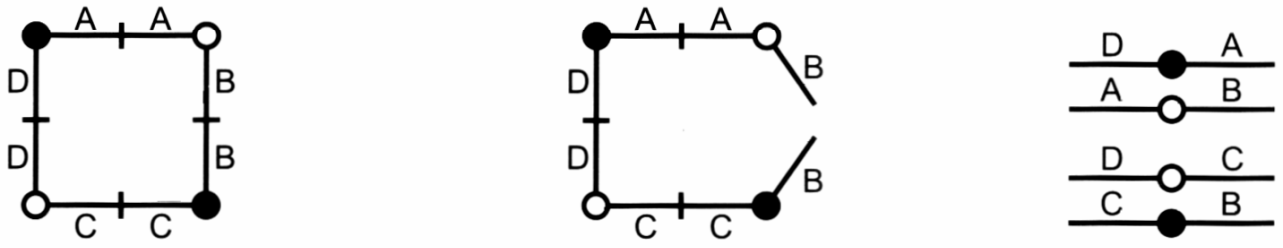


4 chiasmata open ring

3 chiasmata broken ring

inviable

(3) Adjacent-2 segregation



4 chiasmata open ring

3 chiasmata broken ring

inviable

Fig. 4.7. Segregation of interchange quadrivalents

3. Methods

3.1. Chromosome squash preparation

(1) Immature spikes of rye were collected during spring and fixed in Carnoy's solution (6:3:1 mix of ethanol: chloroform: acetic acid).

(2) Remove a spike from the bottle and place immediately in 70% ethanol in a Petri dish so that it does not dry out. Recap the bottle straight away as chloroform is toxic.

(3) Remove a spikelet from the centre of the spike, and dissect out under a dissecting microscope one of the three anthers which are synchronous in development and contain pollen mother cells (PMCs) at the same stage of meiosis.

(4) Add a small drop of aceto-carmine to the anther and macerate the tissue with a brass tapper in the centre of a microscope slide.

(5) Draw away the fluid from the debris with tip of a needle, apply a cover-slip to the macerate and squash very hard between folded filter paper.

(6) View the preparation under a $\times 10$ objective and first identify the PMCs. These are generally larger and usually have homogeneously pink cyto-plasm.

(7) View under $\times 20$ or $\times 40$ and identify the particular stage of meiosis. Please ask for help and/or consult the Fig. 4.2 if you are unsure of the stage.

(8) If the PMCs are too young (pre-meiotic) or too old (pollen grains) select another spikelet higher up or lower down the spike, respectively.

(9) If you fail to find anything after a number of attempts, use the prepared slides provided.

(10) Repeat (3) to (7) until you have observed and can confidently identify all the major phases of meiosis.

(11) We would like you to concentrate particularly upon metaphase I (Fig. 4.3) in which we would like you to perform the following tasks:

(a) Identify a well-spread cell (as in Fig. 4.3) in which all seven individual bivalents can be identified.

(b) Interpret and count the numbers of chiasmata in each pairing configuration and determine the total chiasma frequency for the cell.

(c) What can you say about the distribution of chiasmata?

(d) What is the consequence of this in terms of genetic recombination in this species?

Synaptonemal complex

During leptotene of meiotic prophase chromosomes form axial elements which assemble into a tripartite, proteinaceous structure (the synaptonemal complex, SC) during synapsis. The synaptonemal complex holds homologous chromosomes in close register and facilitates crossing over during pachytene. This structure can only be resolved by electron microscopy. Fig. 4.5 shows the synaptonemal complex complement of rye, in which the chromatin of the bivalents has been dispersed by hypotonic detergent, and the synaptonemal complexes themselves stained with silver.

Study Fig. 4.5 and answer the following questions:

(a) How many synaptonemal complexes are present?

(b) At what stage of meiotic prophase are they, and why?

(c) How would you picture synaptonemal complexes *in vivo?*

4. Interchange rye — introduction

In normal diploid organisms, homologous chromosomes associate only as bivalents during meiosis. However, in an interchange heterozygote four chromosomes can share substantial homology as a result of the reciprocal translocation of segments between two non-homologous chromosomes (Fig. 4.6).

These four chromosomes will still synapse together homologously during meiotic prophase, but instead of forming bivalents will form an interchange quadrivalent at this stage. All other chromosomes of the complement will form homologous bivalents only. The interchange quadrivalent will be consolidated by either three or four chiasmata to form a chain or ring, respectively, at metaphase I. The quadrivalent can adopt one of two orientations on the metaphase I plate (Fig. 4.7):

(a) disjunctional — the chromosomes form a "butterfly" ring (4 chiasmata) or a zig-zag chain (3 chiasmata), an orientation which ensures that normal chromosomes will segregate to one pole, and interchange chromosomes to the other. This orientation will produce viable gametes.

(b) non-disjunctional — the chromosomes form a closed ring (4 chiasmata) or broken ring (3 chiasmata), an orientation which permits interchange and normal chromosomes to segregate to the same poles. This will produce inviable gametes.

The quadrivalent is expected to orientate disjunctionally and non-disjunctionally with equal likelihood, producing 50% viable gametes and resulting in 50% fertility.

5. Scientific problem

The aim of this practical is to determine whether or not there is a relationship between chiasma frequency per cell and the orientation of the interchange quadrivalent at metaphase I.

Light microscopy

(1) Permanent slides are distributed to the course participants. Observe the slides of this material under a $\times 10$ objective. Please treat the slides with greatest respect. They are very precious! Return the slides upon completion of the analysis.

(2) Select a well-spread metaphase I cell in which all or most of the pairing configurations can be identified.

(3) Switch to $\times 100$ oil immersion and carefully sketch each pairing configuration, noting how many chromosomes each comprises and how many chiasmata are involved. The various configurations you will come across have the following numbers of chromosomes and chiasmata:

Configuration	No. chromosomes	No. chiasmata
rod bivalent (II)	2	1
ring bivalent (II)	2	2
chain quadrivalent (IV)	4	3
ring quadrivalent (IV)	4	4

(4) For each metaphase I plate, record the total number of chiasmata and the orientation of the interchange quadrivalent (disjunctional or non-disjunctional).

(5) Repeat (1) to (4) with other suitable metaphase I plates. The more cells you score, the better the analysis.

(6) If you fail to find suitable metaphases on your slide, select another. If you prefer to score from photographs (more difficult), some are available.

(7) Enter your data in the appropriate place in the table on the board.

Analysis

(1) For each chiasma frequency calculate the disjunctional index (DI) as follows:

$$DI = \frac{\text{no. cells with a disjunctional IV} \times 100}{\text{total no. cells}}$$

(2) Comment on the relative numbers of disjunctional and non-disjunctional quadrivalents over all chiasma frequencies and offer a cytological explanation for any deviation from the expected 1:1 ratio.

(3) Statistically analyse with a regression analysis the relationship between chiasma frequency and disjunctional index. This can be done simply and quickly using a PC with appropriate software.

(4) Comment on the results of the analysis and briefly discuss the cytological significance, if any, of the relationship.

6. The research skills you will acquire

Having conducted the above two experiments you should have acquired skills in the following:

- Cytological preparation of meiotic material for light microscopy
- Identification and interpretation of meiotic stages
- Understanding of the nature and cytological consequences of chromosome mutation
- Regression analysis and the interpretation of results.

7. Report

Assessment will be in the form of a report which will have the structure of a short research paper.

References

REES H. and JONES R. N., 1977. Chromosome Genetics. Arnold.

Contents

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A PRACTICAL COURSE MANUAL

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