Investigating Chromosomes

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Introduction

Many fascinating features of chromosome structure and behaviour are clearly described in the books now available for students at school, college and university. These accounts describe how the chromosome accommodates the genetic information, releases this information according to a predetermined sequence during development and transmits it to the next generation while maintaining combinations of genes which interact functionally in an adaptive way. Some who read about these events will wish to investigate them for themselves. Indeed, at a time when there is an increasing demand for cytogeneticists in plant breeding, human clinical genetics and veterinary medicine, such investigations may be for many a necessary component of their practical training or employment. Unfortunately, all too many are defeated by the technique before facing the challenge of interpreting the chromosomes they see. Some become discouraged by the experience of complex and empirical methods with little to show at the end but a murky glimpse of mitosis. For others, too daunted even to try their hand, chromosomes remain as lifeless microscopic components of a bought slide or as photographs or line drawings in a book. Even those who have some success with their preparations could achieve more if encouraged to think beyond the named stages of mitosis and meiosis.

The purpose of this book is to encourage student and teacher to demonstrate and investigate for themselves the structure and behaviour of the chromosome complement as seen down a light microscope. This purpose partly accounts for the bias in selection of material for inclusion. In the first place, the emphasis throughout is on the chromosomes rather than the techniques. Only one basic technique will be described in detail even though many alternatives exist, some of which may be better or even essential for certain specialized purposes. Much can be achieved by relatively inexperienced cytologists, even those with limited time or facilities, by the imaginative use of a simple versatile technique which becomes more familiar with every successful investigation. Little can be expected if every observation requires the selection of the appropriate method from a daunting array of alternatives. The simple method given here has proved successful with complete beginners at school and university, in classes of over 200, under 'field' conditions away from a laboratory, and with plant, animal and human cells.

At least as important as the choice of method is the selection of suitable living material. The second bias in this book is in the choice of this material, which comes mainly from among the flowering plants. There are sound practical reasons for choosing angiosperm material for introductory chromosome studies: a) they are generally easier than animals to maintain alive under laboratory, or at least greenhouse and garden plot, conditions; b) dividing cells of higher plants are often easier to obtain and process, and the chromosomes themselves often larger, than those of animals and lower plants while most of the basic characteristics of chromosome structure and behaviour are equally well represented; and c) when a particular chromosome condition is accompanied by sexual sterility it can only be maintained in asexually reproducing organisms such as vegetatively propagated plants. For these reasons, plant chromosomes are more often used in teaching and, at least in the past, have

been more widely studied, yielding a more extensive literature. Nevertheless, when animal material has an important contribution to make, instructions necessary for its study are included.

This book is also biased in its treatment of the cell because it is concerned only with the chromosome complement and the visible events of nuclear divisions. Although division and segregation of chromosomes are only two of the visible components of cell division (organelle replication and distribution, and cytokinesis or cleavage are equally important for successful cell replication), the additional techniques necessary to study the cytoplasm are beyond the scope of this book.

Finally, the treatment is biased in its emphasis on the practical aspects of material, method and approach. In a book intended to be used primarily in the laboratory, a general outline of each topic is included as background information only to ensure recognition of significant features. For detailed treatment of the underlying explanations and implications of these features in a wider range of material, reference should be made to the reading lists accompanying each section.

Four aspects of the investigation of chromosomes are dealt with in separate chapters. Chapter 1 describes simple techniques for obtaining preparations and recording observations of chromosomes; techniques which with only minor modification are sufficient for all the subsequent sections. Chapter 2 introduces the basic features of chromosome morphology and behaviour to be observed during the mitotic cycle at different stages of the life cycle and during meiosis, together with the methods and materials required to reveal them. Chapter 3 is concerned with cytogenetics, the study of the chromosome complement in relation to the flow of genes between individuals and generations. In particular, this involves application of information from the preceding chapter to the study of the various effects of changes in the size, number or structure of chromosomes on their behaviour at meiosis. In Chapter 4, a change of approach from one of demonstration to one of enquiry is encouraged by outlining several projects selected to indicate the variety of investigations made possible by using the knowledge gained from Chapters 1 to 3.

Thus the chapters are arranged in order of increasing scope and complexity, each building on the content of the one before. By following them in sequence, the beginner can first learn the basic technique and then proceed towards progressively more sophisticated studies of his own preparations. For those requiring information on specific aspects of chromosome structure or behaviour, the contents are listed in detail.

Chapters and sections dealing exclusively with instructions on technique or lists of material for specific purposes are indicated by a tinted box covering the page number. This applies to the whole of Chapter 1 and Appendices 1 and 2. Within Chapters 2 and 3, information on methods and materials relating to each major topic is placed at the end of the sub-section introducing that topic and presented in a sequence which corresponds to the content of the introduction. Thus, for example, chromosome coiling is described in Section 2.2.2 on pages 17–20 while the relevant information on material and techniques to demonstrate it is given on pages 27–28 at the start of the Section 2.2.5. Similarly, aneuploids are described on pages 103–105 in Section 3.4.2h while the examples are listed on page 110 within Section 3.4.4.

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I would like to thank all those who have helped me to enjoy an interest in chromosomes since my introduction to the subject as a student. I am also grateful to everyone who has assisted me in the preparation of this book and in particular to Miss Julia Boardman of Edward Arnold (Publishers) Ltd. for her skill in bringing order out of chaos.

Contents

1 Finding chromosomes – a matter of method

1.1 Introduction

Every cytologist has his favourite technique for chromosome preparations. This may be one of the many published methods or, more likely, an empirically derived minor variation of it. None is ideal for all purposes and many, though successful in practised hands, require considerable experience before they give consistently good results. The secret for success is a simple, reliable technique and practice.

All techniques are designed to fix, stain and preserve the chromosomes so that they can be profitably studied. *Fixation* is an attempt to kill the material rapidly in such a way that the internal structures are preserved in a life-like form. Because fixation involves such processes as denaturation of proteins, some alteration of structure is always induced and care has to be taken in interpretation to recognize artefacts (those features which are induced by the treatment and not present in the living cell). Because the various cell components differ in their fixation properties, methods are chosen for their ability to achieve good fixation of the particular structures being studied, in this case the chromosomes, even though they may not give equally good fixation of other cytoplasmic structures. Good fixation is a rather subjective assessment indicating that the appearance after treatment suggests a structure similar to that deduced from phase contrast images of living cells and from cells fixed by other techniques. The fixative described in Section 1.5 is based on the long known and widely used acetic-alcohol fixative, which causes some chromosome shrinkage but is otherwise acceptable. The inclusion of formalin counteracts the tendency to form 'bubbles' along the arms of large chromosomes, and chloroform helps to remove chlorophyll from green tissues and is claimed to make penetration by the fixative more rapid. In certain cases, the acid solvent of the dye used for staining can act satisfactorily as a simultaneous fixative without prior fixation.

After fixation, material can be stored until required, usually in alcohol, or immediately stained for observation. Storage rarely, if ever, improves the final preparation, frequently accentuating the artefacts and sometimes reducing the staining. A disadvantage of orcein, the dye recommended here, is that staining is sometimes less effective after storage in alcohol. However, for teaching purposes, it is desirable that the student makes his preparation directly from the living organism, rather than from excised fixed tissues, so this property of orcein is rarely a drawback. In any case, the finished preparation can be kept for examination on a later occasion if time is limited. Temporary orcein preparations made by the technique described will keep satisfactorily for days, and sometimes for weeks or even months, while permanent preparations show little or no change after 15 years.

Staining is necessary because without it the colourless chromosomes are difficult to distinguish from the equally colourless cytoplasm. A number of dyes have been used by methods, again usually derived empirically, to give preferential staining of chromosomes by adsorption, taking advantage of their characteristic surface properties. Several naturally occurring dyes, such as carmine and orcein, dissolved in simple

organic acids have been used successfully although they induce some swelling of the chromosomes.

Orcein in propionic acid gives better differential staining than other solutions because, while the chromosomes are deep purple, the cytoplasm shows little or no staining. Counter-staining of the rest of the cell using a second dye with appropriately different adsorption properties is possible, but rarely beneficial for chromosome studies as it inevitably reduces the contrast between the chromosomes and their background.

Stained chromosomes have to be examined by transmitted light using the most highly magnifying objective of the microscope. The preparation must therefore be thin, to transmit sufficient light and to accommodate the shallow depth of focus of these lenses. For a clear image of chromosomes the material must be only one cell thick on the slide, even though most dividing cells occur in relatively massive tissues. There are two main ways of separating cells of such tissues for examination. Thin sections can be cut, but this involves the lengthy and laborious process of wax embedding and microtome sectioning, and while this preserves cells and organelles in their correct relative positions, the chromosomes may be indistinct or, when cut by the knife, even incomplete. A simpler and quicker alternative which is better suited to most chromosome investigations is to treat the cells so that they no longer adhere to one another, and can be separated and spread over a slide. One method is to warm in dilute acid for a short time before breaking up the tissue mechanically to produce a cell suspension. The alternative treatment in concentrated acid at room temperature is more hazardous and unreliable, and tends to give very uneven results within the thickness of the tissue. This maceration inevitably destroys the arrangement of cells within tissues and the technique is unsuitable for anatomical investigations. Carefully restricted pressure on the cover slip will however give a clear image of the cell with its chromosomes

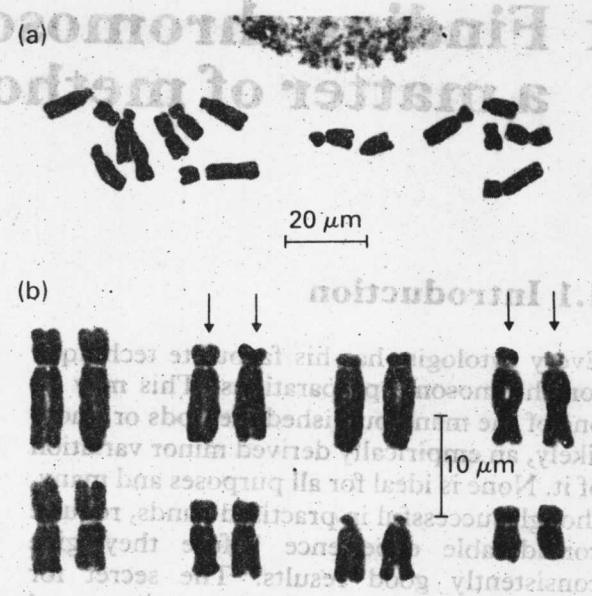

Fig. 1.1 The chromosome complement of the wild hyacinth or bluebell, *Endymion nonscriptus* ($2n = 2x = 16$). **a**, Colchicine pretreated root tip mitosis at metaphase. **b**, Idiogram derived from a second cell showing eight distinguishable chromosomes each represented twice. Two pairs of acrocentric chromosomes (arrowed) have nucleolar organizers in the short arms.

in their natural position, with no more distortion of the organelles than in a section, but with the whole cell intact. Greater pressure, as in the *squash* technique (Section 1.4) will distort their arrangement, but make the individual chromosomes more clearly visible in a complement flattened at one focal level.

The resulting temporary preparation can be made permanent. The decision has to be made, balancing the need for a long-lasting preparation against the risk of losing some cells, at the outset of the preparation, because the slide or coverslip has to be suitably coated with an adhesive. The cell preparation, stuck to a slide or coverslip, is taken through a graded series of alcohol concentrations to a suitable mountant.

The chromosome preparation is then ready for examination. Apart from some basic rules for recording observations, the problems of

interpretation, which depend very much on the species and tissue used, will be covered in subsequent sections. However, there is an additional step, often referred to as *pre-treatment*, which can be inserted into the preparative technique to facilitate the subsequent interpretation of many features of the chromosomal complement in the finished slide. Pre-treatment involves the exposure of living material before fixation to the effects of one of a number of chemicals or physical treatments which inhibit the activity of the spindle during division, resulting in the accumulation of cells with chromosomes scattered through the cytoplasm instead of aggregated on the spindle equator and shorter and straighter than normal due to overcontraction. The result after fixing, staining and squashing is a cell in which the number and morphology of the chromosomes is particularly clear (Fig. 1.1).

The rest of this section describes in detail the equipment and operations required for the technique just outlined.

1.2 Equipment and reagents

The full list of requirements is given in Table 1.1, but some additional comments may be helpful.

Dividing cells can usually be recognized under ×10 objectives, and some rather superficial investigations are possible using a ×40 objective, but any detailed examination of chromosomes requires a ×90 to ×105 oil immersion objective. Much of the preliminary work of preparing and scanning preparations can be done at a lower magnification, so, with careful planning, useful work can be carried out with oil immersion objectives being shared between students although ideally each should have his own. Obviously, the better the optical system, the clearer the chromosomes in a good preparation. However, a typical student microscope is perfectly adequate, the critical factor being that it is correctly set up for maximum optical resolution and illumination according to the manufacturer's instructions. The use of a green filter is often helpful if sufficiently intense illumination is available. The red-purple stained chromosomes will appear black against a green background. Many lenses show least aberration in green light, and the eye is most sensitive to wavelengths in this part of the visible spectrum.

The rod used for separating the cells of the macerated tissue (Section 1.5) should be a piece of 3 mm brass or aluminium rod about 7 cm long, with the cut ends filed and polished. Pieces of plastic knitting needles of comparable size are a tolerable alternative.

An oven or waterbath is required to maintain material for maceration in dilute acid at about 60°C. The waterbath is less convenient than the oven because immersed tubes and bottles tend to float. Most laboratories will have at least one of these items of controlled temperature equipment, but the temperature is not critical and adequate results are possible, though laborious, using mixtures of hot and cold water and a thermometer.

The ridged separating dish is used to float the coverslip away from the inverted slide when making a preparation permanent. While less convenient, any flat container for the diluted acetic acid, with two pieces of glass rod to support the slide above the bottom of the dish, will serve the same purpose.

Species differ in their sensitivity to the pre-treatment chemicals used to clarify the chromosome complements. Colchicine is usually the most successful, but is also unfortunately by far the most expensive and as a possible carcinogen must be handled with care. In many cases saturated solutions of para-dichloro-benzene or alpha-bromonaphthalene are acceptable substitutes and, in those cases where colchicine is found to be inactive or over-active, necessary alternatives. The former is claimed to be parti-

Table 1.1 Equipment and reagents for chromosome preparations. Key: *Required for every student, **required for every 1–6 students, ***required for every 1–20 students.

	Pretreatment	Fixation and Storage	Maceration	Staining	Permanent Mounting
Glass-ware	***100 ml beaker ***Aquarium aerator	*Two 50 mm × 25 mm stoppered specimen tubes **100 ml measuring cylinder	*Two 50 mm × 25 mm stoppered specimen tubes	*Slides and coverslips	**Four solid watch-glasses
Reagents	***200 mls colchicine or *p*-dichloro-benzene or α-bromo-naphalene or 8-hydroxyquinoline	***200 ml abs. ethanol ***200 ml 70% ethanol ***100 ml formalin ***100 ml chloroform ***100 ml glacial acetic acid	**100 ml 1M HCl at 60°C *Distilled water	**Working strength lactopropionic orcein in bottle with bottle pipette	**200 ml abs. ethanol **200 ml 95% ethanol **200 ml 70% ethanol ***200 ml euparal essence **euparal mountant **45% acetic acid
Other equip-ment	**Forceps	**Forceps	***Water bath or oven at 60°C *3″ squares of muslin	*Large sheet white paper on bench *Four 6″ squares of blotting paper *Paper tissues **1 scalpel and 2 mounted needles *Brass rod, 7 cm × 3 mm *Microscope with × 100 objective, immersion oil, lens tissue and green filter **Spirit lamp or microbunsen **Tube glycerin-albumen adhesive **xylol for lens cleaning	**5″ square ridged separating dish

A basic kit for temporary preparations is available from Philip Harris Biological Ltd.

cularly good for leaf material. During the pre-treatment of living material in one of these solutions, bubbling with an aquarium pump is helpful in stirring and aerating the solution. A clean glass tube, with one end drawn out so as to restrict the opening in order to produce a stream of small bubbles, should be clamped so that this end dips into the solution in a beaker. This tube can be connected at the outer end to the aerator, using polythene, *not rubber*, tubing.

In addition to a range of alcohol dilutions, made up by volume with water to the percentages given in the method, a number of solutions have to be prepared. The instructions for these are given below.

PRE-TREATING AGENTS:

Colchicine: Usually used as solutions of 0.01% to 0.2% w/v in water. Batches differ in their activity. Store in a fridge.

8-hydroxyquinoline: 0.002 M solution in water (0.29 g l^{-1}).

p-dichlorobenzene: Used as a saturated solution in water.

α-bromo-naphthalene: As for *p*-dichlorobenzene or 1% aqueous solution of stock solution of 1 ml bromo-naphthalene dissolved in 100 ml absolute ethanol for $\frac{1}{2}$ hour.

FIXATIVE:

Fixative should be freshly prepared for each fixation. It consists of:

Absolute ethanol	10 parts
Chloroform	2 parts
Glacial acetic acid	2 parts
Formalin (40% formaldehyde in water)	1 part

STAIN:

Lacto-propionic orcein: Stock solution: dissolve as far as possible overnight at room temperature 2 g natural orcein (Supplier: George T. Gurr) in a mixture of 50 ml lactic acid and 50 ml propionic acid. Filter.

Working solution: dilute stock solution to between 45% and 60% with water and filter. It is useful to have both 45% and 60% available, as the stronger stain is better for some material. The solution will keep for many months but may require periodic filtration to remove precipitated particles.

1.3 Material

Detailed consideration of the species and tissues required for particular purposes will be dealt with in later sections. Only general consideration of the plant or animal material will be discussed here.

To show chromosomes, the organism or tissue must be alive and healthy and contain actively dividing cells at the time of fixation. To ensure these conditions requires some understanding of the development, anatomy and life cycle of the organism, and of its cultural requirements. If there is any doubt about these, expert advice should be sought.

Organisms which are easy to keep in culture include many of the flowering plants and the most frequently used material for demonstrating dividing chromosomes is the permanently embryonic tissue of growing root tips. These root tips are frequently broken off and lost when plants are dug out of the ground, so it is usually necessary to cultivate the plants in suitable soil or compost in pots. All plants should be repotted at least one month before material is required so that new roots can grow. They are most easily accessible with minimum disturbance to the plant when the root tips have reached the sides of the pot and can be collected from the surface when the plant is tipped out. To obtain younger roots the plant must be washed clean after removing most of the soil by gentle shaking. For most plants, cells are most active if the pot is liberally watered 24 to 48 hours before taking the roots, provided that drainage is good. Alternatively, where a species is most readily obtained as seeds, these can be germinated in pots or on moist blotting paper in Petri

dishes. A number of lateral root tips can usually be obtained from each seed if the emerging radicle is decapitated. Sometimes roots can be obtained from cuttings in aerated mineral culture solution. Healthy growing roots are brittle, translucent and white, with opaque cream to white, gently tapered tips.

When roots are not available, other tissues such as young leaves can sometimes be used. When plants are flowering, the mitotically active ovary or ovule wall of developing flowers or fruits provides a useful substitute which can be treated in the same way as roots after dissecting out appropriately sized pieces of tissue (Section 2.4).

Access to other sources of dividing cells is described in subsequent sections. However, it cannot be over-emphasized that no cell will yield a good preparation unless it is in a healthy condition, and it is a waste of time persisting with any technique if the material is sickly. Correct cultural management of the material up to the time of fixation is at least as important as the subsequent preparative method, and all too often neglected.

1.4 Pre-treatment

Pre-treatment may be required for some investigations, to clarify the morphology of the chromosome complement in the final preparation. This is unnecessary for certain divisions, such as meiosis, mitosis in pollen grains and mitosis during insect spermatogenesis, when chromosomes are normally well contracted and dispersed, but very helpful for root tip mitoses and with other meristematic tissues in plants. Low temperature treatment (e.g. 2°C for 24 hours) of intact tissue is sometimes an adequate alternative.

Washed root tips, either intact on seeds or cut off about 1 cm behind the tip, or other appropriate excised tissues, are immersed in the solution of colchicine or one of its alternatives (Section 1.2) for 4 to 6 hours at room temperatures not exceeding about 18°C. The material has to remain alive and active during this time, and it is important that the glassware and solution used is entirely free from detergent and other noxious chemicals. Continuous agitation and aeration by the bubbles from an aquarium aerator is recommended, particularly if large amounts of material, such as 100 root tips, are being processed simultaneously. At the least, the solution should be shallow with a relatively large surface and periodic stirring should take place throughout the treatment. The material is subsequently transferred to a clean tube for fixation, using forceps in order to leave behind as much as possible of the solution, grit and debris.

1.5 Basic technique

The steps in the technique are summarized in Table 1.2 and Fig. 1.2, and described in more detail below.

Fixation

Start here for smears and squash preparations of all somatic plant tissues. For squash preparations of divisions in fresh material of plant spores and animal cells start at 5.

1) Choose brittle, translucent roots with cream to white tips, or an equivalent healthy meristematic tissue. Immerse 1 cm root tips, or other tissue pieces of comparable volume, for 5 minutes in an excess of freshly prepared fixative in a specimen tube.

The material can be washed briefly in 70% ethanol in a clean tube if it is thought necessary to remove all traces of formalin before immersion in HCl. Formaldehyde can react with HCl vapour to produce a toxic chloromethyl ester. It is therefore also advisable to keep the formalin bottle at a distance

from the heated HCl. Formalin can be omitted from the fixative but inferior fixation, particularly of large chromosomes, may result.

For storage after fixation, the fixative can be subsequently replaced by 70% ethanol, but this may reduce the effectiveness of later orcein staining.

Maceration

2) Transfer the tissue with forceps to M HCl for 5 minutes at a maintained temperature of about 60°C. Any grit or debris should be left behind in the fixative.

3) Transfer the tissue to water. If several tissue pieces are involved, a convenient way to change solutions is to cover the mouth of the tube with a sheet of muslin, and shake the solution of HCl through into a sink with the tap running, leaving behind the plant material. Then, without removing the muslin, pour the water in, washing the tissue pieces back to the bottom of the tube in 2–3 cm of water. Material can be stored for a day or two in water.

Table 1.2 Summary of technique. See pp. 6–11 for details.

PRE-TREATMENT – for karyotype studies of plants only	4–6 hours in aerated pre-treatment solution (e.g. 0.05% colchicine) at room temperature, preferably below 18°C
FIXATION – for all chromosome studies of plant tissues and smears	5 mins in fixative: Absolute ethanol – 10 parts Chloroform – 2 parts Glacial acetic acid – 2 parts Formalin – 1 part Wash briefly in 70% ethanol in a clean tube
MACERATION – for all chromosome studies of plant tissues and smears	5 mins 1M HCl at 60°C. Transfer to water
CELL SUSPENSION – for all material	Tap or tease 1 mm piece of tissue in 5 mm drop of lacto-propionic orcein. Remove visible particles
COATING COVERSLIP – only necessary for intended permanent preparations	Thinly coat coverslip on lower surface with glycerin albumen and dry over a small flame
STAINING – for all material	Add further stain, spread and lower a coverslip. Tap gently on coverslip with needle point to disperse material if necessary. Leave for 10 mins – 24 hrs
SQUASHING – for plant material and perhaps to a lesser extent for animal material	Press down on coverslip, without lateral movement, through blotting paper
Temporary preparation completed	Examine and repeat, or make permanent as required
MAKING PERMANENT	Float off coverslip in 45% acetic acid 5–10 mins 75% ethanol 5–10 mins 90% ethanol 5–10 mins 100% ethanol
Permanent preparation completed	Mount in euparal

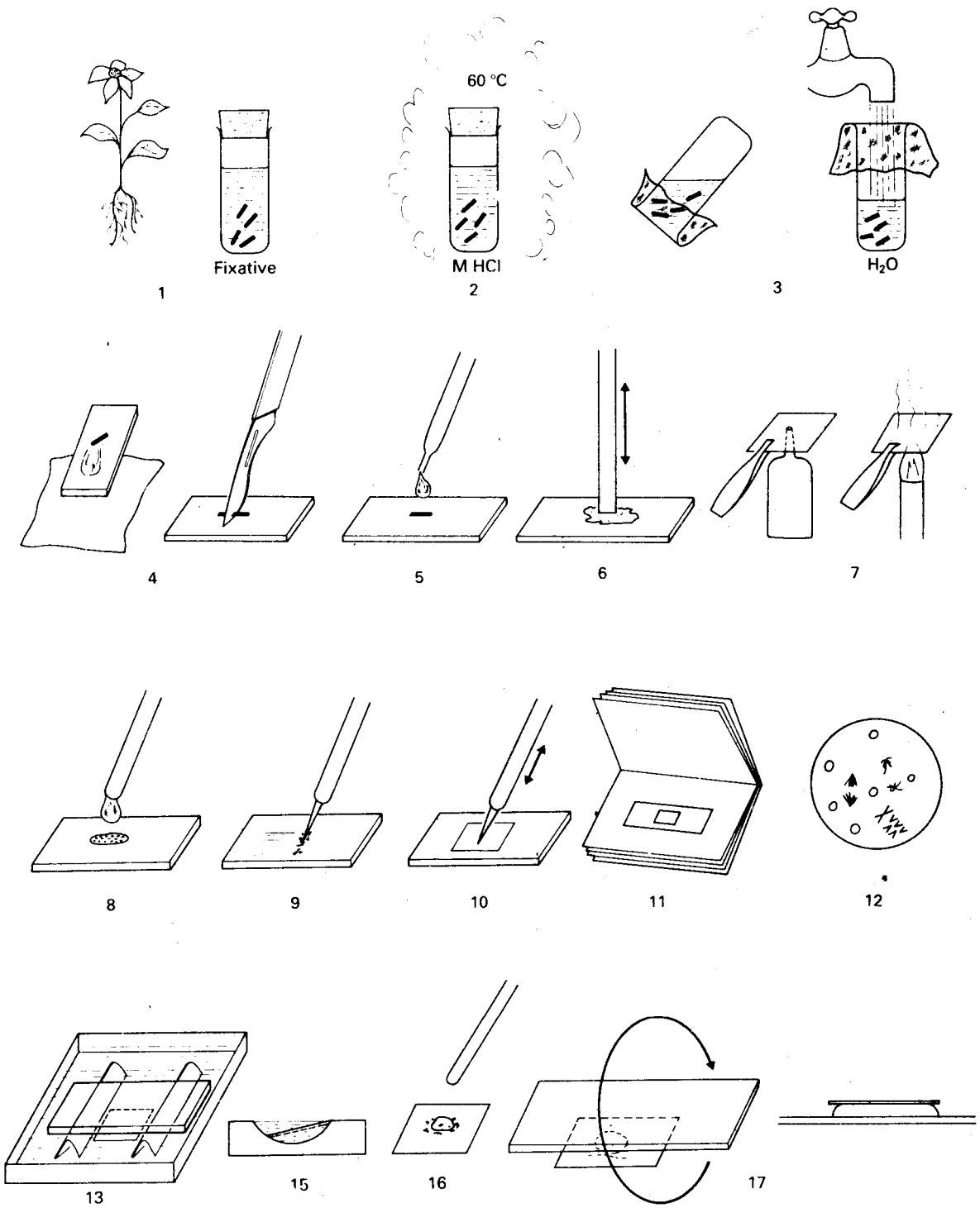

Fig. 1.2 The technique for making preparations of root tip mitosis. The numbered stages refer to those described in Section 1.5.

Preparing the cell suspension

4) Remove one piece of tissue and place it on a slide, sloped to allow excess water to drain on to blotting paper. Cut off the terminal 1 mm of the root tip, or excise an equivalent volume of other meristematic tissue, and discard the rest. Most beginners use too much material.

5) Begin here for squash preparations of plant spores and animal tissues.

   Onto one root tip, anther, ovule, insect testis follicle or equivalent piece of tissue, place one small drop (*c.* 5 mm diameter) of lacto-propionic orcein.

6) Gently tap the tissue in the stain/fixative with the end of the brass rod held like a pen in a vertical position. There should be enough stain to produce a thick suspension, but not enough to allow the material to escape from under the descending rod. Animal material requires little or none of this treatment, teasing out the tissues with needles being sometimes better.

   Plant anthers require only enough pressure to burst the walls and release the pollen cells, which are naturally separated from each other as they develop. Even more massive plant tissues such as root tips, with adhering thick cellulose walls, should, if correctly prepared, only require 10–20 taps, each time lifting the rod 1–2 cm above the slide, to produce a suspension of very small particles, ideally of single, separated cells. Great feats of strength or endurance are not required.

Coating the Coverslip

For intended permanent preparations only. Temporary preparations do not require this step, and the method proceeds direct from 6 to 8.

7) If permanent preparations are required, the coverslip to be used must be first coated with an adhesive. A clean coverslip is given a thin coating of glycerin-albumen which is then dried over a small flame. This is best done by putting a small drop of adhesive on the middle of the *underside* of a horizontally held coverslip from the nozzle of the tube, held upright. With a finger tip this drop is then smeared over the coverslip surface as if in an attempt to remove it all. On drying it over the flame, the albumen will smoke slightly and go cloudy, but a thin layer will be almost invisible. The coverslip should not be obscured by a layer of charred meringue!

Staining

8) Add another drop or two of orcein stain so that the suspension can be uniformly spread over the area to be occupied by the coverslip, but not so much that most of the material is lost when the excess stain is squashed out from under the coverslip when it is lowered. A common fault is to add too much stain, although when this is impressed upon beginners, a few in their zeal will work with an almost completely dry preparation. At no time should the cells dry out. With experience, it is often possible to tell from low power examination at this stage whether the material is good enough to warrant proceeding further with the preparation.

9) Remove any tissue particles large enough to see individually, and ensure that no pieces of grit are on the slide.

10) Lower a clean coverslip, or one coated with adhesive on the lower surface. Any small clumps of cells can be dispersed by gentle tapping on the coverslip with the tip of a mounted needle. Leave for at least 10 minutes to stain. The progress of staining can be followed at low power under the microscope, and it may improve for anything up to 24 hours. In most cases a usable preparation will be available

Table 1.3 Technique faults and remedies.

Symptoms	Possible causes and cures
TEMPORARY PREPARATION	
1) No cells present	Too little material, too much liquid, poor maceration – refer to detailed instruction
2) No divisions present	Unhealthy material – check cultural conditions. Wrong tissue; not meristematic – check anatomy. Wrong development stage; not dividing – check development. Wrong division stage in synchronous system – repeat
3) No divisons seen	As 1 or 2, or divisions present but missed through lack of experience – examine under × 40
4) Anaphase and telophase seen after pretreatment	Material dividing very slowly – check material or increase duration of treatment. Pretreatment ineffective – check temperature, concentration or change chemical
5) Chromosome clumped or sticky	Unhealthy material – check cultural conditions. Pre-treatment ineffective – check temperature and solution or change chemical. Poor fixation – renew fixative, check recipe, cut up tissue into smaller pieces. Wrong maceration treatment – check solutions, temperature, time
6) Cells not sufficiently squashed	Too little pressure – press harder Too much material. } Refer to Material not spread out. } detailed Grit or debris under coverslip. } instructions Poor maceration – use smaller pieces and if necessary give up to 20 mins treatment
7) Cells not sufficiently stained	Squashing too soon – leave longer. Poor penetration – check maceration and suspension of cells, and anatomy of tissue. Stain too weak – check recipe or increase concentration
8) Nuclei distorted, fragmented or 'rolled'	Lateral movement of slide during squashing
9) Broken slide or coverslip	Uneven bench surface. Grit under slide or coverslip
PERMANENT PREPARATIONS 1 to 9 plus:	
10) Coverslip will not float off slide	Too much material, too much adhesive, adhesive not dried off – check detailed instructions
11) Cells lost during dehydration	As 8, or too little adhesive
12) Cells distorted	Cells dried out during processing. Wrong alcohol concentrations or sequence
13) Cloudy mountant	Water in absolute ethanol, possibly from atmosphere – renew

within minutes, but sometimes staining will improve if the preparation is left overnight.

Squashing

11) When examination confirms that staining is adequate, place the slide carefully on a sheet of blotting paper on a completely flat surface and then cover it with two more layers of blotting paper. Avoiding any lateral movement, press the coverslip straight downwards by pushing with the thumbs through the upper layers of blotting paper. Excess stain will be expelled and absorbed and the cells flattened. Well prepared material should not need great pressure, and spores and animal cells usually need very little.

12) Examine again. The most flattened and spread cells are likely to be round the periphery, where the cells were flattened as they moved with the stain to the