CYTOGENETIC STUDIES OF SELECTED GENERA OF THE TRITICINAE

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STATEMENT

To the best of my knowledge and belief this thesis contains original research which has not been submitted previously for any degree at this, or any other, university. No material previously published, or written by any other person, is included in this thesis except where due reference is made in the text.

Signed.

David C. JEWELL

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SUMMARY

The development of, and the problems associated with, chromosome banding studies in plants were reviewed. The N-banding technique was confirmed as a technique capable of differentially labelling 9 of the 21 chromosomes of hexaploid wheat (Triticum aestivum L.). The critical procedures in the N-banding technique were identified. The N-banding technique was found to be reliable for the identification of meiotic chromosomes as well as mitotic chromosomes.

The genetic nomenclature assigned to the 9 N-banded wheat chromosomes was independently confirmed. The N-banding technique was used in this thesis to identify both alien chromosomes and structural chromosome rearrangements present in selected aneuploids of wheat and in stocks containing a range of abnormal chromosome types within the sub-tribe TRITICINEA.

It was demonstrated that, on the basis of their individual N-banding patterns, all 14 chromosomes of Aegilops variabilis could be identified and differentiated from the 9 wheat chromosomes that exhibit banding patterns. N-banding analysis was therefore used for the identification of all the available addition lines of Aegilops variabilis, Aegilops umbellulata and Aegilops longissimum to hexaploid wheat. This study demonstrated that, as yet, none of the above addition line series are complete. The hexaploid wheat cultivars Blaukorn and Orlando were used to demonstrate that rye chromosomes could be identified in substitution lines, and the cultivars Aurora and Transfed, and lines 256/75 and 486/77 were used to demonstrate the power of

N-banding analysis to detect translocations between wheat and rye chromosomes. The hexaploid wheat cultivar Poso was used to demonstrate the precision with which translocations involving the banded chromosomes of wheat can be identified using the N-banding technique. It was also possible to analyse the wheat complement of the available tetraploid triticales and to identify some spontaneous chromosome rearrangements present in other stocks. In many cases, chromosome banding studies represent the best presently available technique for detailed chromosome analysis.

Natural polymorphisms of banding patterns were detected for all 9 of the N-banded chromosomes in wheat by analysing different cultivars. These polymorphisms were not observed to affect the identification of chromosomes that exhibit banding. Furthermore, it was deemed possible to use these variations in banding pattern to detect the end product of homologous chromosome exchange in the progeny of hybrids containing known polymorphic differences in banding pattern for a pair of homologous chromosomes.

A simplified C-banding technique was developed for comparison with the N-banding technique and the relative benefits of each technique were evaluated for chromosome identification in the sub-tribe TRITICINEA. It was concluded that the N-banding technique is more specific than the C-banding technique, and a possible biochemical basis for the formation of N-bands was proposed.

N-banding was observed to be a powerful cytogenetic tool for the identification of individual chromosomes with a high level

of precision. This technique should have many applications to cytogenetic studies in wheat and related genera.

SECTION 1. GENERAL INTRODUCTION

Individual chromosome identification is an integral part of wheat cytogenetics. While some wheat chromosomes have been identified on the basis of general morphology, a karyogram of general applicability has not been satisfactorily established.

Cytogenetic studies have been considerably enhanced by new chromosome staining techniques which have been developed in the last 11 years. These staining techniques result in differential banding of mitotic metaphase chromosomes. These methods have enabled the individual identification of all the chromosomes in man, mouse and many other members of the animal kingdom. Unfortunately, the application of these techniques to plant chromosomes has not been particularly successful and no general chromosome banding technique has, as yet, been developed for plant chromosomes. The application of banding techniques to plant chromosomes has been hampered by many problems such as the preparation of whole cell metaphase plates without the normal aid of hydrolysis, the need to remove the coverslip before treatment of the preparations, the existence of natural variation (Polymorphisms) in the banding pattern of chromosomes in different cultivars, and the lack of understanding of the mechanism(s) of chromosome banding.

Two chromosome banding techniques have, however, been applied to wheat chromosomes - the C-banding technique and the

N-banding technique. The C-banding technique, although banding all 21 chromosomes of wheat, has not been universally successful for the identification of wheat chromosomes, as the results have not been repeatable in different laboratories. There have been only 5 or 6 wheat chromosomes which can easily be identified in somatic cells on the basis of their morphology and Giemsa band pattern. Yet, in all cases the hexaploid wheat cultivar Chinese Spring has been used as a reference. The N-banding technique (the second banding technique applied to wheat chromosomes) was demonstrated to differentially stain 9 of the 21 wheat chromosomes. The N-banding technique was, however, reported by the initial workers to specifically stain the nucleolar regions of chromosomes in other genera. No banding technique has yet been used for the identification of meiotic chromosomes of wheat, although the identification of meiotic chromosomes in a few other plant genera has been achieved.

The concern of this thesis is to evaluate the N-banding technique as a potentially universal technique for the individual identification of mitotic and meiotic chromosomes of wheat and related genera and to establish the relative benefits of the N-banding and C-banding techniques for this purpose. Selected aneuploids from within the sub-tribe TRITICINEA have been chosen for N-banding analysis in order to confirm the genetic nomenclature of the 9 N-banded chromosomes of wheat. These and a range of abnormal chromosome types have been chosen for N-banding analysis to determine the power of this cytogenetic tool for the identification of related alien material and structural

chromosomal rearrangements. These are discussed in detail.

A study to determine the critical steps of the N-banding technique was also carried out. Furthermore, as a result of the procedures used in the N-banding technique and a comparison of the N-banding regions of the chromosomes with specific satellite DNA locations on the chromosomes of wheat and the related species Aegilops variabilis, a suggestion is made as to a possible biochemical basis of N-band formation.

SECTION 2. LITERATURE REVIEW

2.1 The early development of chromosome banding

The pioneering studies of Caspersson *et al.* (1968) constituted the introduction of chromosome banding techniques to cytogenetics. Caspersson *et al.* (1968) demonstrated that fluorescent dyes such as Quinacrine Mustard (QM) affect selective, discrete fluorescent labelling in both plant and animal chromosomes. The intense or reduced fluorescence at localised regions of chromosomes gave a banded appearance and is known as fluorescence banding or more specifically "Q-banding". The technique simply involved the exposure of the chromosome preparations to QM stain, followed by microscopic examination using ultraviolet light. It was concluded, by comparison with spectrophotometric studies of DNA content, that increased fluorescence at specific regions along the chromosomes cannot be attributed to parallel variations in DNA content. The banding was postulated to reflect differences in chemical reactivity. The fluorescent alkylating agent used was presumed to bind preferentially with DNA characterised by high GC/AT ratios; however, the possibility of preferential accessibility of DNA to the agent on different regions of the chromosomes could not be excluded.

Caspersson *et al.* (1969a, 1969b) tested a number of

fluorescent alkylating agents and demonstrated that the preferential localisation of these DNA binding dyes was more or less in agreement with the presence and location of chromosomal segments showing cold-induced "starvation" of DNA (see Darlington and La Cour, 1940). Vosa (1970) proposed that there were at least four main types of heterochromatin showing all possible combinations of positive and negative cold effects ("starvation") and enhanced or reduced fluorescence.

Although the mechanism of the differentiation along the chromosomes was far from understood, it was suggested that chromosome identification and investigations of chromosomal polymorphisms and phylogenetic relationships connected with the various types of heterochromatin could be achieved at a level hitherto impossible. Caspersson et al. (1970a, 1970b, 1971) were able to identify each of the human chromosomes on the basis of their characteristic banding patterns and hereby proved the power of this new cytogenetic tool. The main disadvantage of this technique was that the fluorescence faded with exposure to ultraviolet light, thus necessitating photographic records for study. Horn and Walden (1971) demonstrated fluorescent banding in Zea mays L.; however, their evidence showed that some regions thought to be euchromatic also exhibited increased fluorescence thus casting doubts on the explanation of the banding advanced by Caspersson et al. (1969a, 1969b).

The first non-fluorescent banding technique employing the Giemsa stain was developed from the studies of Pardue and Gall

(1970), who observed denser centromeric regions of the mouse chromosome complement after *in situ* hybridisation with complementary RNA of mouse satellite DNA and subsequent Giemsa staining. The method consisted of denaturation of chromosomal DNA in metaphase chromosome preparations by heat or alkali treatment, reannealing it in suitable buffer, followed by staining in Giemsa. The specific banding of the centromeric regions in this way was called "C-banding" (staining of centromeric type constitutive heterochromatin).

It was soon apparent that by employing a number of technique modifications it was possible to obtain differential staining of the constitutive heterochromatin in the chromosome arms of mammalian and human metaphase chromosomes (G-banding). This resulted in the important step of "standardisation" in human cytogenetics (Paris Conference 1971) and with the aim of improved communication in the field of human cytogenetics a standardised system of designating individual chromosomes, chromosome regions and bands was proposed.

Though the initial report on banding with QM was from *Vicia faba*, the subsequent major developments in methodology and staining techniques stem mainly from experiments with mammalian chromosomes. The ease with which the mammalian chromosome preparations can be made seems likely to have been the primary reason for development in this field. Through minor changes in the basic theme of denaturation and incubation prior to staining and the use of proteolytic enzymes such as urea and trypsin, many

simplified techniques were developed for mammalian and human metaphase chromosomes. A comprehensive bibliography of this early literature is given by Nilssen (1973) and a review of the human and mammalian techniques is given by Kato and Moriwaki (1972) and Comings et al. (1973).

These new techniques led to a prolification of nomenclature viz. "ASG technique" (Arrighi and Hsu, 1971) - Acid Saline Giemsa; "BSG technique" (Sumner, 1972) - Barium hydroxide Saline Giemsa; "R-banding" (Dutrillaux and Lejeune, 1971) - reverse Giemsa banding; "N-banding" (Matsui and Sasaki, 1973) - nucleolus organiser banding; and "cd staining" (Eiberg, 1974) - centromeric dots.

The development of methodology and staining techniques was, however, much slower in plant material. Vosa and Marchi (1972) were the first to apply Giemsa staining to plants (Vicia faba, Allium, Scilla, Zea mays and three species of Tulbaghia) and they compared the results of Giemsa and fluorescent staining and found a close correspondence between the segments strongly stained with Giemsa and those segments differentiated with Quinacrine. An important difference was however noted: the Giemsa method was not observed to discriminate between segments with intense and slight fluorescence, but stained both in the same way. The obvious advantages of using ordinary light microscopy equipment and working on permanent preparations was also noted. As a result of investigations to modify the Giemsa technique for animal chromosomes (used on plants by Vosa and Marchi (1972)), Schweizer

(1973) published a Giemsa technique that was used in full or in part depending on the species. Although Schweizer's results again demonstrated that Giemsa could be used to differentially stain plant chromosomes, he chose plants that had known heterochromatic (H) segments revealed by cold treatment, as had most previous authors. Sarma and Natarajan (1973) were also interested in the association between the staining and the H segments of rye Secale cereale L.), in this case known to be late replicating. However, they also found that a new fluorescent dye, Hoechst 33258, could be used to obtain differential staining in contrast to the staining reaction with Quinacrine Mustard and Quinacrine dihydrochloride which gave no differential staining. Although most of the early research into chromosome banding in plants was directed at revealing the nature of the banding, other possible ramifications were discussed. Sarma and Natarajan (1973), for example, observed some banding of a non-terminal nature in wheat chromosomes and some variation in the rye chromosomes from different sources. Merker (1973b), using again a slight modification of Giemsa staining, supported the findings of Sarma and Natarajan (1973) and speculated that chromosome banding may be used to extend the field of cereal cytology and, perhaps, to make karyotype analysis, which previously had depended on measurements of length, arm ratios and general morphology, more exact.

The next major advancement in the use of chromosome banding in plants was the identification of interchanges and inversions in various stocks of Vicia faba (Dobel et al. 1973). Using a Giemsa method (Berger, 1971; cf. Shiraishi and Yosida,

1972 - see Dobel *et al.* 1973), which involved urea as the denaturing agent, Dobel *et al.* (1973) were able to confirm previous karyotypes of banded chromosomes of *Vicia faba* and to identify four reconstructed karyotypes. These experiments clearly demonstrated the value of banding as a cytological tool, not only for chromosome identification but also for the identification of structural changes in plant chromosomes.

One of the important problems in the banding of plant chromosomes was to obtain a squashed preparation of the mitotic material. It is necessary for the tissue to be softened prior to squashing so as to obtain a suspension of cells. The usual technique for achieving such a result is hydrolysis with 1 Normal hydrochloric acid (1N HCl), either before or during staining. This form of treatment normally interferes with the production of bands either with Giemsa or fluorochromosomes. The significant development that overcame this problem was the use of snail gut cytase (Gill and Hornby, 1973). This was used to enzymatically soften the tissue and thus allow whole cell metaphase preparations to be made and stained without significantly reducing the fluorescent patterns of the chromosomes. Until the latter development, mild hydrolysis or pretreatment of fixed root tips in 45% or 60% acetic acid had been used together with considerable pressure when squashing the preparations. Vosa (1970) had previously reported that, when squashed, the cells normally break, resulting in spreads of single or small groups of chromosomes, and the resulting lack of whole cell preparations made karyotyping

difficult. Shortly afterwards, Cataldo et al. (1974) reported a cellulase-facilitated squash technique. Gill and Kimber (1974a) achieved softening with a mixture of pectinase and cellulose and again demonstrated that normal staining could be achieved except when the root-tips had been subjected to normal hydrolysis (1N HCl).

Verma and Rees (1974) and Vosa (1974), using different varieties of rye, both published an idiogram for the identification of the seven pairs of C-banded rye chromosomes. These idiograms were based on the total length and arm ratios of the chromosomes in association with their C-banded patterns. This basis for identification was also proposed by Hadlaczky and Koczka (1974). Gill and Kimber (1974a) also reported a karyotype and idiogram of the seven pairs of rye chromosomes. However, they numbered the karyotype on the basis of their proposed homoeology to the wheat chromosomes (see Appendix 1 for explanation of chromosome homoeologous relationships). This appeared to be a much more satisfactory basis for the karyotype in view of the lack of accuracy in chromosome measurements, especially in different varieties (Herold, 1972 - see Schlegel and Mettin, 1978). Gill and Kimber (1974b) also reported a C-banding technique for cereal chromosomes, specifically wheat, rye and barley, and also (1974c) demonstrated the individual identification of all the wheat chromosomes in the cultivar Chinese Spring on the basis of their C-banding patterns.

Further evidence for more than one type of heterochromatin was put forward (Stack et al. 1974; Filion, 1974; and Fiskesjo, 1974) and cast further doubt on the method of chromosome banding and its uses to distinguish all types of heterochromatin. Differential Giemsa staining was seen to be possible in both mammalian (McKay, 1973) and plant (Yamasaki, 1973; Merritt and Burns, 1974), chromosomes with no treatment prior to staining. This, however, was definitely not the case in other plants such as rye (Sarma and Natarajan, 1973; Verma and Rees, 1974; Vosa, 1974) and tulip (Filion, 1974). The lack of one standard Giemsa staining method for plant chromosomes was further highlighted by the findings of Fiskesjo (1974) who demonstrated a difference in the terminal and centromeric Giemsa bands of Allium :- the terminal and centromeric bands appeared to require the preparations on the slides to be stored for different times ("maturation") before they could be differentiated by staining. At about the same time Matsui and Sasaki (1973) and Matsui (1974a, 1974b, 1974c) introduced the N-banding technique which involved acid extraction of DNA, RNA, and Histone proteins followed by staining in Giemsa. This method was alleged to stain the nucleolar regions in both mammalian and plant chromosomes. When Greilhuber (1974) demonstrated that hydrochloric acid could be used to induce chromosome banding with Giemsa (Hy-banding), it became even more evident that a number of techniques could be used to reproducibly stain plant chromosomes differentially.

The last important adaptation of Giemsa stain to plant chromosomes was its application to meiotic chromosomes to achieve

differential staining (Marks, 1974). Marks (1974) had shown that Giemsa bands similar to those seen in mitotic chromosomes are discernible at all principal stages of meiosis. It was also concluded that these bands were not the product of the Giemsa procedure since they can be seen in unstained preparations, using phase contrast optics, as chromocenters (condensed chromatin) in interphase nuclei and as condensed regions in prophase chromosomes. Marks (1974) was therefore in agreement with McKay (1973) and Merritt and Burns (1974) in concluding that Giemsa techniques only serve to display differential regions which are present without treatment. Although this conclusion is in doubt (see prior discussion), Marks (1974) had now paved the way for research into plant meiosis at an unprecendented level due to his conclusion that mitotic and meiotic banding patterns were similar.

2.2 Further advances and uses of chromosome banding in plants

2.2.1 Additional research into the basis of chromosome banding in plants

Having established the fact that chromosome banding could be applied successfully to plants, a limited amount of research was continued into the possible mechanisms of the differential linear staining in plant chromosomes. Greilhuber (1975) carried out extensive studies on the comparison of Hy- and C-bands in Vicia faba. These studies prompted two generalisations:

1) Since a clear-cut distinction in banding patterns, as in G- and C-bands in vertebrates, has never been demonstrated in plants, all Giemsa bands in plants should be regarded as C-bands (constitutive heterochromatin), in this case being defined according to Brown (1966) and redefined by Greilhuber (1977) as representing "unconvertible chromosome segments which do not decondense during telophase, so forming chromocenters during interphase, including all such very small bodies (heterochromomeres), which are consubstantial with chromocenters as revealed by staining behaviour or cytochemical tests". This definition closely resembles the classical understanding of heterochromatin (Heitz, 1932).

2) Although previous studies of Vicia faba revealed the same basic C-banding pattern, more or less completely, it was

postulated that this would depend on whether more or less optimal procedures had been chosen.

Greilhuber (1975) was able to substantiate the assertion that Hy-banding provided a different pattern from C-banding. However, each technique was assumed to be selectively staining constitutive heterochromatin with differing properties. It was assumed that, on the basis of the acid treatment required to produce Hy-bands, certain classes of non-histone proteins associated with DNA could be responsible for the staining differentiation. This assumption is in agreement with the role proposed previously for histones and non-histones in the C- and N-banding techniques (Comings et al. 1973; Utsumi and Takehisa, 1974; Matsui, 1974a). Limin and Dvorak (1976) also favoured an important role of proteins when they tested various times, temperatures and concentrations of SSC (saline/sodium citrate buffer) together with treatment in Barium Hydroxide, $Ba(OH)_2$. It was shown that $Ba(OH)_2$ treatment alone resulted in no C-band staining in rye chromosomes and that C-bands could be produced after treatment in $Ba(OH)_2$ with incubation in SSC for as little as 1 minute at a temperature of $0^{o}C$. It was therefore suggested that $Ba(OH)_2$ alters the chemical structure of nucleoproteins in heterochromatin, rendering them insoluble in SSC. It was considered unlikely that SSC functions as a DNA reassociating agent in the production of C-bands, and regarded more likely that SSC acts as a leaching agent for soluble nucleoproteins. This proposed mechanism was, however, placed in doubt by the findings of Sachan and Tanaka (1976) that in maize chromosomes C-banding could be achieved by using $Ba(OH)_2$ treatment either before or after treatment in SSC.

Schweizer (1976) used two fluorescent DNA binding (guanine-specific) antibodies in conjunction with AT base pair specific fluorochromes to demonstrate both normal and reverse pattern chromosome banding in plants. These findings seemed to provide new evidence for banding being the result of variations in DNA base composition along chromosome arms.

The possibility that banding could also be caused by regions of DNA being made preferentially accessible to the dyes, perhaps as a result of coiling, also could not be disregarded.

It has not been possible since Giemsa banding techniques have been applied to plant cytology to demonstrate banding which is comparable with G-bands in higher vertebrates (G-banding being the visualization of chromosome segmentation in contracted dividing chromosomes along their whole length, and consequently also in regions other than those occupied by constitutive heterochromatin - G-banding usually also requires a less severe extraction technique). The inability to demonstrate G-bands in plants could be due to a number of reasons, for example as a consequence of the different mode of chromosomal preparations in plants, or a genuine absence of G-banding chromatin in plants (Greilhuber, 1974), or, in terms of molecular biology (Nagl, 1976). It was also proposed that the lack of G-bands might simply be due to the high degree of contraction in plant chromosomes (Greilhuber, 1977), thus making the G-bands optically indetectable. Holm (1976) and Kongsuwan and Smyth (1977) both demonstrated the fluorescent banding, with Quinacrine compounds, differentiated many more sites than did C-banding in Lilium

chromosomes. These results could, however, be explained by Greilhuber's hypothesis (discussion above) in that the two procedures may stain different types of constitutive heterochromatin.

It is apparent from the presently available literature that the mechanisms of chromosome banding in plants is not yet understood and that no single hypothesis, as yet advanced, can satisfactorily explain the chromosome banding observed. A combination of all the factors discussed in this section may indeed be involved.

2.2.2 Availability of a general technique for chromosome banding in plants

On the basis of presently available literature it does not appear possible to have a universal method for differential chromosome staining of plant chromosomes. Several researchers have used many variations of the original Giemsa technique of Pardue and Gall (1970) to band plant chromosomes and this also is evidence for the lack of one general technique.

Gill and Kimber (1974b) and Kimber *et al.* (1976) reported a general Giemsa technique for cereal chromosomes and noted that the Giemsa techniques reported previously failed to produce any consistent banding in cereal chromosomes. Tanaka and Taniguchi (1975) also reported a banding method for plant chromosomes having been unable to obtain reliable results from previously published techniques. It was noted by Iordansky *et al.* (1978b) that in the

work of Merker (1973, 1973a, 1975) on analysis of triticale and of triticale-wheat hybrids, he was unable to karyotype wheat chromosomes because of insufficient differentiation. Bennett and Smith (1975) were unable to confirm the identity of the wheat chromosome involved in the wheat-rye translocation present in the wheat cultivars 'Aurora' and 'Caucasus' for the same reason, and yet Gill and Kimber (1974a, 1974c), using a very similar technique, were able to identify all the wheat and rye chromosomes on the basis of their individual C-banding patterns.

The non-universal applicability of chromosome banding in plants was not confined to C-banding techniques; for example, Sarma and Natarajan (1973) were unable to achieve chromosome banding with Quinacrine compounds used on rye chromosomes, as also have been later authors.

The N-banding procedure (Matsui and Sasaki, 1973; Matsui, 1974a, 1974b, 1974c) was improved by Funaki et al. (1975) and applied to 27 types of material including mammals, a marsupial, birds, amphibians, fish, an insect and plants, and from the available cytological and biochemical data it was concluded that it specifically stained the neucleolar regions of chromosomes. Thus is appeared that the N-banding technique was a good candidate for a universally applicable banding technique for both plants and animals. However, Pimpinelli et al. (1976) demonstrated that N-banding did not correspond to the nucleolar organising regions of Drosophila chromosomes, but induced extensive staining in the heterochromatic portions of the chromosomes and in some species gave good criteria for chromosome differentiation and identification. These findings were supported by Gerlach (1977)

who found the nucleolar sites of wheat chromosomes unstained, although the N-banding technique diagnostically labelled 9 of the 21 pairs of chromosomes. The conflicting results found in the staining specificity of the N-banding technique are discussed more fully in the body of this thesis.

It appears that not only is the mechanism of chromosome banding not understood, but also that no single banding technique is yet applicable to plant chromosomes.

2.2.3 Chromosome banding polymorphisms and studies into taxonomy and evolution of plants

Giemsa C-banding techniques have been used to demonstrate band heteromorphy between homologous chromosomes. The heteromorphy has revealed that banding pattern polymorphisms are widespread within species. Although this condition has been most extensively studied in man and animals, it has also been found to apply to plants. After the early reports (Schweizer, 1973; Vosa, 1973; Filion, 1974; Marks and Schweizer, 1974; Darvey and Gustafson, 1975; Hadlaczky and Belea, 1975) the first large study of variation was carried out on Leopoldia comosa (Liliaceae) by Bentzer and Landstrom (1975) followed by four more large taxonomic and evolutionary studies on a broad scale in the Liliaceae and Asteraceae (Schweizer and Ehrendorfer, 1976; Greilhuber and Speta, 1977, 1978; Ehrendorfer et al. 1977).

These studies demonstrated that the heteromorphy may consist of the size of the bands, their number, or both, and that

the various "banding styles" (the overall appearance of the banding patterns) could be used to taxonomically separate species. A high degree of banding polymorphism was also exhibited within species (Kenton, 1978; Lelley et al. 1978; Linde-Laursen, 1978; Zurabishvili et al. 1978). In the case of rye and wheat, the polymorphisms resulted in difficulty in identifying the chromosomes (Lelley et al. 1978; Zurabishvili, 1978). With barley, however, in no case was the variation in banding patterns found to be so great as to preclude the recognition of one common basic karyotype or the common basic banding pattern in the particular chromosomes.

Thus it has proved important, in investigations of evolutionary significance and in chromosome identification within a species, to have an understanding of inherent variation of banding patterns. These studies have also importantly indicated that the banding patterns of chromosomes are heritable.

2.2.4 The stability of the banding patterns throughout the cell cycle

While performing chromosome banding studies on plant chromosomes, associations between the number of chromocenters in interphase nuclei were often observed. These chromocenters, together with the kinetochores, were seen to be polarised to opposite regions of the nucleus (see Fussell, 1977; Stack et al. 1977; and Tanaka and Tanaka, 1977, for reviews). Observations by Thomas and Kaltsikes (1976a) and Mogford (1979) of the "bouquet" arrangement of chromosomes at prophase, together with the above

report on the polarisation effect, led to the following conclusions:

1) the C-banded material does indeed remain discernible right throughout the mitotic cell cycle.

2) chromosomes maintain their non-random telophase configuration through interphase as observed by Heitz (1932). (Fusion of these structures was also observed and thought to be due to somatic association - Singh et al. 1976).

Although these findings in themselves are useful, it takes on an importance when looked at in the light of meiotic studies of chromosome banding patterns. Marks (1974) achieved banding of meiotic chromosomes in plants for the first time, but the question remained as to whether, or not, the same patterns appear constantly at the particular regions of chromosomes in various types of somatic and meiotic cells. Tanaka and Komatsu (1977) were indeed able to demonstrate this using the chromosomes of Crepis capillaris. As yet very few studies have been carried out to determine chromosome banding in plants at meiosis - these have mostly been with rye and triticale for the purpose of identifying rye bivalents, univalents and non-homologous pairing of chromosomes (Singh and Lelley, 1975; Lelley, 1975; Kranz, 1976; Thomas and Kaltsikes, 1976b; Dhaliwal et al. 1977; Schlegel and Weryszko, 1979). Apparently chromosomes do indeed maintain their unique banding patterns throughout the cell cycles which allows chromosome identification outside the normal confines of metaphase or prophase preparations.

Thus chromosome banding in plants has developed as an invaluable cytological tool for the investigation of cytological

and taxonomic/evolutionary problems, as well as allowing chromosome identification in many plants (see Table 2-1).

TABLE 2-1

Plant genera within which mitotic chromosome banding studies have been reported.

Monocotyledons	Dicotyledons
# Allium	Anacyclus
Asparagus	* Anemone
Avena	Arabidopsis
Brodiaea	Beta
Cymbidium	Chrysanthemum
Gibasis	* Crepis
Heterotropa	Cucumis
Hordeum	Haplopappus
Leopoldia	Hepatica
Lilium	Lotus
Ornithogalum	Lycopersicon
Scilla	Nicotiana
#* Secale	Nigella
Spiranthes	Paeonia
Trillium	Phaseolus
#* Triticale	Ranunculus
Triticum	Rosa
Tulbaglia	Solanum
Tulipa	Spinacia
Zea	# Vicia
Zebrina	
Zingiber	

* indicates that both meiotic and mitotic banding studies have been reported.

# indicates the genera within which the majority of chromosome banding studies have been attempted.

2.3 The main problems in chromosome banding in plants

Many of the problems associated with the banding of plant chromosomes have been mentioned above. However, they will be briefly summarised here in order to provide an overview:

a) Due to the presence of the cell wall in plants, chromosome preparations are required to be squashed, which in turn requires the tissue to be softened. Hydrolysis in 1 normal hydrochloric acid (normal practice) cannot, in most cases, be used without a detrimental effect on chromosome banding. Also, the material has to be squashed under a coverslip which has inherent problems as the coverslip must be removed to perform differential staining. This can be achieved by freezing the material (Conger and Fairchild, 1953) in either liquid nitrogen or with dry ice, or by floating it off in ethanol. Again in this procedure there is the risk of "losing" cells by the removal of the coverslip. Therefore, some techniques involve the "subbing" of slides (see Darvey and Gustafson, 1975).

b) The purity of some chemicals has been deemed important (Merker, 1973a; Darvey and Gustafson, 1975).

c) A lack of knowledge of the mechanism of chromosome banding in plants (see section 2-2-1) has probably resulted in the lack of universally applicable techniques. This, together with the apparent failure of techniques in other laboratories (Gill and Kimber, 1974a, 1974b; Sarma and Natarajan, 1973; Pimpinelli et al. 1976), has made this one of the most important problems.

d) The lack of standard karyotypes for plant species and the well documented phenomenon of banding polymorphisms has also made communication of results difficult.

e) Poor publications (for example, Raicu et al. 1975; Kordan and Uncle, 1975; Lee and Hanneman, 1976; Sekioka et al. 1978) and confusion of nomenclature has also been detrimental to adequate communication of techniques for general application. Nomenclature in this thesis is as follows: all chromosome banding techniques utilising the Giemsa stain are regarded as staining constitutive heterochromatin according to the definition of Greilhuber (1977) and hence publications referring to G-banding in plants are assumed to be a misunderstanding of the meaning of the term. All other nomenclature is used according to the Paris Conference (1971) except where nomenclature has been introduced at a later date (it is then used according to the author's original definition).

2.4 The present state of chromosome banding in the TRITICINAE

The TRITICINAE is a subtribe (Hutchinson, 1934) of the tribe TRITICEAE Dumont, and incorporates the genera Agropyron Gaertn, Triticum L. em Thell, Aegilops Eig, Secale L., and Haynaldia Schur. C-banding has been observed in examples of all genera in the TRITICINAE (see Kimber et al. 1976, Gill and Kimber, 1977). However, karyotypes have been published for Triticum (Gill and Kimber, 1974c; Iordansky et al. 1978a; Gerlach, 1977; Zurabishvili, 1978), and Secale (Verma and Rees, 1974; Vosa, 1974, Hadlaczky and Koczka, 1974; Gill and Kimber, 1974a; and others) only.

Triticale (X Triticosecale Wittmack) has also been the subject of chromosome banding studies; however, these studies were largely concerned with the identification of the rye component.

The chromosomes of the genus Secale are the most studied genus of the TRITICINAE. In the TRITICINAE the relationships of chromosomes have been investigated in several ways and a universal system of numbering has been developed (Sears, 1966 - see also explanation in Appendix 1). Darvey (1973) summarised the available literature on the homoeologous relationships of the rye chromosomes to the hexaploid wheat complement (T. aestivum cv. Chinese Spring) and was able to assign a numbering system based on these relationships (Darvey and Gustafson, 1975). This method of standardising the rye chromosome nomenclature not only offers the advantage of overcoming the inaccuracy of chromosome measurements (see section 2-1), but also allows the identification of

chromosomes in an homoeologous context.. If this system of chromosome numbering is adhered to, the tentative identification of D genome chromosomes in the integrated hexaploid triticales (which contain a mixture of D genome and rye genome chromosomes) can be postulated. Although it is becoming more popular, this system of nomenclature has not been accepted universally in banding studies with rye chromosomes.

Earlier reports of polymorphism within a species have been confirmed by larger studies (Darvey and Gustafson, 1975; Lelley *et al.* 1977) and have led to difficulties in the identification of chromosome 2R, 3R and 7R, both within and between species. In most cases, chromosome 2R can be distinguished from 3R and 7R and chromosomes 1R, 4R, 5R and 6R are easily identified due to their relatively standard pattern (Lelley, 1975).

The identification of *Secale* chromosomes has made possible the re-evaluation of the interspecies relationships in the *Secale* genus (Shchapova and Kobylyanskii, 1976; Singh and Robbelen, 1977). Although several karyotypes of rye are now available, absolute chromosome identification is, however, still in doubt. De Vries and Sybenga (1976), for example, used chromosome banding in an attempt to correlate the identification system of chromosomes in diploid rye and the added rye chromosomes to wheat (addition lines) with that based on a rye translocation tester set. Zeller *et al.* (1977) also identified individual chromosomes in rye trisomics by C-banding and corroborated their results by crosses to the same translocation tester set. In only two cases did the work of de Vries and Sybenga (1976) agree with that of Zeller *et al.* (1977).

Rye chromosomes have also been observed with reduced telomeric heterochromatin (heterochromatin in the distal portion of chromosomes) (Merker, 1976; Roupakias and Kaltsikes, 1977). Most of the characteristic C-bands in rye are of a telomeric nature. This "loss" of heterochromatin may further hamper chromosome identification. Rye chromosomes exhibit similar C-banding patterns at meiosis (Lelley, 1975, and other authors - see Schlegel and Weryszko, 1979, for review), thus supporting the hypothesis that the chromosome banding pattern remains constant throughout both the mitotic and meiotic cell cycles.

Gill and Kimber (1974c) and Iordansky et al. (1978a) both reported differential C-banding patterns for all 21 chromosomes of T. aestivum (hexaploid wheat). Iordansky et al. (1978a) concluded that the karyotypes of the two cultivars studied ("Chinese Spring" and "Diamant") differ drastically both in total structural heterochromatin content and its localisation on the nine morphological homologous chromosomes. The other twelve pairs of chromosomes showed no morphological similarity.

Comparing the differential banding of the cultivars "Diamant" and "Chinese Spring" (Gill and Kimber, 1974c), Iordansky et al. (1978a) found a similarity between only 5 chromosomes and, by comparing their Chinese Spring banding patterns with those of Gill and Kimber (1974c), only a general similarity between nine of the chromosomes.

In these two studies, the proposed nomenclature was also different. Gill and Kimber's identification was based on the

genetic nomenclature (correlated with the homoeologous relationships) from the results of a study of the ditelocentric lines, whereas Iordansky et al. used length and banding patterns in a similar way to that used in human material (Paris Conference, 1971).

Subsequent research, using further cultivars of hexaploid, tetraploid and diploid wheats, enabled Zurabishvili et al. (1978) to propose that the karyotype of each polyploid wheat species consists of two groups of chromosomes. The first one is formed by ten pairs of "constant" chromosomes occurring almost in all species and the second by all the remaining "variable" chromosomes that are either fully species specific or occur only in a few species.

Gill and Kimber (1977), subsequent to their identification of all 21 chromosomes of wheat, stated that in wheat "at least 11 chromosomes, including 4A, 5A, 6A, 1B, 2B, 4B, 6B, 1D, 4D and 5D, are diagnostically banded and can be easily identified". In view of these conflicting results the identification of wheat chromosomes with C-banding can at best be tentative. The results of Schlegel and Mettin (1978) show that while a great number of wheat chromosomes have been measured and categorised, these fail to fit a karyogram of general validity. It was further concluded that only five or six chromosomes can easily be identified by Giemsa banding, hence the identification of all other chromosomes remains subject to error. These conclusions were formed in the apparent unawareness of the findings of Iordansky et al. (1978a) and Zurabishvili et al. (1974, 1978).

In view of the above results of C-banding studies with the genus Triticum it would appear that other scientists using C-banding to explain genome origins of wheat chromosomes (Natarajan and Sarma, 1974; Hadlaczky and Belea, 1975), and for the identification of wheat chromosomes (Gustafson and Krolow, 1978), may have reached erroneous conclusions.

Gerlach (1977) was able to apply the N-banding technique to T. aestivum cv. Chinese Spring and some closely related species. Gerlach (1977) demonstrated that N-banding did not stain the Neucleolar Regions of wheat but gave differential banding on 9 of the 21 pairs of chromosomes in the cultivar "Chinese Spring". There was a similarity between the banding pattern of these 9 chromosomes and the same 9 chromosomes banded with C-banding (Gill and Kimber, 1974c), and yet, the banding was not identical. Gerlach (1977) also chose to number the N-banded chromosomes according to their homoeologous nomenclature and identified the banded chromosomes on the basis of the banding patterns present in the singly or doubly ditelocentric tester lines of the cultivar Chinese Spring.

The N-banding patterns of the wheat karyotype have also been achieved by Islam (1980) (see also section 4 of this thesis). The N-banding technique may therefore be a reliable technique for the study of some chromosomes in the TRITICINAE. The main advantage of this technique may be that a smaller number of chromosomes are banded than with the C-banding technique, thus rendering these chromosomes easier to identify even if polymorphisms of banding patterns are exhibited in different cultivars.

The Giemsa C-banding technique has been used with numerous modifications by those who have reported banding in the TRITICINAE. Indeed chromosome banding has been observed in all the genera of the TRITICINAE even to the extent of identifying translocations (Gill and Kimber, 1977). Clearly the establishment of more karyotypes and further research towards a standardised and universally applicable technique can only enhance investigations of the genetics, cytology and evolution of this important taxonomic group.

SECTION 3. MATERIALS AND METHODS

3.1 Seed stocks

3.1.1 Hexaploid wheats

Triticum aestivum L. cv. Chinese Spring

T. aestivum cv. Aurora

T. aestivum cv. Blaukorn (K601)

T. aestivum cv. Federation

T. aestivum cv. Kite

T. aestivum cv. Orlando

T. aestivum cv. Transfed

T. compactum cv. Poso

The above cultivars were provided for study from stocks held at the Waite Agricultural Research Institute.

3.1.2 Aneuploids of hexaploid wheat

Addition lines A, B, C, D, E, F, G, H, I, J, L, M, N, O and P of *Aegilops variabilis* to *T. aestivum* cv. Chinese Spring.

Addition lines A, B, C, D, E, F and G of *Ae. umbellulata* to *T. aestivum* cv. Chinese Spring. Seed of the *Ae. variabilis* addition lines was kindly supplied by Prof. C.J. Driscoll and the seed of the *Ae. umbellulata* addition lines A, B, C, D, E and

G (isolated by Kimber, 1967) was supplied by Dr. K.W. Shepherd. Seed of Ae. umbellulata addition line F was provided by Prof. R. Riley.

Addition lines B, D, E, F and G of Ae. longissimum to T. aestivum cv. Chinese Spring were provided by Dr. M. Feldman.

Line 256/75 (a 1R/1B translocation in a T. aestivum cv. Gabo background) provided by Dr. K.W. Shepherd.

Line 486/77 (a 1R/1B translocation and a 1R/1D translocation present in a T. aestivum cv. Gabo background) provided by Dr. K.W. Shepherd.

The following aneuploids in a Chinese Spring wheat background were also provided for study by Dr. K.W. Shepherd.

Nulli tetra 3A/3B, Nulli tetra 7B/7A, Nulli Tetra 2D/2B,

Tetra 3B, Tetra 4B, Tetra 4A, AND

Double Ditelo 4A

Seed of the tetraploid male sterile T. durum isolated by Mr. A.M. Hossain from the male sterile line Cornerstone (Driscoll, 1977) and crossed to T. monococcum by Mr. A.M. Hossain was also examined.

3.1.3 Triticale

The cultivars of triticale listed below were provided by Prof. C.J. Driscoll from stocks held at the Waite Agricultural Research Institute.

T.236 (hexaploid)

T.240 (hexaploid)

T.507 (hexaploid) - Coorong

T.937 (hexaploid) - Beagle

T.1343 (hexaploid) - Drira

4 x 2 4a - 1090 (tetraploid)

4 x 3 4a - 1089 (tetraploid)

4 x 5 4a - 1091 (tetraploid)

3.1.4 Other stocks

Aegilops variabilis;

F_1 seeds of Ae. variabilis and T. aestivum cv. Chinese Spring; BC1 seeds (1st Backcross) of the F_1 between Ae. variabilis and T. aestivum cv. Chinese Spring backcrossed to Chinese Spring; seed of Secale cereale L. cv. Imperial from stock held at the Waite Agricultural Research Institute.

3.2 Mitotic metaphase preparations for chromosome banding

Seeds were germinated at 27°C on damp filter paper in a petri dish. When the seminal roots were 1-2 cm in length, they were immersed in 0.05% to 0.07% colchicine solution for four hours. The root tips were then excised and fixed in 3:1 ethanol-acetic acid and stored at -15°C until examined. The seedlings can be grown into normal plants following the colchicine treatment if immediately and thoroughly washed. Root tips were dissected in 45% v/v acetic acid and all debris removed. An 18 mm x 18 mm coverslip was placed on the preparation and it was then squashed between folded filter paper after mild heating. Coverslips were removed following freezing (see Conger and Fairchild, 1953) in liquid nitrogen and the slides were immediately placed in 95-100% ethanol at room temperature and stored overnight at -15°C.

3.3 Meiotic metaphase preparations for chromosome banding

One anther from each floret was used to check the meiotic stage. The remaining two anthers were fixed in 3:1 ethanol-acetic acid and stored at -15°C until used. Anthers were macerated in 45% v/v acetic acid and the debris removed. The procedure for mitotic preparations was then followed.

3.4 The N-banding method

The standard N-banding method used in this thesis varied only slightly from that described by Funaki *et al.* (1975) and Gerlach (1977). After removal from ethanol, slides were air dried for 30 min. and treated in 1 Molar NaH_2PO_4 (Univar-analytical reagent), pH 4.2 at 92 ± 1°C, for 4 to 6 min. Slides were rinsed in distilled or deionised water and stained in 7.3% v/v Gurrs Improved R66 Giemsa stain in 0.03 Molar Sorensen's phosphate buffer (pH 6.8) for 30 to 40 min. After staining, the slides were rinsed in distilled or deionised water, air dried and mounted in Zeiss immersion oil (containing PCB) for microscopic examination. The coverslip is ringed with nail varnish if the slides are required to be kept. For banding of meiotic preparations the hot acid treatment time was reduced to 1 to 2 min. Undertreated preparations show near uniform staining; these slides can be rinsed in 95-100% ethanol followed by water to remove the oil, and retreated for a slightly longer period. Overtreated preparations show empty looking, unstained chromosomes and banding cannot be achieved by retreatment.

3.5 The C-banding method

After removal of the coverslip (see section 3-2), the preparations were dehydrated on a warm hot plate instead of using 95-100% ethanol. The slides were then placed in saturated $Ba(OH)_2$ for 5-7 min., at 25°C in a sealed container. The slides were then rinsed in distilled or deionised water, incubated in 2 x SSC (saline/sodium citrate) for 1 hour at 60°C, and finally rinsed and stained as for N-banding. This C-banding method is a simplification of many similar methods published for C-banding in plants.

3.6 The preparation of solutions

a) Prefixative solutions

0.5% colchicine (5 mg/10 ml distilled water) was made fresh each time, and 10 ml of solution was used with one filter paper for each 11 cm petri dish.

Where ∝ bromonaphthalene is used, it was prepared as a 1% tap water solution of a stock solution consisting of 1ml of ∝ bromonaphthalene dissolved in 100 ml of absolute ethanol (Linde-Laursen, 1975). The emulsion so formed was more stable than the standard method of shaking 2ml of ∝ bromonaphthalene in 100 ml of tap water, as well as being more economical. The stock solution could be stored for an indefinite period at room temperature and the working solution was made fresh each time.

b) The one molar sodium orthophosphate (NaH_2PO_4) solution can be used for at least five banding runs. However, a fresh solution was made if the solution was more than one week old.

c) Giemsa Staining Solution

Stock solutions of 0.5 molar Na_2HPO_4 and 0.5 molar NaH_2PO_4 were made and can be kept at room temperature for about 1 month. The working solution of 0.03 molar Sorensen's buffer and 7.3% Giemsa was prepared freshly each time by taking 1.5 ml of each stock buffer solution and adding it to approximately 20 ml of distilled water, and 1.8 ml of Gurrs Improved Giemsa R66 was added

to the solution just prior to use.

d) Saturated Barium Hydroxide

About 6gm of $Ba(OH)_2$ was added to 100 ml of distilled water and stirred for approximately 5 min. The solution was then allowed to settle for about 1 min. and then the film of barium carbonate was scraped off the surface and the treatment container completely filled. This solution was made fresh for each banding run.

e) 2 x SSC (saline/sodium citrate)

A stock solution of 20 x SSC (17.43 gm of sodium chloride and 8.82 gm of sodium citrate in 100 ml of distilled water) can be stored indefinitely at $-15^{o}C$. The working solution of 2 x SSC (0.3 Molar NaCl + 0.03 Molar $Na_3C_6H_5O_7$) was prepared by taking a 1 in 10 dilution of the stock solution. The 2 x SSC solution can be used for several banding runs, but was made fresh if more than 1 week old.

f) Other solutions.

The 3:1 ethanol-acetic acid fixer was always made just prior to use, and the 45% acetic acid solution was made as required. Fresh ethanol was always used for the dehydration of preparations.

3.7 Photography

All photomicrographs were taken using an automatic Zeiss camera and Recordak film (KODAK). Photomicrographs of banded preparations were taken using a green and yellow filter combination to increase contrast. The film was developed in 50% v/v DK50 (KODAK) for 10 min. at 20°C and printing was on gloss Kodak paper. The photomicrographs were cut and karyotypes mounted on blank photographic paper and rephotographed by the photographic section.

SECTION 4. A STUDY AND DEVELOPMENT OF THE N-BANDING TECHNIQUE

4.1 Introduction

The Giemsa N-banding technique as developed by Matsui and Sasaki (1973) and Matsui (1974a, 1974b, 1974c) involved the use of 5% trichloroacetic acid and 0.1 M hydrochloric acid, followed by Giemsa staining to produce a dark staining (positive) band in the nucleolar region of chromosomes. Funaki *et al.* (1975) developed an improved N-banding technique using 1M NaH_2PO_4, instead of the 5% trichloroacetic acid and 0.1M hydrochloric acid procedures, and demonstrated specific banding in the nucleolar organiser regions of chromosomes in 27 materials including mammals, a marsupial, birds, amphibians, fish, an insect and plants. The latter authors observed that in some species small bands distributed over many chromosomes were present and were thought to be dispersed nucleolar material.

The method of Funaki *et al.* (1975), with slight modifications, was applied to hexaploid wheat by Gerlach (1977) who demonstrated that 9 of the 21 pairs of wheat chromosomes exhibited unique banding patterns which enabled their individual identification to be made. However, he did not observe banding in the nucleolar regions. This observation of differential staining

not localised in the nucleolar organiser regions supported the findings of Pimpinelli *et al.* (1976), who found that the positive N-banding regions in *Drosophila* chromosomes did not correspond to the nucleolar regions, but induced extensive staining in some of the known heterochromatic regions of the chromosomes.

In view of these conflicting results, a study was designed to:

(i) determine the relative importance of each procedure in the N-banding technique

(ii) to identify the important procedures and to determine the possibility of manipulating these procedures to produce reliable staining of the nucleolar regions in the 1B and 6B chromosomes of hexaploid wheat

(iii) to attempt to reproduce the results of Funaki *et al.* for the exclusive staining of the nucleolar regions of rye and to confirm the results of Gerlach (1977) for the differential staining of 9 of the 21 pairs of wheat chromosomes.

4.2 Modifications to the N-banding technique

The following modifications were made to the standard technique (sections 3-2 and 3-4) and tested on the hexaploid wheat cultivar Chinese Spring:

i) ∝Bromonaphthalene in place of colchicine;

ii) After removal of the coverlsip, slides were dehydrated on a warm hot plate in place of the normal dehydration in ethanol;

iii) The time the slides were left in 95-100% ethanol after the coverslip was removed was varied from 1 hour to 1 week. This was done at room temperature (about 21°C), at 4°C and at -15°C;

iv) Slides were air-dried (after dehydration in ethanol) and left at room temperature for up to a week;

v) 1M NaH_2PO_4 was used over a range of pH (pH 2.0 - pH 6.5);

vi) 1M NaH_2PO_4 was replaced by each of a 50/50 mixture of 2M H_3PO_4 and 2 M NaOH, 1M KH_2PO_4, 1M $NH_4H_2PO_4$, phosphoric acid diluted to pH 4.2 (approximately 0.0008M) and a 2 x SSC solution adjusted to pH 4.2 with hydrochloric acid. The original N-banding method (Matsui and Sasaki, 1973) involving incubation in 5% trichloroacetic acid at 90°C and 0.1M hydrochloric acid at 60°C was also examined;

vii) The incubation time in 1M NaH_2PO_4 was varied from 0.5 min. to 1hour;

viii) The temperature of the 1M NaH_2PO_4 buffer was varied from 80°C to 99°C;

ix) The effect of staining the slides in Giemsa buffered at slightly differing pH's and monotoring the banding during the staining period was examined;

x) The possibility of retreating preparations with 1M NaH_2PO_4 if the initial staining was unsatisfactory was examined;

xi) Depex (Gurr B.6970 Searle Diagnostic, High Wycombe, Bucks, England) and Eupral (GBl Labs Limited, Heatons Mills, Denton, Manchester, Made in England) were used to mount slides in lieu of immersion oil.

4.3 Results

Placing the root tips in 45% acetic acid for approximately 2 min. prior to dissection in 45% acetic acid was found to be satisfactory for softening the material. However, the epithelial cells (see figure 4-1) were observed to be very difficult to squash. The dissection of root tips was therefore important not only to obtain a single cell suspension but also to minimise the number of epithelial cells present in the preparations, as these cells severely limited the number of good quality single cell metaphase squashes.

The standard N-banding method used in this thesis (section 3-4) resulted in differential staining of 9 of the 21 pairs of chromosomes in the hexaploid wheat cultivar Chinese Spring (see figure 4-2). Only complete cells were used for analysis. The banding patterns on these 9 chromosomes correspond almost exactly with the patterns observed by Gerlach (1977) with the exception of a minor band in the subterminal region of the long arm of chromosome 7A, a minor band in a proximal position to the centromere in the long arm of chromosome 2B, and the two minor bands in the distal region of the long arm of chromosome 6B. An idiogram illustrating the relative sizes and positions of the N-bands in the cultivar Chinese Spring is presented in figure 4-3. Occasional diffuse banding was observed in the nucleolar regions of chromosomes 1B and 6B. These regions are indicated by an asterisk in figure 4-2.

The use of α Bromonaphthalene as a pre-fixative and the

FIGURE 4-1 Unstained epithelial cells of hexaploid wheat in a squashed preparation (viewed with phase contrast optics). These cells are difficult to squash and as such they have not spread well.

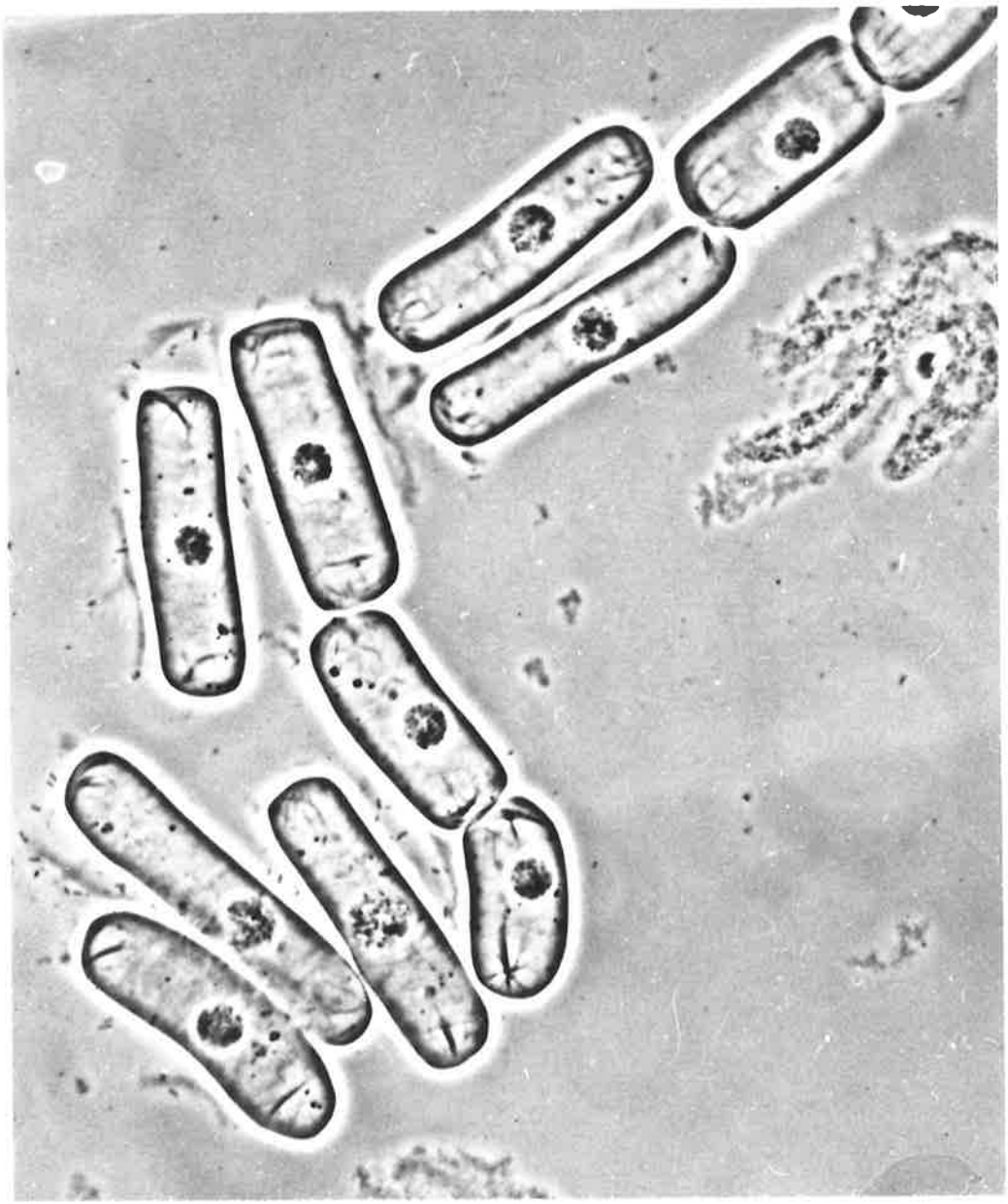


FIGURE 4-2 An N-banded karyotype of the hexaploid wheat cultivar Chinese Spring. The nucleolar regions of chromosomes 1B and 6B are indicated by an asterisk.

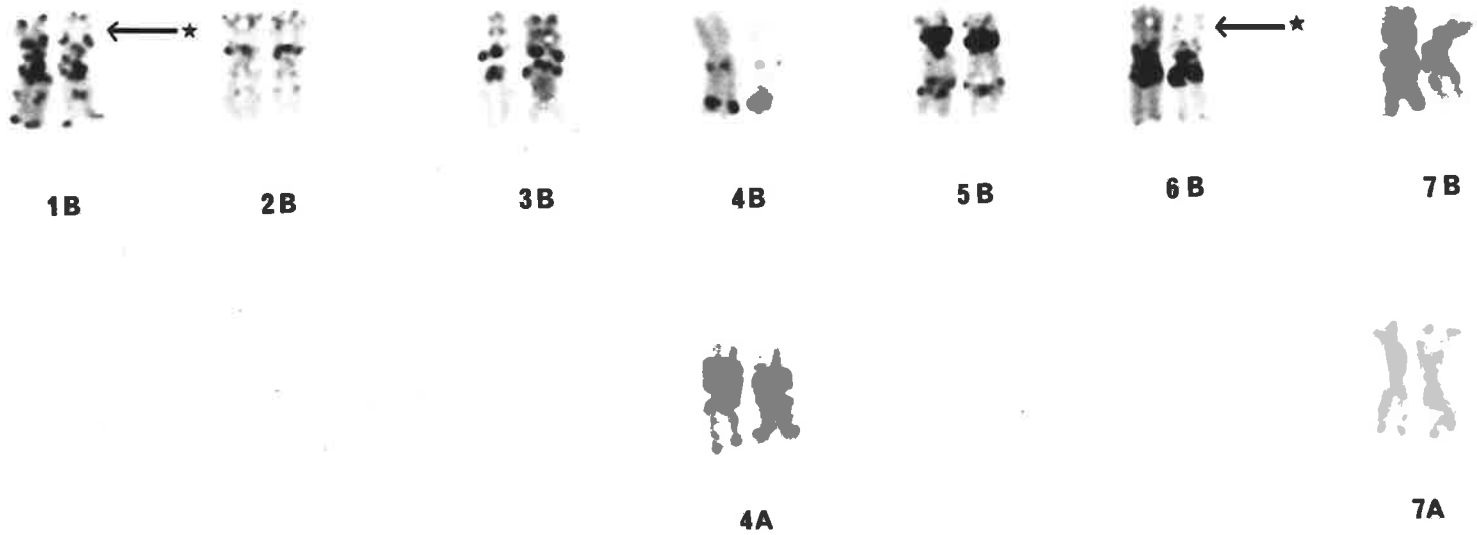
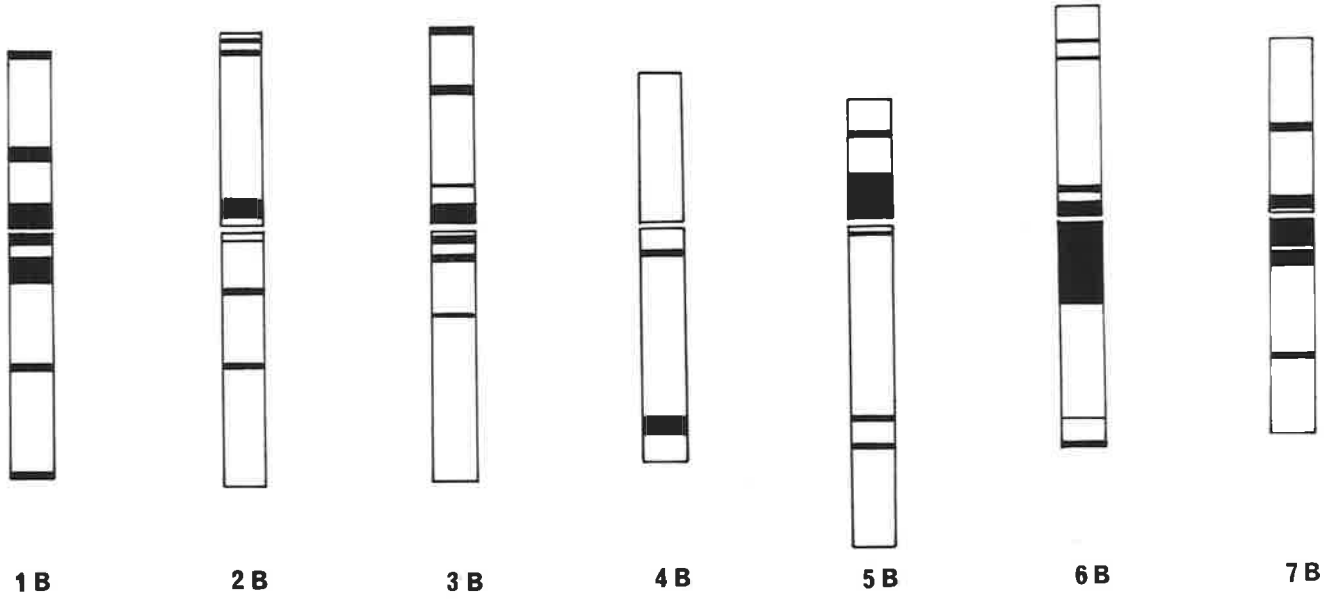

1B
2B
3B
4B
5B
6B
7B
4A
7A

FIGURE 4-3 An idiogram of the chromosomes of the hexaploid wheat cultivar Chinese Spring, illustrating the relative size and position of the N-banded regions. The relative lengths and arm ratios of the chromosomes are those of Sears (1954).


1B
2B
3B
4B
5B
6B
7B


4A
7A

use of a warm hot plate to dehydrate slides instead of ethanol did not alter the N-banding. The time spent in ethanol, however, was observed to effect the N-banding - the longer the slides spent in ethanol, the less the required time in 1M NaH_2PO_4 to achieve N-banding. The quality of the preparations also declined with longer storage in ethanol. The storing of slides in ethanol at higher temperatures had a similar effect to storage for longer periods at $-15^{o}C$.

Air-drying slides for up to a week also necessitated a reduction in the time of the 1M NaH_2PO_4 treatment and resulted in a parallelled reduction in the quality of banding.

The pH of the 1M NaH_2PO_4 was also found to effect banding. A trace of N-banding was found as low as pH 3 and as high as pH 6, and good banding was observed between pH 3.5 and pH 4.5.

The 50/50 mixture of 2M H_3PO_4 and 2M NaOH, 1M KH_2PO_4, 1M $NH_4H_2PO_4$ and the 2 x SSC solution adjusted to pH 4.2 all gave satisfactory N-banding, though not of the quality of 1M NaH_2PO_4 (Univar). The trichloroacetic / hydrochloric method of Matsui and Sasaki (1973) produced the same N-banding patterns as the 1M NaH_2PO_4 method on wheat chromosomes. However, the quality of banding and the general morphology of the chromosomes was inferior. Different brands of 1M NaH_2PO_4 were observed to give variable results and the Univar-analytical reagent was selected as the brand giving the best results. Dilute phosphoric acid gave no banding.

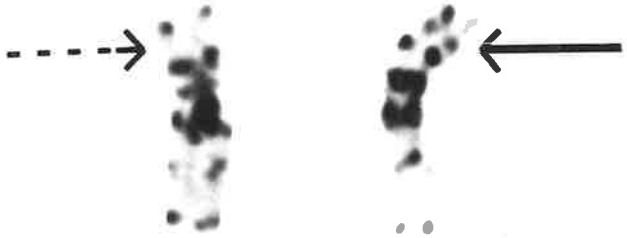
The incubation time in 1M NaH_2PO_4 was observed to be very important. After 0.5 min. in the hot acid buffer, chromosomes were uniformly dark, and after 4 - 5.5 min. in the buffer, the optimum banding definition was reached. After 30 min. only traces of banding were visible. Temperature was also very important. At 96°C and higher, N-banding was achieved. However, structural damage was caused by the removal of pieces of the cell from the slide and at lower temperatures a longer treatment time was required, resulting in some loss of chromosome definition.

Small changes in the pH of the Giemsa stain and the use of Depex and Eupral for mounting slides were observed to have no effect on the banding.

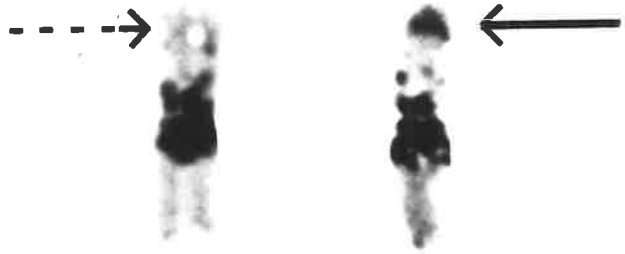
The most satisfactory conditions for making visible the nucleolar bands in the 1B and 6B chromosomes of wheat (see figure 4-4) involved the use of 1M NaH_2PO_4 at 90°C for 3 min. after immersion for 3 to 5 hours in ethanol at -15°C. The same conditions, but using the 1M NaH_2PO_4 for 1 - 2 min., were the most satisfactory for the N-banding of rye chromosomes (see figure 4-5). An idiogram illustrating the size and position of the N-bands in the rye chromosomes is presented in figure 4-6.

The N-bands on the rye chromosomes were largely of the same diffuse type as the nucleolar bands in the 1B and 6B chromosomes of wheat, and resembled closely the C-banding pattern (see figure 4-5). The karyotype of rye chromosomes presented in figures 4-5 and 4-6 are based on that of Gill and Kimber (1974a) and later publications by numerous authors. The classification of chromosomes 4R and 7R is however in accordance with the findings

FIGURE 4-4 N-banded chromosomes 1B and 6B of hexaploid wheat with and without positive staining in the nucleolar regions. The "solid" arrows indicate the nucleolar band and the "broken" arrows indicate the position of the nucleolar region or secondary constriction in preparations that do not exhibit a band in this region.



1B



6B

10 μ

FIGURE 4-5 A comparison of the N-banded and C-banded karyotype of rye chromosomes (cultivar Imperial). The chromosome nomenclature is based on that of Gill and Kimber (1974a) and later publications. The classification of 4R and 7R is, however, in accordance with the findings of Koller and Zeller (1976).

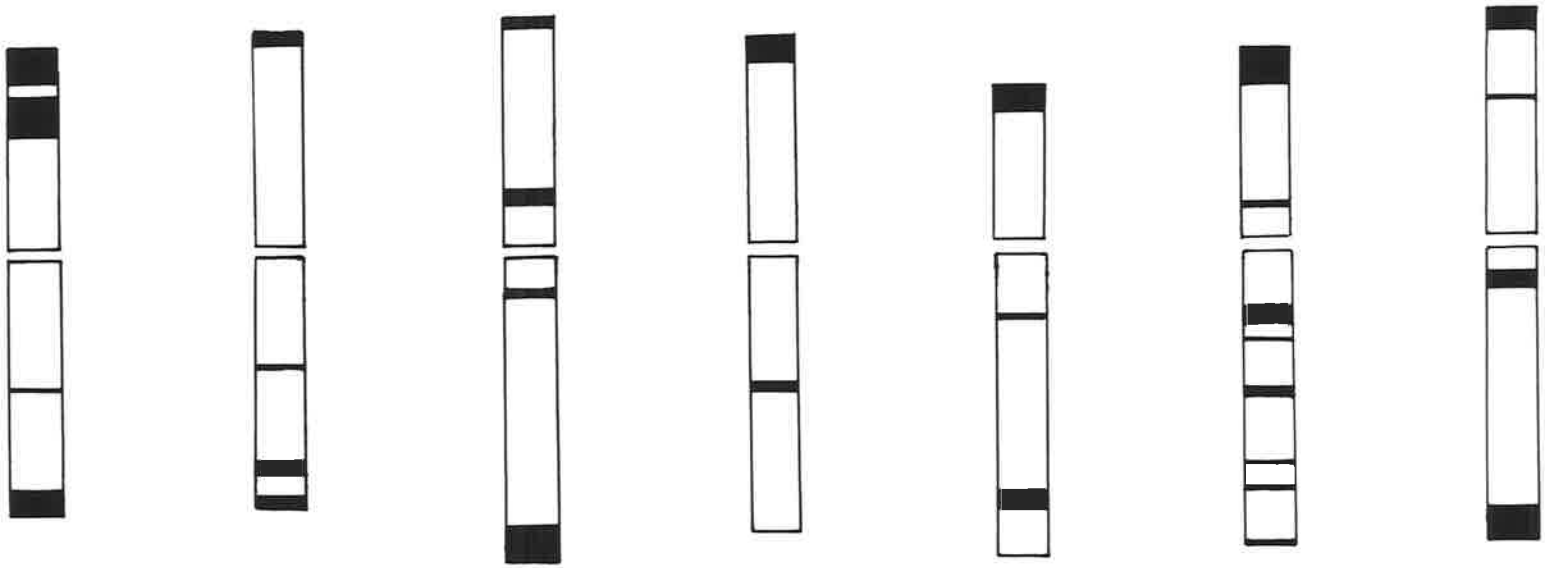
N-BANDING

1R 2R 3R 4R 5R 6R 7R

C-BANDING

1R 2R 3R 4R 5R 6R 7R

FIGURE 4-6 An idiogram of the chromosomes of Imperial rye illustrating the relative size and position of the N-banded regions. The relative lengths and arm ratios of the chromosomes are the averaged measurements from the analysis of 10 cells.


1R
2R
3R
4R
5R
6R
7R

of Koller and Zeller (1976).

The retreatment, in 1M NaH_2PO_4 for a slightly longer period, of undertreated preparations of wheat chromosomes (uniformly stained) was observed to produce N-banding. However, it was not possible to obtain differential N-banding of overtreated chromosomes by retreatment in 1M NaH_2PO_4 buffer. Neither the rye bands nor the nucleolar bands on 1B and 6B were present after retreatment of preparations even with a reduced treatment time.

It was not possible, in this laboratory, to stain exclusively the nucleolar regions of either rye or wheat chromosomes using any of the modifications to the technique described in this study.

4.4 Discussion

N-banding using 1M NaH_2PO_4 is a simple technique and the results for wheat chromosomes are repeatable within and between laboratories. The patterns obtained for the nine identifiable Chinese Spring wheat chromosomes were essentially identical to those found by Gerlach (1977) using the same technique in a different laboratory.

Following the modification of each step in the N-banding technique, two procedures are considered to be critical for reliable banding of good quality; firstly, temperature and time of treatment in the hot acid buffer, and, secondly, the degree of dehydration. Other steps, although essential, did not require precise control.

The best results were achieved using NaH_2PO_4 produced by Ajax Chemicals (Univar - analytical reagent). Other brands gave variable results or no banding. This may be due to differences in the levels of impurities. It also appears important that the immersion oil used for mounting the slides contain PCB (polychlorinated biphenyles). Some new immersion oils do not contain PCB and mounting in these oils results in deterioration or loss of bands within 48 hours. Banded preparations mounted in oil containing PCB have been kept at least a year without loss of staining definition. Slides mounted in Depex or Eupral have also been kept for periods greater than 12 months without loss of staining definition. However, the advantage of using immersion oil is that the slides can be scanned and photographed immediately

after staining and before mounting.

The 1M NaH_2PO_4 could satisfactorily be replaced by 1M NaH_2PO_4 prepared by mixing equal quantities of 2M H_3PO_4 and 2M NaOH as well as by 1M KH_2PO_4, 1M $NH_4H_2PO_4$ and a 2 x SSC solution adjusted to pH 4.2. It was also demonstrated that the original N-banding method utilising trichloroacetic acid and hydrochloric acid (Matsui and Sasaki, 1973) produced N-banding patterns identical to those produced by 1M NaH_2PO_4 in wheat chromosomes. The failure of dilute H_3PO_4 to produce N-bands is presumably due to this solution's weak buffering capacity.

The use of the substitute hot acid buffers demonstrated that neither the metal ion nor the phosphate ion were prerequisites for N-banding, and further served to emphasise that a critical factor in the technique is the temperature and time of treatment in an acid solution buffered at approximately pH 4.

Figure 4-4 illustrates that an extra band in the nucleolar region of chromosomes 1B and 6B of wheat can be reliably identified by manipulation of the time and temperature of the 1M NaH_2PO_4 treatment. The bands in the nucleolar regions are of a diffuse nature in contrast to the sharp 'dot-like' appearance of the other N-bands in wheat chromosomes.

The standard N-banding technique resulted in little or no banding on rye chromosomes. However, if the temperature and time of treatment in 1M NaH_2PO_4 are reduced, banding can be achieved in rye chromosomes. It is most likely that the subterminal band in the short arm of chromosome 1R of rye represents the nucleolar

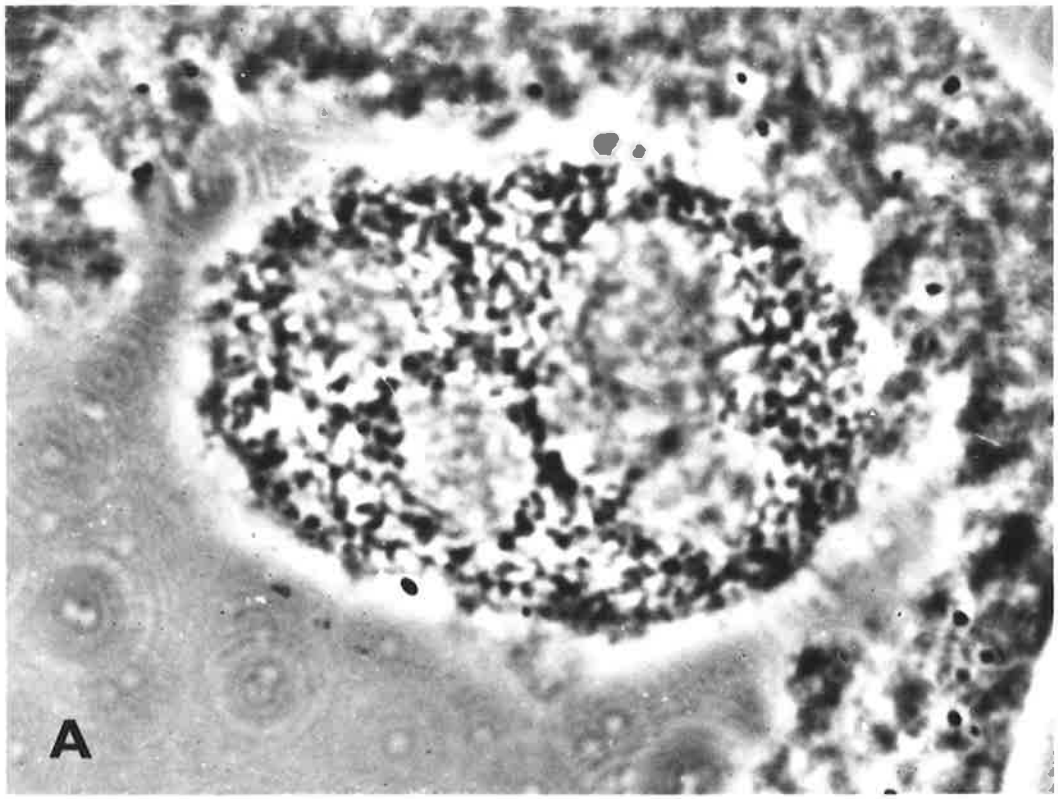
region. Nearly all the bands in rye exhibit a diffuse nature and, as with the nucleolar bands on chromosomes 1B and 6B of wheat, are not stable on retreatment of preparations in hot acid buffer. The difference in appearance of these bands, the fact that the hot acid treatment needs to be reduced to obtain them, and the fact that these bands are not stable after retreatment provides evidence that they represent a different heterochromatin or heterochromatin-protein complex. The reliable identification of the nucleolar regions of chromosomes 1B and 6B is in contrast to the findings of Gerlach (1977). The conclusion that nucleolar regions of wheat chromosomes or related species do not stain with this technique is false. More correctly, nucleolar regions are not specifically stained by this technique. It is further proposed that the less severe acid treatment renders the N-banding technique less specific and thus allows these additional banding sites on the rye chromosomes and in the nucleolar regions of chromosomes 1B and 6B to stain positively.

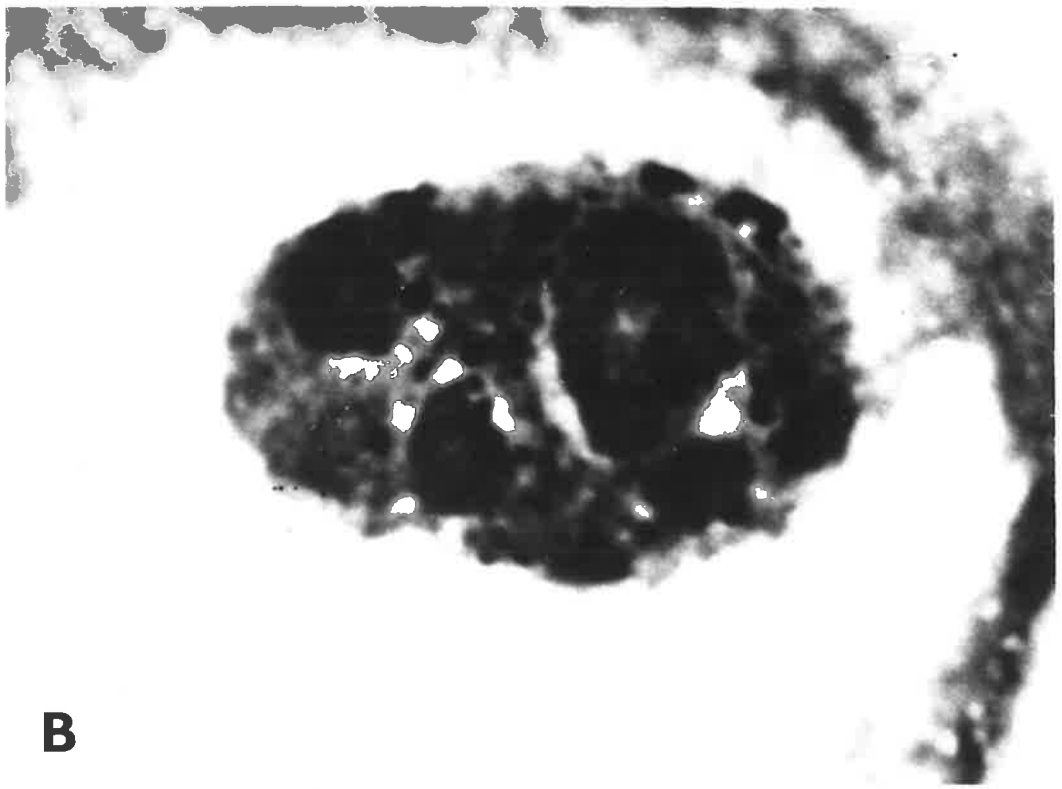
The nucleolar regions can also be stained in interphase nuclei (see figure 4-7b). However, these regions are not specifically stained. The positive staining of the nucleolar regions in interphase cells is more stable to retreatment than the nucleolar regions on metaphase chromosomes, and may be due to the large size of the nucleolar bodies in the interphase cells.

In unstained interphase nuclei of wheat cells, the nucleolar bodies are the only discernible feature which is in contrast to the appearance of unstained interphase nuclei of rye where large heterochromatin regions are evident. This observation of the difference in the rye and wheat nuclei both before and

FIGURE 4-7a An unstained interphase nucleus of hexaploid wheat viewed with phase contrast optics.

FIGURE 4-7b The same cell as in figure 4-7a following staining with the N-banding technique. The nucleolar regions are densely stained and correspond to the opaque regions in the unstained cell. The nucleolar regions are not the only positively stained regions of this interphase cell.


A


B

after staining (see figures 4-7 and 4-8) is evidence that the definition of constitutive heterochromatin given by Greilhuber (1977) is the most satisfactory, as it allows for the differentiation by banding techniques of both the discernible ("natural") and indiscernible heterochromatin present in unstained interphase nuclei.

From available cytological and biochemical data it was suggested by Funaki et al. (1975) that the N-bands represent certain structural non-histone proteins specifically linked to the nucleolar regions in various eukaryotic chromosomes. The fact that the nucleolar regions in wheat and rye could not be exclusively stained with the standard N-banding technique, or any of the modifications examined in this section, does not suggest that the proposed involvement of non-histone proteins (Funaki et al. 1975) is incorrect. It would, however, appear that these structural non-histone proteins are not only specifically associated with the nucleolar regions of chromosomes. The possible biochemical basis of the N-banding technique is discussed further in section 6-1.

The development of a simplified and rapid C-banding technique for rye chromosomes enabled the comparison of the C-bands and N-bands of the same variety / clone of rye chromosomes. A high correspondence between N-bands and C-bands was exhibited although the banding patterns were not identical (see figure 4-5). Some similarity between N-banding and C-banding in wheat was found by Gerlach (1977) and again in barley by Islam (1980). Thus it is to be expected that there should be some similarity in the banding patterns produced by C-banding and

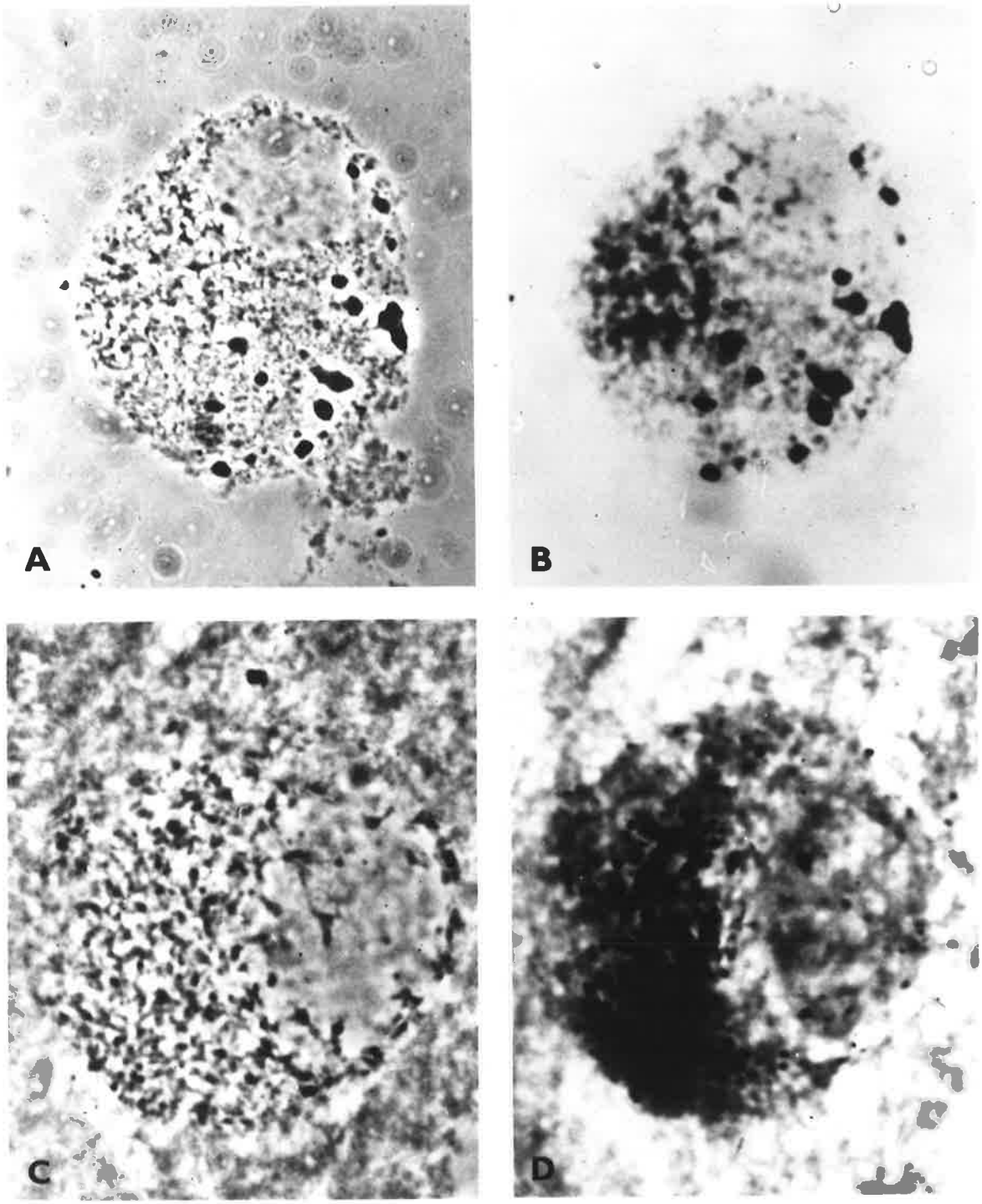
FIGURE 4-8 A comparison of unstained and N-banded interphase nuclei of wheat and rye.

a An unstained interphase nuclei of diploid rye (cultivar Imperial) viewed with phase contrast optics. Large chromocenters are evident in this unstained cell.

b The same cell as figure 4-8a following staining with the N-banding technique. The large chromocenters are seen to stain positively, so too is the centromeric heterochromatin. The polarisation of the centromeric regions and telomeric regions to opposite sides of the cell is evident.

c An unstained interphase nuclei of hexaploid wheat (cultivar Chinese Spring) viewed with phase contrast optics. No large chromocenters are apparent.

d The same cell as figure 4-8c following staining with the N-banding technique. Many small positive staining regions are differentiated. As wheat does not exhibit much telomeric banding, most of the densely stained material is polarised to the same side of the cell as the centromeric heterochromatin.


A
B
C
D

N-banding of rye chromosomes. However, the good compatability of the C-bands and N-bands in rye may be, at least in part, due to the less stringent conditions required in order to obtain the N-bands on rye chromosomes.

All the modifications tested with the N-banding technique in the present study could not reproduce the exclusive staining of the nucleolar regions in either wheat or rye chromosomes. It is possible to explain the results of Funaki et al. (1975) for materials other than rye by assuming that the N-banding heterochromatin or heterochromatin-protein complex is only present in blocks adjacent to the nucleolar regions; or perhaps that the non-histone proteins are only associated with the nucleolar regions in these materials. In rye, however, no plausible explanation for the specific staining of the nucleolar band achieved by Funaki et al. (1975) is evident as this result could not be substantiated using the standard N-banding technique or any of the modifications tested. The N-banding technique can be used to successfully and reliably stain the nucleolar regions of 1B and 6B of wheat and 1R of rye by manipulation of the critical procedures in the technique. However, the banding is not exclusive to the nucleolar regions. With the exception of the banding in these nucleolar regions and the identification of some minor bands, the results of Gerlach (1977) for the N-banding of wheat chromosomes in the cultivar Chinese Spring were found to be reproducible in this laboratory. Subsequently Islam (1980) was also able to reproduce an identical N-banding pattern for wheat chromosomes and to recognise nucleolar bands in barley chromosomes (not exclusive staining of the nucleolar regions).

The N-banding technique is therefore a good candidate for universal application to hexaploid wheat and near relatives for the purpose of reliable chromosome identification of those chromosomes that exhibit diagnostic N-banding patterns.

SECTION 5. SPECIFIC APPLICATIONS OF THE N-BANDING TECHNIQUE IN THE TRITICINAE

5.1 Introduction

The banding patterns of plant species are accepted as being heritable, and Gill and Kimber (1974a) demonstrated the bands to be unchanged in the presence of the chromosomes of another species by way of identifying the individual addition lines of rye chromosomes added to wheat. In experiments with the chromosomes of *Vicia faba*, Dobel *et al.* (1973) also demonstrated the ability of chromosome banding studies to identify structurally rearranged chromosomes. As discussed in the literature review (section 2-4 in particular), it was observed to be possible, in a few cases, to identify translocations in wheat and rye chromosomes using C-banding. It was also possible to identify some rye chromosomes at meiosis in both rye and triticale.

In section 4 it was demonstrated that the N-banding technique could be reliably used for the identification of some chromosomes in wheat and its relatives. The aims of this section were therefore to assess the value of the N-banding technique for the following:

i) the identification of alien material added to wheat;

ii) the identification of substitution and translocation lines;

iii) as an indirect result of aims i) and ii), it is possible to investigate the effect on chromosome identification of natural polymorphisms in banding patterns;

iv) the possibility of the identification of meiotic chromosomes of wheat;

v) to analyse different tester stocks from those used by Gerlach (1977) in order to verify the nomenclature assigned to the 9 N-banded chromosomes in the hexaploid wheat cultivar Chinese Spring by Gerlach (1977).

Complete cells of at least 3 seeds of each seed stock were analysed and, where possible, in addition lines exhibiting a chromosome modification, seeds from a sister line or parent line were also examined to confirm the finding.

5.2 The identification of alien material added to wheat

Seventeen addition lines containing 21 pairs of wheat chromosomes and one chromosome from the related species *Ae. variabilis* (genome constitution CuCu $S^{V}S^{V}$) were made available by Driscoll (1974) and arbitrarily assigned the letters A to Q. *Ae. variabilis* is a tetraploid species (2n = 28), and thus there is a maximum of 14 single chromosome addition lines. The 17 isolated lines must therefore include duplicates and, further, may not include all 14 possible addition lines. Chromosome banding could be instrumental in clarifying the exact number of addition lines isolated.

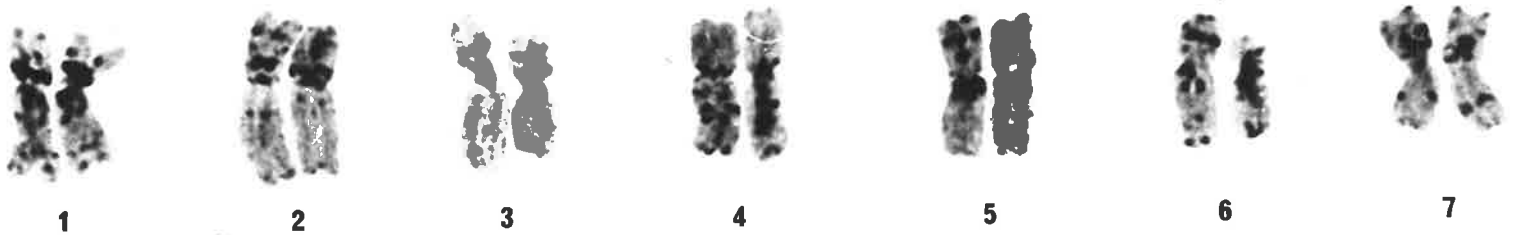
Kimber (1967) isolated 6 of the 7 possible addition lines of *Ae. umbellulata* (a diploid species with the genome constitution CuCu) and Riley isolated the one remaining addition line (Chapman and Riley, 1970). The 7 chromosomes of the Cu genome are combined with the S^{V} genome of unknown origin, but are considered to be closely related to the S genome of *Ae. speltoides*, in the tetraploid species *Ae. variabilis*. Feldman (personal communications) considers that *Ae. variabilis* is a complex species which derived from hybridisation between several initial amphiploids, these arising from crosses of *Ae. umbellulata* with various species of the sub-section Emarginata, ie. *Ae. longissima*, *Ae. searsii*, *Ae. sharonensis* and *Ae. bicornis*. N-banding of the *Ae. umbellulata* addition lines and the seven tentative addition lines of *Ae. longissimum* (kindly supplied by Dr. Feldman) could therefore be valuable in assessing the genome origins in *Ae. variabilis*.

The 14 chromosomes of Ae. variabilis each exhibit a unique N-banding pattern (see figure 5-1). These differ from the 9 hexaploid wheat chromosomes that band with this technique. The available addition lines of Ae. variabilis, Ae. umbellulata and Ae. longissimum to the hexaploid wheat cultivar Chinese Spring therefore provide excellent material with which to examine the identification of alien material added to wheat and also to examine the relationship between the two diploid Aegilops species and the tetraploid Aegilos species.

Figure 5-2 shows the N-banded karyotype of the F_1 seed of Chinese Spring wheat crossed with Ae. variabilis and demonstrates that all 14 Ae. variabilis chromosomes and the 9 Chinese Spring chromosomes that exhibit N-banding retain their unique banding patterns in the hybrid. It is therefore concluded that the N-banding patterns are heritable and unchanged by the presence of alien chromosomes and cytoplasm. An idiogram illustrating the relative sizes and positions of the N-bands in Ae. variabilis is presented in figure 5-3. An occasional diffuse band was observed in the short arm of chromosomes 4, 10 and 11 of Ae. variabilis and where the acid treatment has presumbly been more severe, the bands are not evident. However, the major bands alone are sufficient to classify, unambiguously, all the chromosomes in any preparation. The N-banded chromosomes of Ae. variabilis were assigned numbers 1 to 14 on an arbitrary basis.

Seed of the monosomic (single) addition line of chromosome N of Ae. variabilis to Chinese Spring wheat was used to determine whether the presence of all the Ae. variabilis chromosomes was

FIGURE 5-1 An N-banded karyotype of tetraploid *Aegilops variabilis*.


1
2
3
4
5
6
7


8
9
10
11
12
13
14

FIGURE 5-2 An N-banded karyotype of the F_1 hybrid between Chinese Spring wheat and Aegilops variabilis. All 9 N-banded wheat chromosomes and all 14 N-banded Aegilops variabilis chromosomes exhibit the same banding patterns that were observed in the two parents of the F_1 seed.

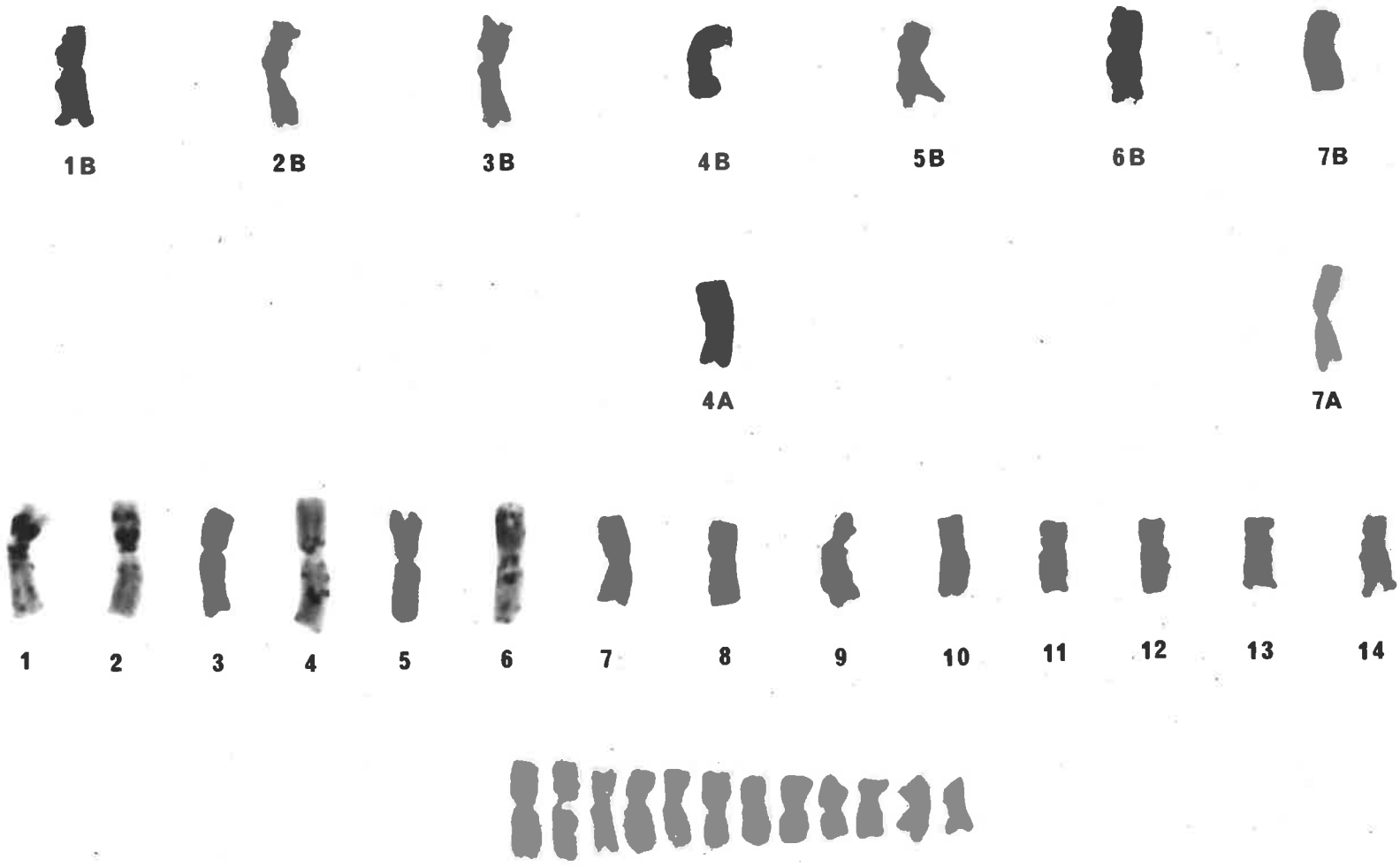
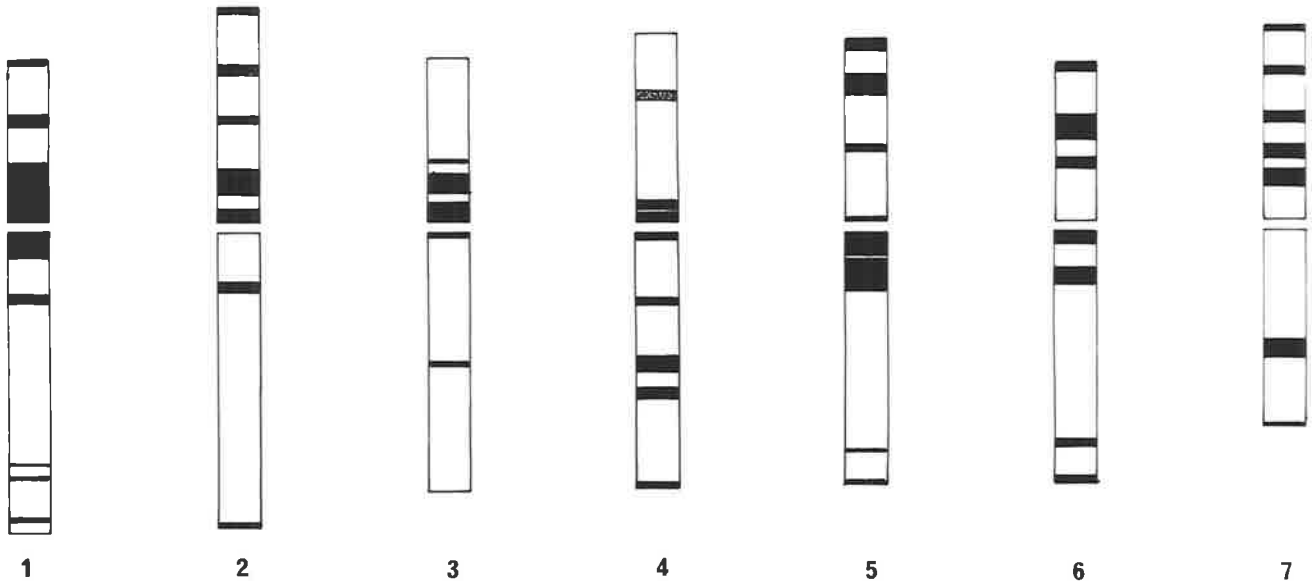

1B
2B
3B
4B
5B
6B
7B
4A
7A
1
2
3
4
5
6
7
8
9
10
11
12
13
14

FIGURE 5-3 An idiogram of the chromosomes of Aegilops variabilis illustrating the relative size and position of the N-banded regions. The stippled regions in chromosomes 4, 10 and 11 represent the diffuse banding sites in this species. The relative lengths and arm ratios of the chromosomes are the averaged measurements from the analysis of 10 cells.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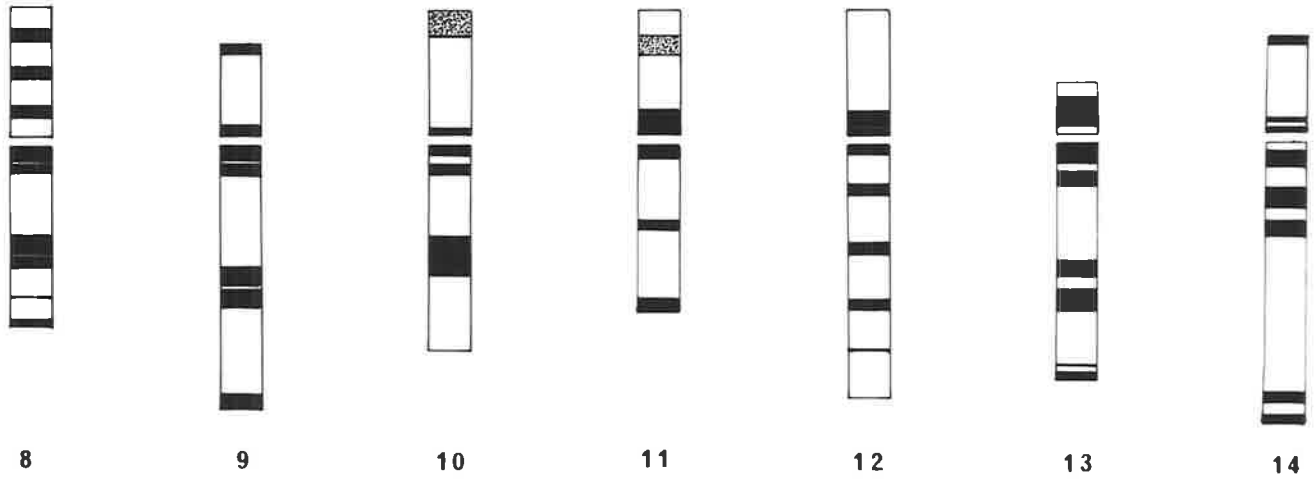
3
4
5
6
7


8
9
10
11
12
13
14

required as a prerequisite for the N-banding of individual Ae. variabilis chromosomes. Figure 5-4 illustrates a whole cell N-banded metaphase preparation (2n = 43) and a karyotype of this preparation is presented in figure 5-5. The N-banding pattern of chromosome N can be equated to that of chromosome 13 in the Ae. variabilis karyotype (see figures 5-1, 5-2 and 5-3) and clearly demonstrates that the banding pattern is heritable and independent of the other Ae. variabilis and Chinese Spring chromosomes and cytoplasm. It was therefore concluded that the N-banding technique could be reliably used to identify the single addition lines of Ae. variabilis chromosomes to wheat.

N-banding analysis was successfully attempted on 14 of the remaining 16 Ae. variabilis addition lines isolated by Driscoll (1974). The Ae. variabilis addition lines K and Q could not be re-isolated from the stocks isolated by Driscoll (1974). The results of N-banding analysis of addition lines of the Ae. variabilis chromosomes A, B, C, D, E, F, G, H, I, J, L, M, O and P are as follows;

Addition line A. The alien chromosome present was observed to be a complete chromosome addition of chromosome 11 of the parental Ae. variabilis karyotype.

Addition line B. The alien chromosome present was observed to be a complete chromosome addition of chromosome 3 of the parental Ae. variabilis karyotype.

Addition line C. The alien chromosome present was observed to be a structurally modified chromosome 3 of the parental Ae. variabilis karyotype. This chromosome was observed to be structurally altered by a terminal deletion of part of the long arm with the break point being just distal to the band in the

FIGURE 5-4 A whole cell N-banded mitotic metaphase preparation of the Aegilops variabilis monosomic addition line N (2n = 43). The chromosome N of Aegilops variabilis is indicated.

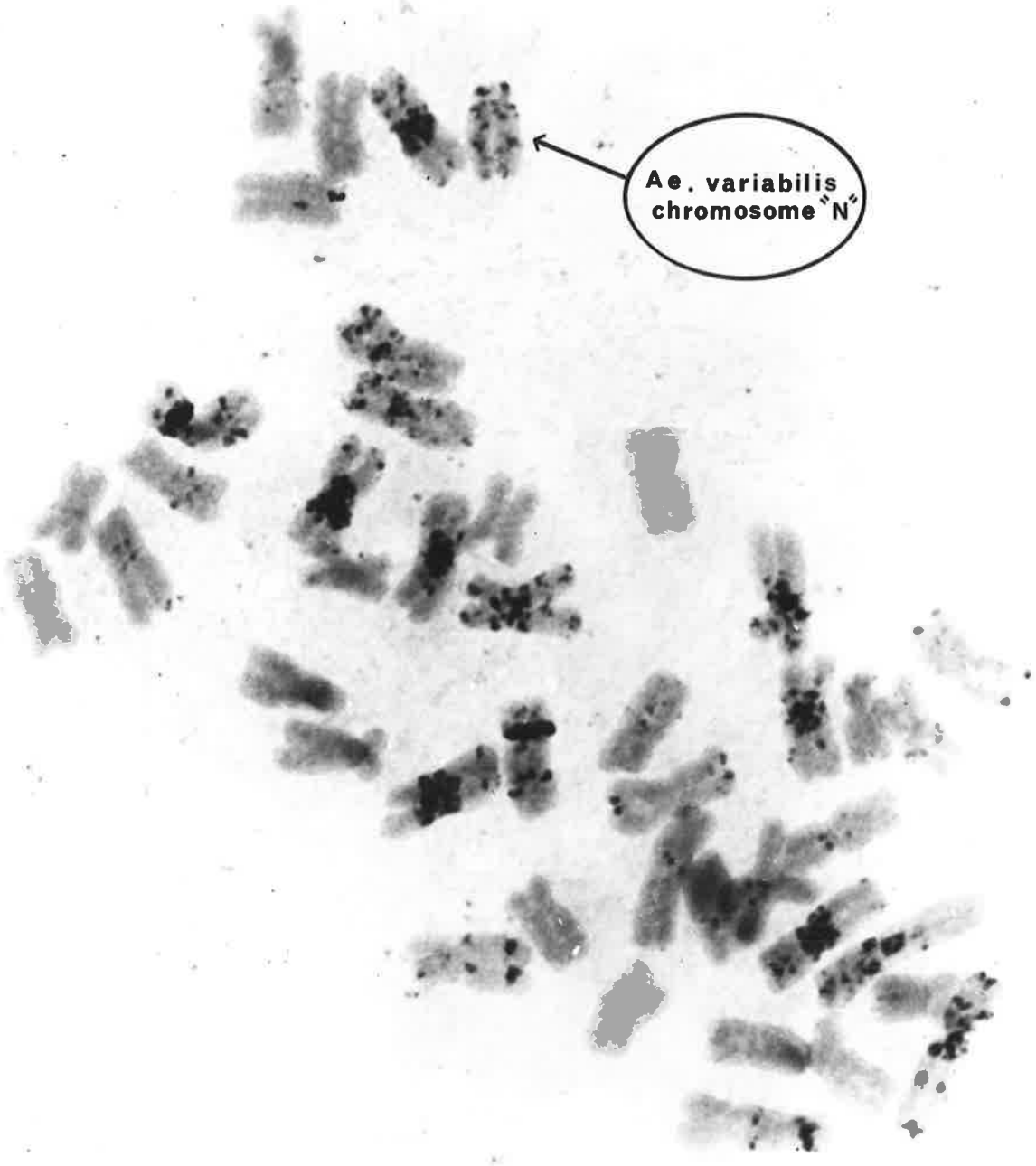
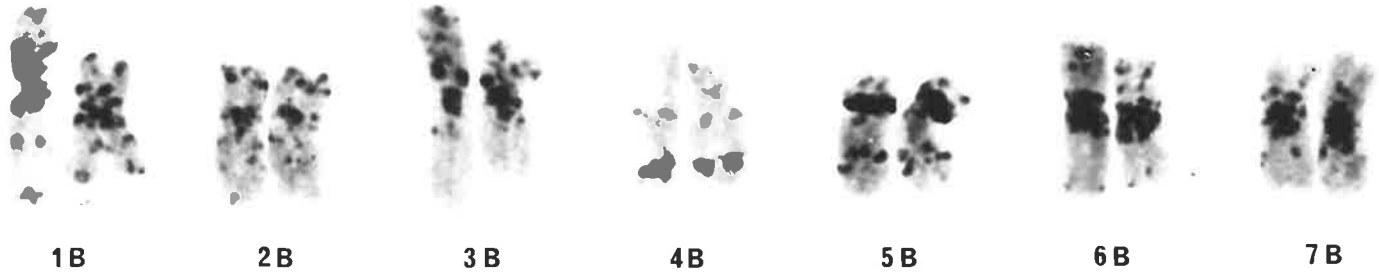

Ae. variabilis
chromosome "N"

FIGURE 5-5 An N-banded karyotype assembled from the metaphase preparation of the Aegilops variabilis addition line N which is presented in Figure 5-4.



1B 2B 3B 4B 5B 6B 7B

4A 7A

Ae.v. + "N"

median position of the long arm.

Addition line D. The alien chromosome present was observed to be a complete chromosome addition of chromosome 3 of the parental Ae. variabilis karyotype.

Addition line E. The alien chromosome present was observed to be a structurally modified chromosome 3 of the parental Ae. variabilis karyotype. This chromosome was observed to be structurally altered by a terminal deletion of just over half of the long arm with the break point being in a position just proximal to the band in the median position of the long arm.

Addition line F. The alien chromosome present was observed to be a complete chromosome addition of chromosome 5 of the parental Ae. variabilis karyotype.

Addition line G. The alien chromosome present was observed to be a complete chromosome addition of chromosome 4 of the parental Ae. variabilis karyotype.

Addition line H. Two sister lines of seed were examined as in all stocks where a structurally modified chromosome was detected. However, in this instance a difference between the sister lines was observed. The first line was observed to contain an isochromosome of the short arm of chromosome 10 of the parental Ae. variabilis karyotype. The second line analysed was observed to be a complete chromosome addition of chromosome 10 of the parental Ae. variabilis karyotype.

Addition line I. The alien chromosome present was observed to be a complete chromosome addition of chromosome 8 of the parental Ae. variabilis karyotype.

Addition line J. The alien chromosome present was observed to a structurally modified chromosome 8 of the parental Ae. variabilis karyotype. This chromosome was observed to be stucturally altered

by a terminal deletion of most of the long arm with the break point being in a position just distal to the two closely associated bands proximal to the centromere.

Addition line L. The alien chromosome present was observed to be a complete chromosome addition of chromosome 13 of the parental Ae. variabilis karyotype.

Addition line M. In this instance, three different karyotypes were detected in the sister lines analysed. A complete chromosome addition of chromosome 6 of the parental Ae. variabilis karyotype was identified (figure 5-6). An addition line involving the complete chromosome 6 of the parental Ae. variabilis karyotype as well as a substitution of a heterozygous translocation product involving the short arm of chromosome 6 and an unbanded wheat chromosome were also detected (figure 5-7). Lastly, an addition line involving an isochromosome of the long arm of chromosome 6 together with a substitution of a heterozygous translocation product involving the short arm of chromosome 6 and an unbanded wheat chromosome was also observed (figure 5-8).

Addition line O. The alien chromosome present was observed to be a complete chromosome addition of chromosome 3 of the parental Ae. variabilis karyotype.

Addition line P. The alien chromosome present was observed to be a complete chromosome addition of chromosome 12 of the parental Ae. variabilis karyotype. In the wheat complement of this addition line, chromosome 3B was observed to differ slightly in the N-banding pattern and this is discussed in section 5-4.

It was therefore concluded that after analysis of the 15 addition lines of Ae. variabilis available for study, 9 of the 14 possible single addition lines of this species had been isolated.

FIGURE 5-6 An N-banded karyotype of the Aegilops variabilis monosomic addition line M (2n = 43).

1B 2B 3B 4B 5B 6B 7B

4A 7A

Ae.v.+"M"

FIGURE 5-7 An N-banded karyotype of the Aegilops variabilis monosomic addition line M (2n = 43). This particular plant was also observed to contain a heterozygous translocation product that has resulted in the short arm of chromosome M of Aegilops variabilis being present on an unbanded wheat chromosome. Using standard mitotic metaphase techniques this plant would be extremely difficult to differentiate from the normal addition line M.

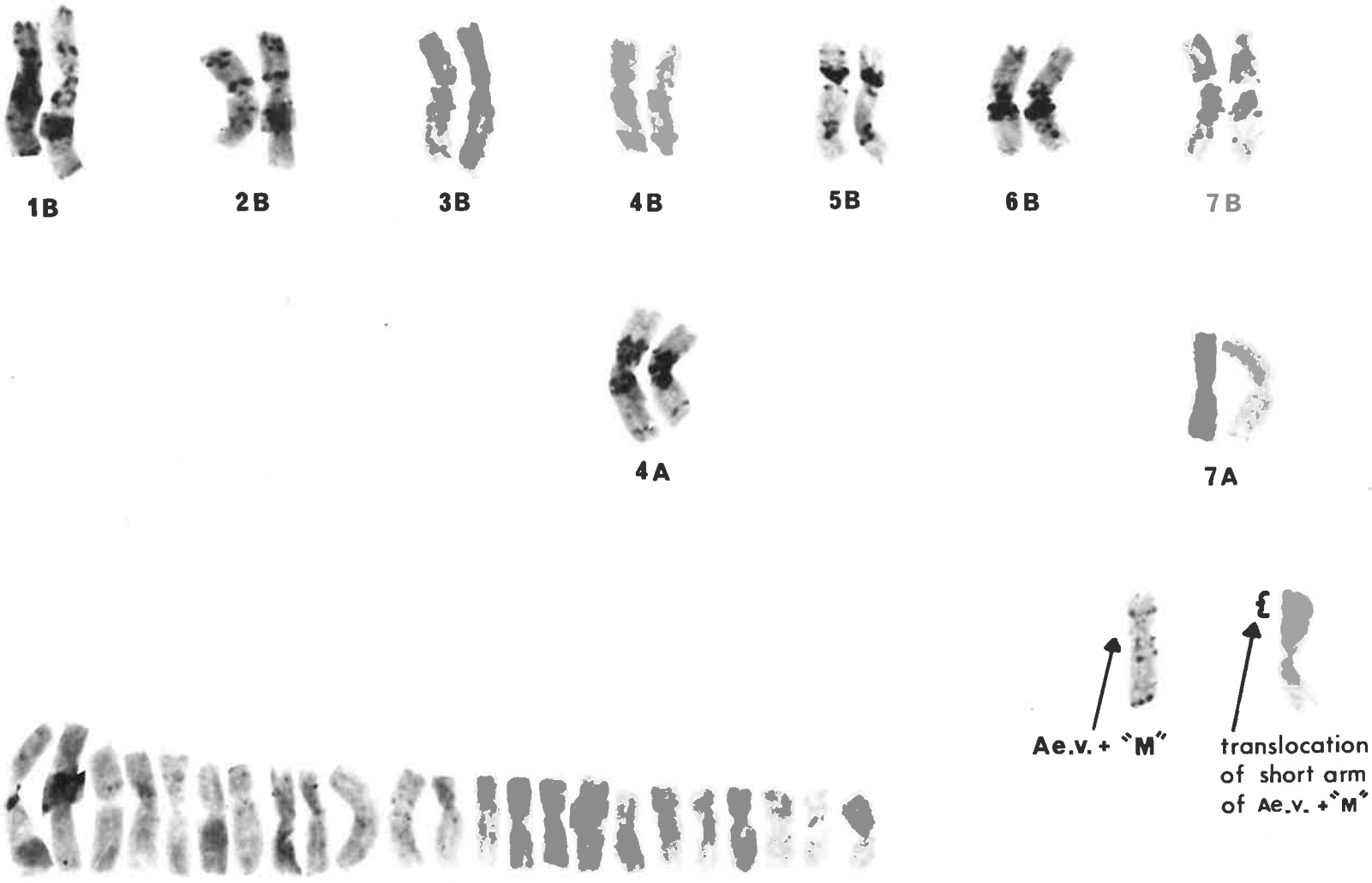
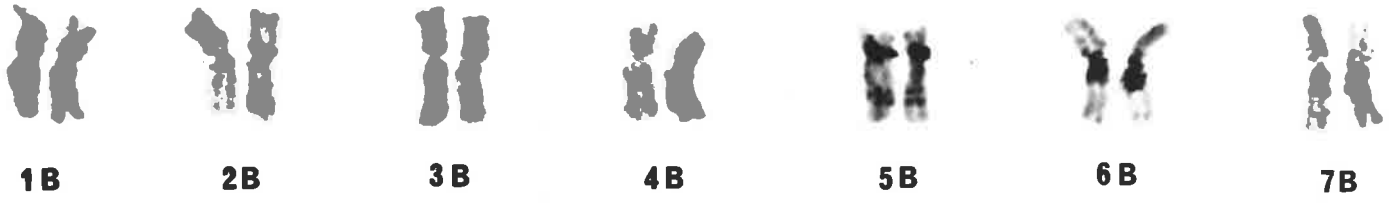

1B
2B
3B
4B
5B
6B
7B
4A
7A
Ae.v. + "M"
translocation of short arm of Ae.v. + "M"

FIGURE 5-8 The N-banded karyotype of a plant (2n = 43) isolated from the Aegilops variabilis monosomic addition line M (2n = 43). This plant was observed to contain the same heterozygous translocation chromosome as present in figure 5-7 and to contain a single isochromosome of the long arm of the Aegilops variabilis chromosome M. Using standard mitotic metaphase techniques this plant would be extremely difficult to differentiate from the normal addition line M.



1B 2B 3B 4B 5B 6B 7B

4A 7A

isochromosome of long arm of Ae.v. + "M"

translocation of short arm of Ae.v. + "M"

Figure 5-9 illustrates the N-banded patterns of the 9 Ae. variabilis chromosomes isolated as chromosome additions to Chinese Spring wheat.

The N-banded patterns of the alien chromosomes present in the 7 addition lines of Ae. umbellulata and the 5 tentative addition lines examined of Ae. longissimum are presented in figure 5-10. The addition line of Ae. longissimum chromosome B was the only line not observed to be a complete alien chromosome addition with a normally N-banded Chinese Spring wheat complement. Addition line B of Ae. longissimum was observed to contain an alien chromosome disomically substituted for chromosome 6B of the wheat complement and to contain an N-banded telocentric chromosome (see figure 5-10). It was also evident from these studies that addition lines E and G of Ae. umbellulata were identical.

All the alien chromosomes identified in the Ae. longissimum and Ae. umbellulata addition lines could be related to similarly banded chromosomes in the N-banded karyotype of Ae. variabilis with the exception of the Ae. umbellulata chromosome present in addition line A and the telocentric chromosome present in the Ae. longissimum line B (see table 5-1). The correspondence of the banding patterns of the Ae. umbellulata and Ae. longissimum lines with those of the Ae. variabilis karyotype was not exact. However, most of the differences observed can satisfactorily be accounted for by assuming the existence of variation in the size of the N-banding regions and relying on the position of the bands for chromosome identification. It was apparent that the Ae. longissimum chromosomes present in the addition lines had banding patterns more closely correlated with

FIGURE 5-9 The N-banded patterns of the complete *Aegilops variabilis* chromosomes isolated as addition lines to Chinese spring wheat. The numbers represent the number assigned to these chromosomes in the parent *Aegilops variabilis* karyotype.

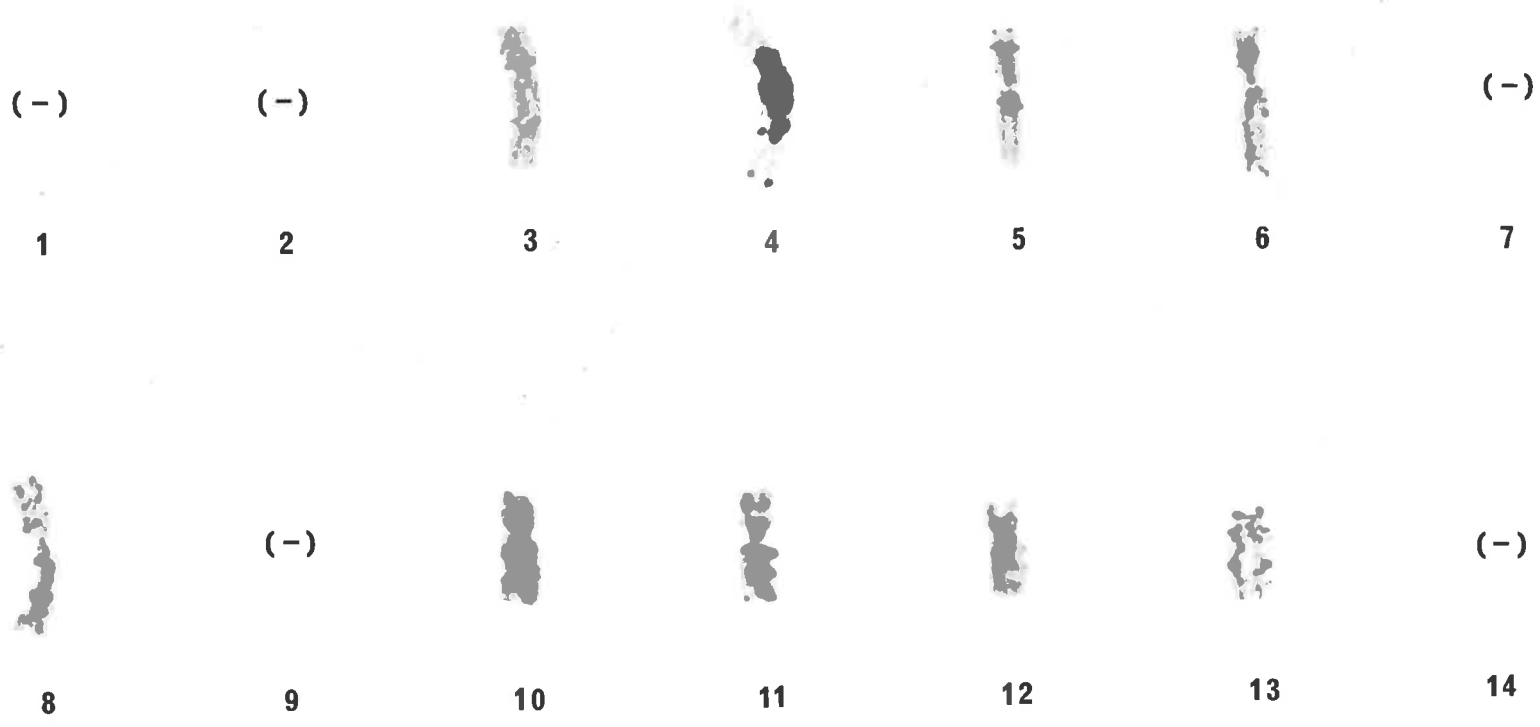
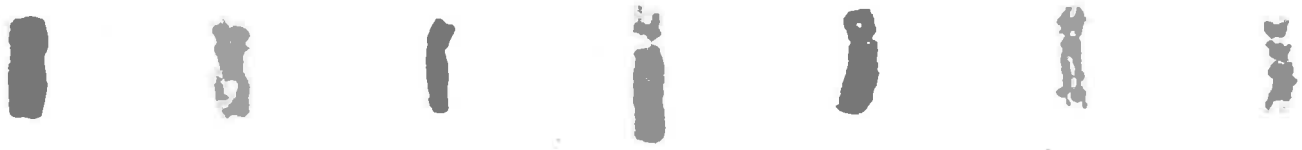

(–)
(–)
(–)
1
2
3
4
5
6
7
8
(–)
10
11
12
13
(–)
9
14

FIGURE 5-10 The N-banded patterns of the Aegilops umbellulata and Aegilops longissimum chromosomes identified in the addition lines of these species added to Chinese Spring wheat. Addition line B of Aegilops longissimum was the only addition line not observed to be a complete alien addition with a normally N-banded wheat complement. Aegilops longissimum B was observed to contain an alien chromosome disomically substituted for chromosome 6B of wheat and to contain an N-banded telocentric chromosome. Addition lines E and G of Aegilops umbellulata were observed to contain identically N-banded chromosomes.

Ae. umbellulata Addition Lines



A B C D E F G

Ae. longissimum Addition Lines

(–) (–)

A B C D E F G

TABLE 5-1

The addition lines of Aegilops variabilis, Aegilops umbellulata and Aegilops longissimum and their relationship to the N-banded karyotype of Aegilops variabilis.

CHROMOSOME NO. 1	CHROMOSOME NO. 8
	Ae. variabilis I and J*
Ae. longissimum D	
CHROMOSOME NO. 2	CHROMOSOME NO. 9
Ae. longissimum G	Ae. umbellulata E and G
CHROMOSOME NO. 3	CHROMOSOME NO. 10
Ae. variabilis B, C*, D, E* and O	Ae. variabilis H
Ae. longissimum F	
CHROMOSOME NO. 4	CHROMOSOME NO. 11
Ae. variabilis G	Ae. variabilis A
	Ae. umbellulata B
CHROMOSOME NO. 5	CHROMOSOME NO. 12
Ae. variabilis F	Ae. variabilis P
CHROMOSOME NO. 6	CHROMOSOME NO. 13
Ae. variabilis M	Ae. variabilis L and N
Ae. umbellulata F	Ae. umbellulata D
CHROMOSOME NO. 7	CHROMOSOME NO. 14
Ae. longissimum E	Ae. umbellulata C

* indicates that the alien chromosome is modified and not a complete addition line.

Addition line C of Ae. longissimum not available for study.

Addition line B of Ae. longissimum was a substitution of chromosome 7 for 6B of wheat and also contained a telocentric that could not be identified.

Addition line A of Ae. umbellulata could not be matched to the Ae. variabilis karyotype.

Ae. variabilis chromosomes than did the chromosomes of Ae. umbellulata.

Chromosomes 3, 6, 11 and 13 of the N-banded karyotype of Ae. variabilis were identified in addition lines of Ae. variabilis as well as in either Ae. umbellulata or Ae. longissimum. Addition line A of Ae. variabilis and addition line B of Ae. umbellulata, for example, were both assumed to be chromosome 11 of the N-banded karyotype of Ae. variabilis on the basis of their individual N-banding patterns. It is therefore possible to compare the plant phenotype of the addition lines in these four specific cases (plants were glasshouse grown in 25 cm pots containing a peat-sand-fertilizer mixture).

In the case of chromosome 3, the Ae. variabilis addition lines containing this chromosome were observed to exhibit an open or lax head type, reduced plant height and reduced fertility. Ae. longissimum addition line F which was also observed to contain this chromosome exhibited an open or lax head type, reduced plant height and slightly reduced fertility.

In the case of chromosome 6, the plants of Ae. variabilis addition line M which contained this chromosome exhibited no apparent phenotypic marker. However, the Ae. umbellulata addition line F which contained this chromosome exhibited reduced head size, and mild grass clumping. Plants containing two doses of this chromosome (disomic addition line F,2n = 44) exhibited strong grass clumping and died before heading occurred.

In the case of chromosome 11, the Ae. variabilis addition line A which contained this chromosome exhibited black glumes and an otherwise normal phenotype. However, the Ae. umbellulata addition line B which contained this chromosome had no apparent

morphological marker.

In the case of chromosome 13, the Ae. variabilis addition lines containing this chromosome exhibited awn promotion and an otherwise normal phenotype. The Ae. umbellulata addition line D which contains this chromosome does exhibit awn promotion. However, the head shape also differs and is thin and reduced in length.

Although chromosome A of Ae. variabilis and chromosome B of Ae. umbellulata do not exhibit the same phenotypic marker, both these chromosomes have been substituted for the group 1 chromosomes of Chinese Spring wheat (see Driscoll, 1975). The substitution of chromosome B of Ae. umbellulata for chromosome 1B exhibits black glumes, whereas the Ae. variabilis addition line A and all three Group 1 substitutions of this chromosome exhibit black glumes, thus demonstrating at least a gene strength difference in these two chromosomes.

These two addition lines have also been crossed in order to elucidate their relationship. Chromosome A of Ae. variabilis and the chromosome B of Ae. umbellulata were observed to pair but only in 54% of meiocytes (Driscoll, personal communications).

The observed difference between addition lines of the same chromosome isolated from the tetraploid Ae. variabilis and the diploids may be explained by evolutionary change in the tetraploid or may simply be a reflection of interspecies variation. The differences observed in the banding patterns of the diploid chromosomes and the corresponding chromosomes present in the tetraploid karyotype may therefore reflect actual genetic

differences in these chromosomes and not merely the amplification and deamplification of inert heterochromatin.

The identification of the three different karyotypes of Ae. variabilis addition line M and the disomic substitution present in Ae. longissimum addition line B demonstrate the value of analysing whole cell preparations. It would not, for example, be possible to differentiate between a monosomic substitution of Ae. longissimum chromosome B for chromosome 6B and a normal addition line unless whole cells were analysed to determine the dosage of each chromosome.

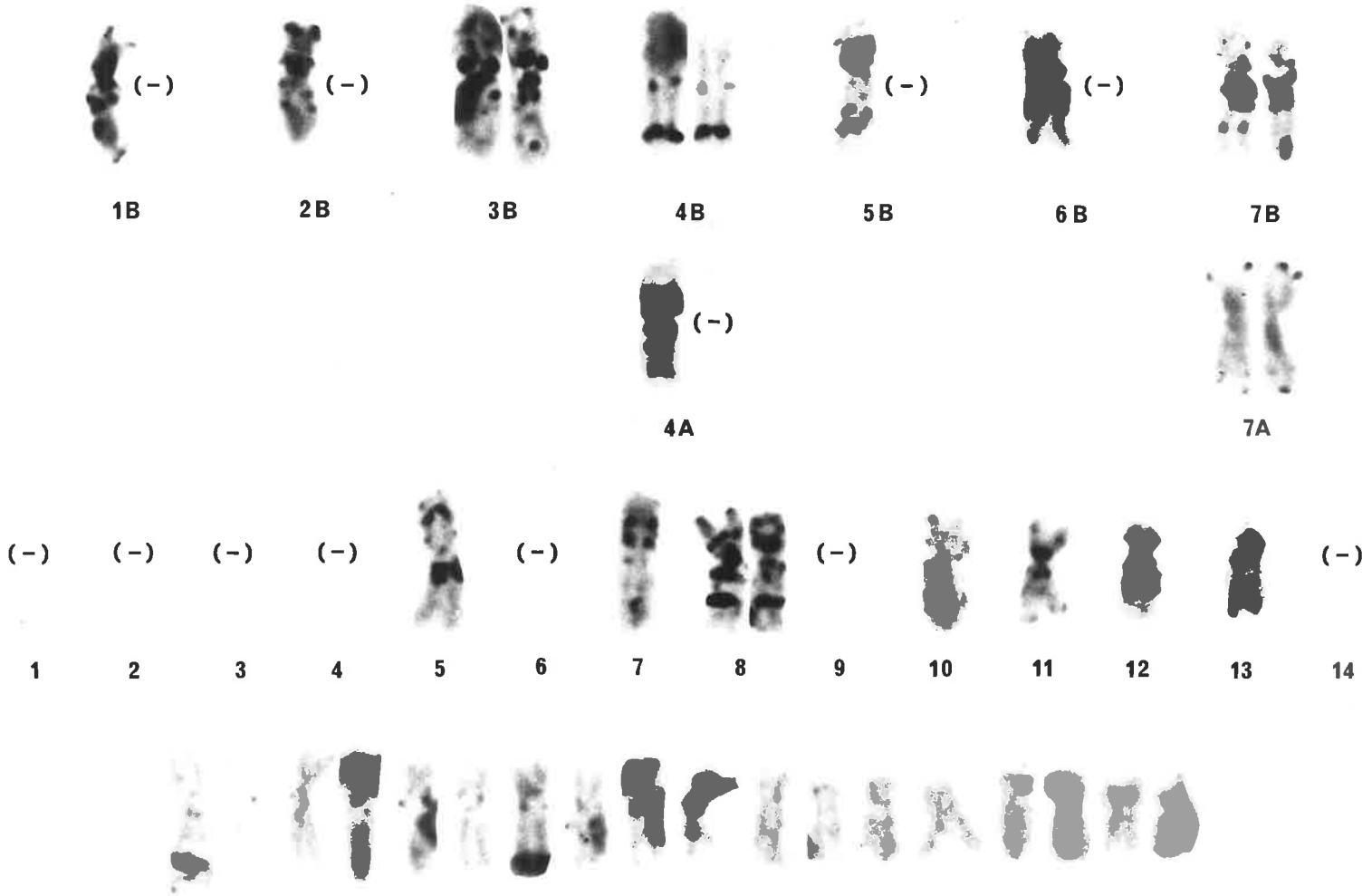
The fact that whole cell preparations can be analysed also provides a method of analysing, to a limited extent, chromosomal constitution in some plants that hitherto could not be analysed. For example, in a backcrossing program designed to isolate the missing Ae. variabilis addition lines, the first backcross plants (BC1) of Chinese Spring by Ae. variabilis exhibited a range in chromosome number from 39 to 63, together with a corresponding range in head type on the resulting plants (see figure 5-11). The backcross program was undertaken in order to obtain seed from unreduced eggs in the F_1, as the F_1 plant is sterile and chromosome doubling could not be induced with colchicine. If an unreduced egg cell was fertilised by backcrossing with Chinese Spring pollen the resulting seed would be expected to contain 21 pairs of wheat chromosomes and 14 univalents from Ae. variabilis, thus having a total of 56 chromosomes.

Figure 5-12 demonstrates the karyotype of a BC1 plant containing 39 chromosomes. If it is assumed that 21 of these

FIGURE 5-11 A selection of differing head types present in the BC1 plants of Chinese Spring by Aegilops variabilis by Chinese Spring. Each of the 15 examples of head type were from different BC1 plants. The chromosome number of these plants ranged from 39 to 63. The first five examples of head type were all taken from BC1 plants containing the expected chromosome number of 56.

0
cm
5
0
cm
5
0
cm
5

FIGURE 5-12 The N-banded karyotype of a BC1 plant (Chinese Spring by Aegilops variabilis by Chinese Spring) containing 39 chromosomes instead of the expected 56 chromosomes.


(-)
(-)
(-)
(-)
(-)
(-)
1B
2B
3B
4B
5B
6B
7B
(-)
4A
7A
(-)
(-)
(-)
(-)
(-)
(-)
(-)
1
2
3
4
5
6
7
8
9
10
11
12
13
14

chromosomes came from the wheat pollen parent, then it is evident that the egg cell contained only 10 of the 21 wheat chromosomes present in the F_1 and only 7 of the possible 14 Ae. variabilis chromosomes - the extra chromosome being a second number 8 chromosome from Ae. variabilis. The presence of two number 8 chromosomes of Ae. variabilis is presumably due to non-disjuction. Driscoll and Quinn (1968) demonstrated that the F_1 between Chinese Spring and Ae. variabilis contained 35 chromosomes and formed less than one chiasmata per cell at Metaphase I of meiosis and this fact, together with the above observations of presumed non-disjunction and univalent loss, leads to the conclusion that unreduced eggs are not the only fertile eggs in a male sterile F_1 and further rules out zygotic chromosome elimination as a possible alternative explanation for BC1 plants containing less than 56 chromosomes. If zygotic chromosome loss was to occur the F_1 plants would not contain 35 chromosomes.

5.3 The identification of chromosome substitutions and translocations

5.3.1 Identification of substitution lines

It was demonstrated in section 5-2 that the N-banding technique could be reliably used to identify the addition of chromosomes of Ae. variabilis, Ae. umbellulata and Ae. longissimum to Chinese Spring wheat. In addition line B of Ae. longissimum a substitution for chromosome 6B was detected by the absence of the N-banding pattern of chromosome 6B from the whole cell metaphase chromosome complement. Two further hexaploid wheat cultivars containing reported chromosome substitutions were examined to establish the value of the N-banding technique for the identification of substitution lines in hexaploid wheat.

The cultivar Blaukorn (line W70a86) was reported to contain a disomic substitution of chromosome 5R for chromosome 4A of wheat (Zeller and Baier, 1973) and the cultivar Orlando was reported to contain a disomic substitution of chromosome 1R for chromosome 1B of wheat (Mettin et al., 1973; and Zeller, 1973). The reported absence of chromosome 4A in the cultivar Blaukorn and chromosome 1B in the cultivar Orlando was confirmed by N-banding analysis, and the presence of chromosome 5R in Blaukorn and chromosome 1R in Orlando was confirmed by using the N-banding and C-banding techniques. It was therefore concluded that wheat stocks containing chromosome substitutions, where the chromosomes involved exhibit unique N-banding patterns, can indeed be

identified.

The tetraploid triticales isolated by Krolow (1973) contain the full rye complement and the remaining 7 pairs are constituted by a mixture of the A and B genome chromosomes of wheat. The three tetraploid triticales (4 x 2, 4 x 3 and 4 x 5) isolated by Krolow were analysed by C-banding (Gustafson and Krolow, 1978) and the wheat chromosome complement of each was tentatively assigned as follows:

4 x 2 carries 1A, 2B, 3B, 4B, 5B, 6A and 7B;

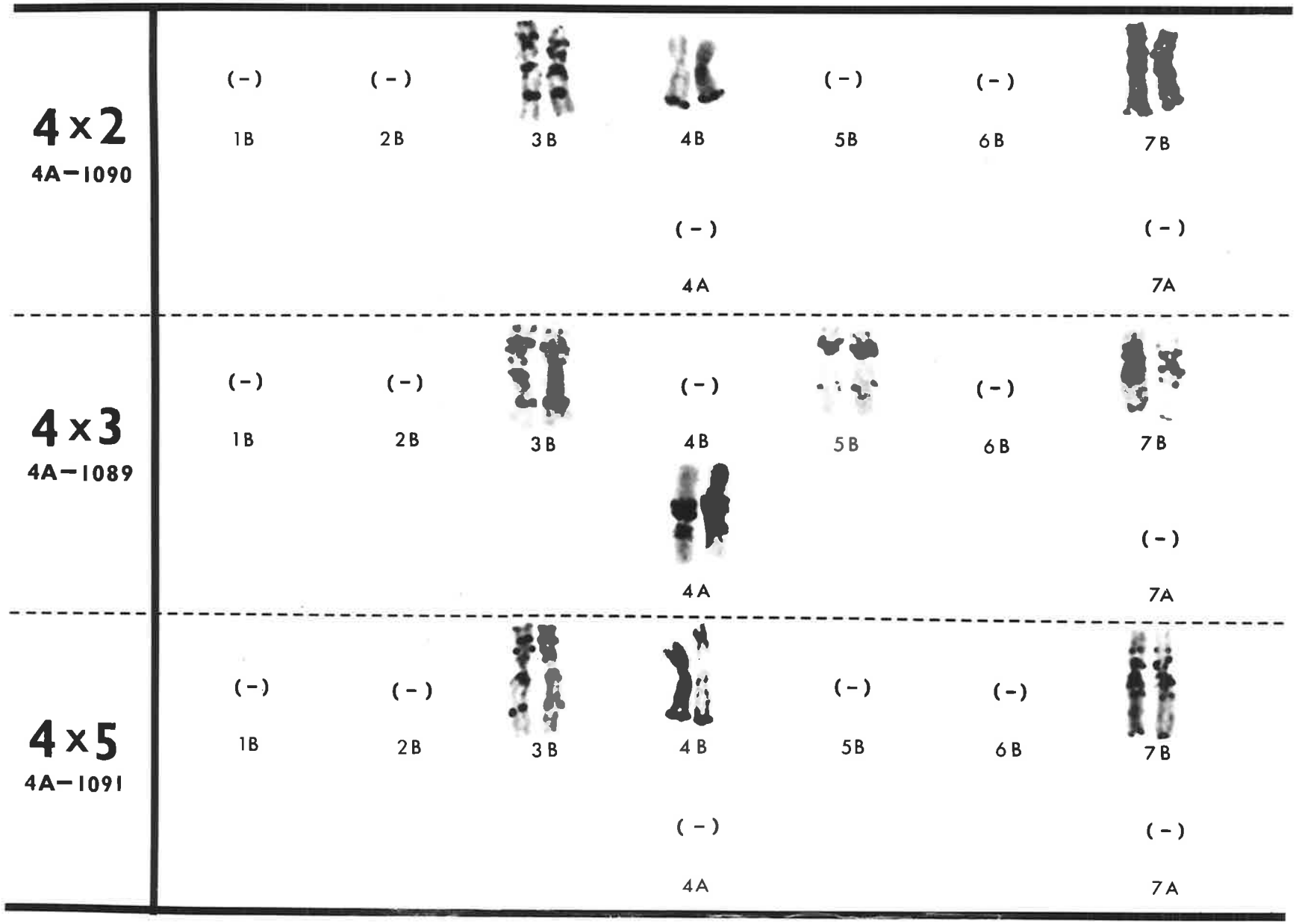
4 x 3 carries 1A, 2B, 3A, 4A, 5B, 6A and 7B; while

4 x 5 carries 1A, 2B, 3B, 4A, 5A, 6A and 7B.

As all 7 B genome chromosomes and 4A and 7A can be identified by N-banding and, as demonstrated, substitution lines can also be identified by N-banding, the three tetraploid triticales therefore represent excellent material for N-banding analysis in order to confirm the results of Gustafson and Krolow (1978). Chromosome 4 and 7 exhibit N-banding in both the A and B genomes. However, in the case of chromosomes 1, 2, 3, 5 and 6 of the wheat complement in the tetraploid triticales, the absence of an N-banded B genome chromosome implies the presence of the homoeologous unbanded A genome chromosomes. Thus the chromosome identity of the whole wheat complement of the tetraploid triticales can be inferred with the N-banding technique.

Figure 5-13 illustrates the results of N-banding analysis of the wheat complement in the three tetraploid triticales. The N-banded wheat chromosomes of the tetraploid triticales could be identified with confidence, although some variation in the banding

FIGURE 5-13 The N-banded wheat chromosomes present in the three tetraploid triticales analysed. Line 4 x 2 and 4 x 5 were observed to be identical. The symbol (-) indicated the absence of a banded chromosome. It is assumed that where a banded chromosome is absent, the homoeologous chromosome is present; for example, line 4 x 3 is assumed to contain chromosomes 1A, 2A, 3B, 4A, 5B, 6A and 7B on the basis of those N-banded chromosomes present.


4x2
4A–1090
(–)
1B
(–)
2B
3B
4B
(–)
5B
(–)
6B
7B
(–)
4A
(–)
7A
4x3
4A–1089
(–)
1B
(–)
2B
3B
(–)
4B
4A
5B
(–)
6B
7B
(–)
7A
4x5
4A–1091
(–)
1B
(–)
2B
3B
4B
(–)
4A
(–)
5B
(–)
6B
7B
(–)
7A

patterns of these chromosomes was observed when compared with the Chinese Spring wheat N-banding patterns. The results of N-banding analysis of the wheat complements in these tetraploid triticales did not agree with the tentative identifications proposed by Gustafson and Krolow (1978). In line 4 x 3 the only discrepancy was in the identification of the group 2 and 3 chromosomes. In lines 4 x 2 and 4 x 5 the wheat complement was observed to be identical and would indicate an error in the seed stocks examined, as the two lines should have been different. This was confirmed by Gustafson (personal communications) in that the same seed was sent to Professor C.J. Driscoll and one of the packets was mistakenly mislabelled.

The further discrepancies observed between my analysis based on N-banding (figure 5-13) and the results of Gustafson and Krolow are confused by the lack of knowledge as the actual identity of the seed stock labelled 4 x 2 and 4 x 5. It also appears that the difference in the C-banding patterns observed in the tetraploid triticales (Gustafson and Krolow, 1978) compared with those published for Chinese Spring chromosomes (Gill and Kimber, 1974c) may have precipitated erroneous chromosome identification. For example, the chromosome designated as 6A by Gustafson and Krolow (1978) appears to be similar to that designated as 4B by Gill and Kimber (1974c). The fact that only 9 chromosomes need to be distinguished from each other in the N-banding analysis, compared to 14 in the C-banding study by Gustafson and Krolow (1978), simplified the effect of banding pattern polymorphisms on chromosome identification (see further discussion in section 5-4).

5.3.2 Identification of translocation lines

In section 5-2 it was demonstrated that structurally modified chromosomes of Ae. variabilis could be identified in addition lines C, E, H, J and M of Ae. variabilis, including a translocation product involving the short arm of chromosome M, using the N-banding technique. The hexaploid wheat cultivars Aurora, Poso and Transfed, together with lines 256/75 and 486/77, were chosen to further illustrate the power of the N-banding technique for the identification of chromosome translocations.

The cultivar Aurora was reported to contain a 1B/1R translocation chromosome (Mettin et al., 1973; Zeller, 1973); the cultivar Poso was reported to have a homozygous reciprocal translocation involving chromosomes 5B and 7B (Sears, 1953; see Quinn and Driscoll, 1970); and the cultivar Transfed was reported to contain a 2R/4A translocation in the background of the cultivar Federation (see Driscoll, 1975). The translocation chromosome present in the cultivar Transfed was reported to involve the β arm chromosome 4A however, the arm of chromosome 2R involved is unknown (Driscoll and Bielig, 1968). Line 256/75 contains a 1R/1B translocation and line 486/77 contains the same 1R/1B translocation together with a 1R/1D translocation (Shepherd, personal communication).

In section 4 it was demonstrated that by relaxing the stringency of the N-banding procedure, characteristic banding in rye chromosomes could be achieved. The same less stringent conditions were also necessary for the formation of a positive band in the nucleolar region of chromosomes 1B and 6B. The presence of a nucleolar band is proposed as a marker band for the

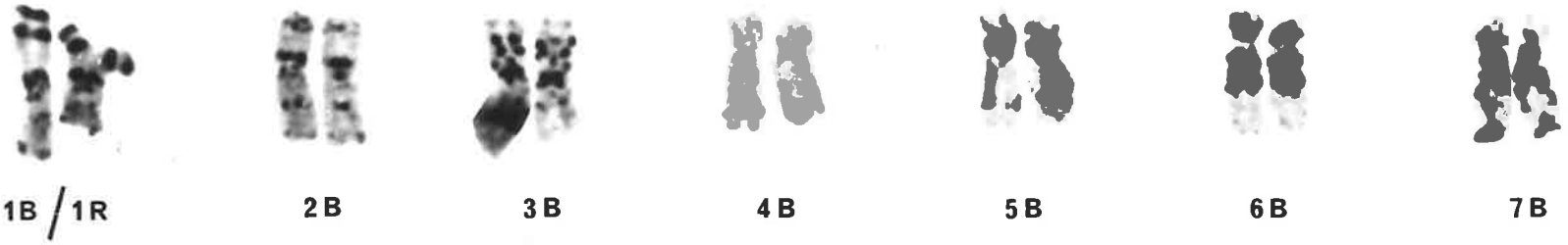
presence of banding on rye segments (ie. if the nucleolar bands are present, the banding of any rye chromosome in that particular preparation should also be apparent).

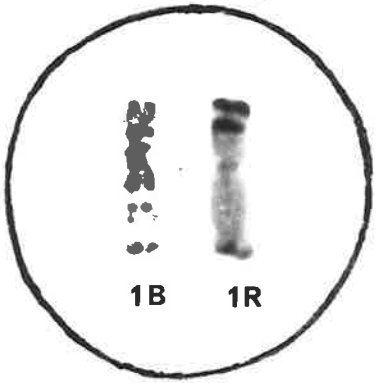
The benefit to chromosome identification of being able to identify both types of heterochromatin is illustrated in the karyotype of the cultivar Aurora (figure 5-14) where the 1R/1B translocation chromosome can be clearly identified and the nucleolar region on chromosome 6B is positively stained. The 1R/1B chromosome can be visualised as a centromeric translocation containing the whole of the short arm of 1R and the whole of the long arm of the chromosome 1B. It is not possible to determine which centromere is present using this technique. In fact it may be a hybrid centromere.

Using the same methods of karyotype analysis, the same 1R/1B translocation chromosome was observed in lines 256/75 and 486/77. However, the 1R/1B chromosome was observed to be lacking most of the terminal band on the short arm of 1R. In line 486/77 the presence of the short arm of 1R (also lacking most of the terminal band) was observed to be translocated to an unbanded chromosome which would confirm the findings of Shepherd (unpublished data) who proposed this chromosome to be 1D. However, on the basis of N-banding, the translocation break point cannot be located.

The 2R and 4A translocation chromosome present in Transfed was also confirmed as a translocation involving part of the long arm of chromosome 2R together with the α arm, and a small segment of the β arm of the median chromosome 4A. The arm of chromosome

FIGURE 5-14 An N-banded karyotype of the hexaploid wheat cultivar Aurora demonstrating the 1R/1B translocation chromosome. The insert illustrates the normal N-banded pattern of chromosome 1B in Chinese Spring wheat and of chromosome 1R in Imperial rye.


1B / 1R
2B
3B
4B
5B
6B
7B


1B
1R

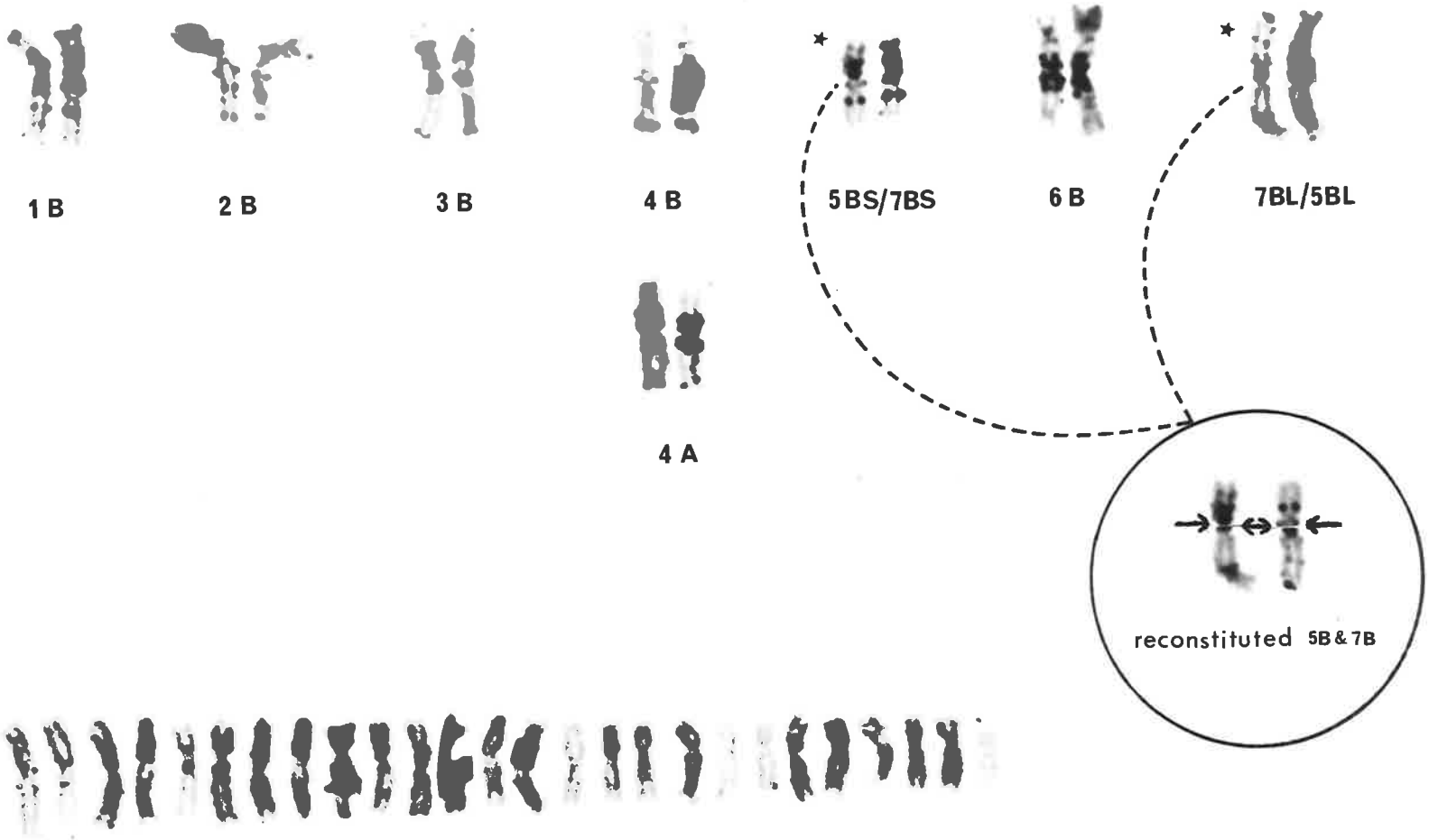

4A
7A

2R involved in this translocation chromosome was not previously identified.

Figure 5-15 illustrates the N-banded karyotype of the cultivar Poso and clearly demonstrates two reciprocal translocation chromosomes involving a centric or near centric break in chromosomes 5B and 7B. One of the translocation chromosomes is seen to contain the short arm of chromosome 5B and the short arm of chromosome 7B, while the other translocation chromosome contains the long arm of chromosomes 5B and 7B. The exact translocation break point cannot be determined using the N-banding technique, due to the intrinsic nature of the pericentric banding on chromosomes 5B and 7B. However the results are in close agreement with the results of Quinn and Driscoll (1970).

Minor differences in the chromosome banding patterns of the N-banded chromosomes (compared with the Chinese Spring karyotype) in the cultivars analysed in this section were observed. However, only in the case of chromosome 7A was chromosome identification affected. In the cultivar Poso, for example, chromosome 7A could not be identified. Most of the observed variations in chromosome banding could be accounted for by increases or decreases in the size of each band. Thus, if the size of the three bands on 7A were decreased enough they would not be apparent with the N-banding technique and the absence of these bands would preclude the identification of chromosome 7A.

FIGURE 5-15 An N-banded karyotype of the hexaploid wheat cultivar Poso demonstrating the 5B/7B translocation chromosomes. The asterisks indicate the chromosomes that were cut through the centromeres to reconstitute the normal 5B and 7B patterns illustrated in the insert.


1 B
2 B
3 B
4 B
5BS/7BS
6 B
7BL/5BL
4 A
reconstituted 5B&7B

5.4 Variation in the N-banding patterns of wheat chromosomes

It was indicated in section 2-4 that the well documented phenomenon of banding polymorphisms in rye chromosomes had made the identification of some rye chromosomes difficult. Three lines of hexaploid triticale (T 236, T 240 and the cultivar Beagle - T 937) were examined in order to confirm the existence of C-banding variation in rye chromosomes. Line T 236 and the cultivar Beagle were observed to contain all 7 pairs of rye chromosomes and line T 240 was observed to have 6 pairs of rye chromosomes with the missing rye pair being identified as 2R. Variations in the C-banding patterns were observed in all three lines, and when the karyotype of the C-banded rye chromosomes of Beagle (figure 5-16) is compared with the C-banded karyotype of Imperial rye (figure 2-6) the variation in the banding patterns is clearly apparent in all chromosomes. Using the C-banding technique, difficulty was experienced in distinguishing chromosome 3R from chromosome 7R which is in agreement with the results of other C-banding studies with rye chromosomes (Lelley, 1975).

C-banding studies in hexaploid wheat have demonstrated unique banding patterns on all 21 chromosomes. However, the variation in banding patterns in different cultivars has resulted in C-banding being only useful for the reliable identification of between 5 and 10 of the 21 chromosomes (see section 2-4). N-banding has resulted in the banding of only 9 of the 21 chromosomes of hexaploid wheat. Thus a banded chromosome has only to be distinguished from 8 other banded chromosomes in this instance instead of 20 others when using the C-banding technique.

FIGURE 5-16 The C-banded rye complement of the hexaploid triticale cultivar Beagle. The chromosome nomenclature is based on that of Gill and Kimber (1974a) and later publications. The classification of 4R and 7R is, however, in accordance with the findings of Koller and Zeller (1976).


1R
2R
3R
4R
5R
6R
7R

Variation in the N-banding patterns of wheat chromosomes has been observed in chromosomes present in the cultivars analysed in sections 5-2 and 5-3. Two further wheat cultivars (Kite and Federation) together with two hexaploid triticales (Drira and Coorong) were chosen in order to enlarge the number of cultivars analysed with N-banding and thereby to assess the effect on chromosome identification of the banding polymorphisms observed.

Figures 5-17 and 5-18 illustrate the variation observed in the N-banding patterns of chromosome 3B and chromosome 7B in the cultivars examined. The N-banding patterns were observed to be constant and reproducible. Thus the variations in pattern cannot be considered to be a result of N-banding procedural variations. Most of the variation in the N-banding patterns observed in the same chromosome could be related to different intensities of banding in a particular banding position and this observation can be satisfactorily explained by assuming that the relative size of these banding regions can vary between cultivars. Some of the bands observed in the long arm of chromosomes 3B and 7B in different cultivars are not observed in the Chinese Spring cultivars (see figures 5-17 and 5-18). However, the hypothesis that banding sites can vary in size would again be a satisfactory explanation of these results, as the size of the bands present in some cultivars may be too small to be detected in the Chinese Spring karyotype.

In the cultivars studied, variation in the banding patterns of all 9 N-banded chromosomes was detected. However, the position of the bands in the Chinese Spring karyotype was observed

FIGURE 5-17 An illustration of the polymorphic variations in the N-banding pattern of chromosome 3B in different cultivars. The chromosome banding patterns were observed to be constant within a cultivar as illustrated by the ten Chinese Spring 3B chromosomes taken from different cells. The 3B chromosomes exhibiting a pattern that varied from chromosome 3B of Chinese Spring wheat are indicated by the letters a to g, and are as follows:

a chromosome 3B present in the hexaploid wheat cultivar Poso

b chromosome 3B present in the hexaploid triticale cultivar Coorong

c chromosome 3B present in the tetraploid triticale line 4 x 5

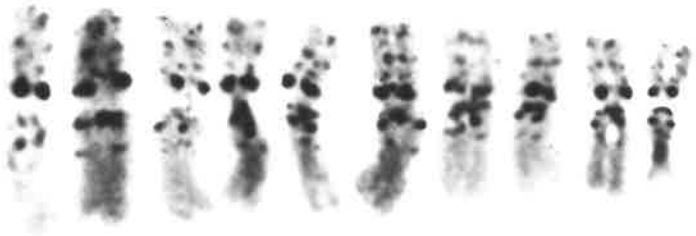
d chromosome 3B present in the hexaploid wheat cultivar Federation

e chromosome 3B present in the hexaploid wheat cultivar Gabo

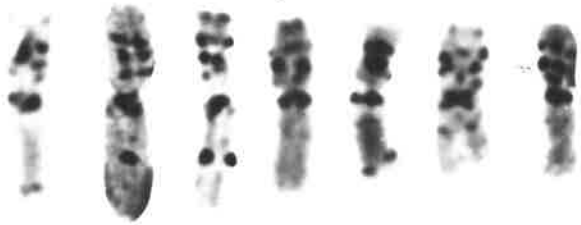
f chromosome 3B present in the hexaploid wheat cultivar Aurora

g chromosome 3B present in the hexaploid triticale cultivar Drira

CHINESE SPRING PATTERN



PATTERN VARIATION IN OTHER STOCKS



a b c d e f g

FIGURE 5-18 An illustration of the polymorphic variations in the N-banding pattern of chromosome 7B in different cultivars. The chromosome banding patterns were observed to be constant within a cultivar as illustrated by the ten Chinese Spring 7B chromosomes taken from different cells. The 7B chromosomes exhibiting a pattern that varied from chromosome 7B of Chinese Spring wheat are indicated by the letters a to f, and are as follows:

a chromosome 7B present in the tetraploid triticale line 4 x 5

b chromosome 7B present in the hexaploid wheat cultivar Kite

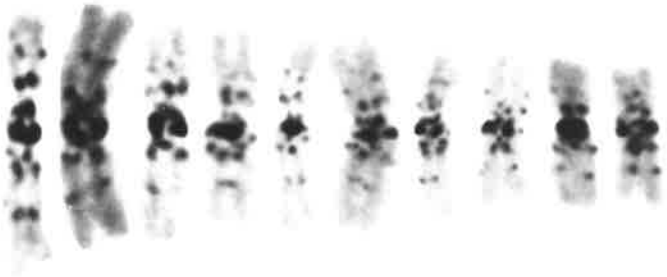
c chromosome 7B present in the hexaploid wheat cultivar Orlando

d chromosome 7B present in the hexaploid triticale cultivar Coorong

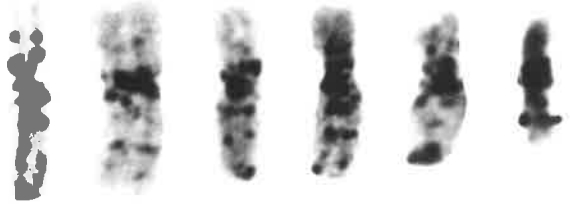
e chromosome 7B present in the hexaploid wheat cultivar Aurora

f chromosome 7B present in the hexaploid triticale cultivar Drira

CHINESE SPRING PATTERN



PATTERN VARIATION IN OTHER STOCKS



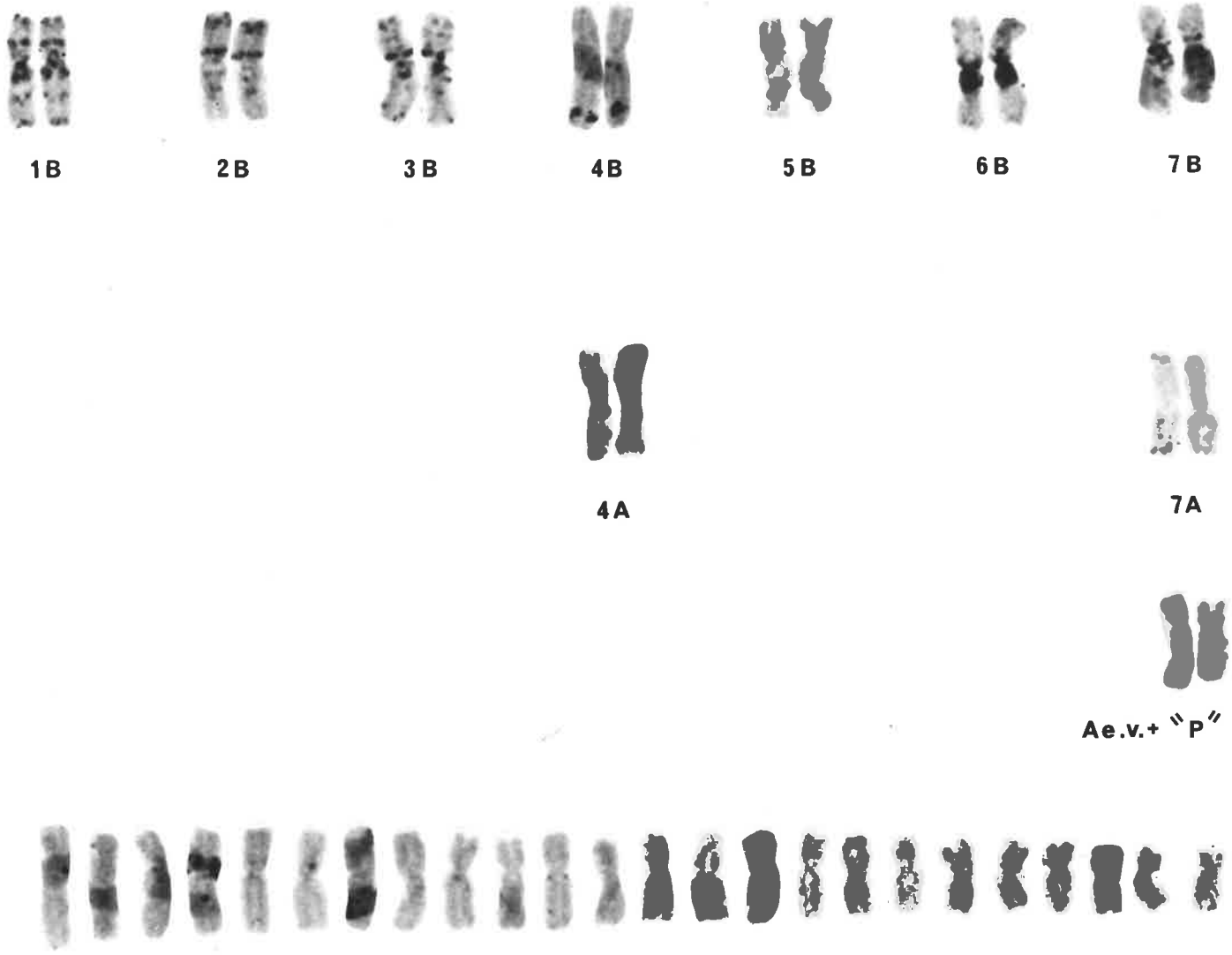
a b c d e f

to be sufficient criterion for the identification of all the banded chromosomes in the other cultivars examined. The only chromosome that could not be routinely identified on the basis of its N-banding pattern was chromosome 7A. For example, as indicated in section 5-3, the cultivar Poso has only 8 N-banded chromosomes (see figure 5-15) instead of 9 and the missing N-banding pattern is that of 7A. This can be explained by assuming that the N-banding sites on this chromosome in this variety are too small to be differentiated by the N-banding technique. This hypothesis can also be used to explain the results of Gerlach (1977) for two different lines of T. dicoccoides where a 7A pattern was observed in one line and not the other.

The fact that the position of the N-bands in the Chinese Spring karyotype appears to be a sufficient criterion for the identification of all N-banded wheat chromosomes so far analysed with the exception of chromosome 7A (where it is unbanded), together with the observation that the banding patterns are heritable, makes possible the cytological identification of crossover products in heterozygous plants where the two homologues exhibit different N-band polymorphisms. Such a situation was detected in the Ae. variabilis addition line P.

The Ae. variabilis addition lines, although in a predominantly Chinese Spring wheat background, all have the cultivar Poso present in their pedigrees (see Appendix II). In the Ae. variabilis addition line P, chromosome 3B was observed to exhibit a subterminal band in the long arm when analysed by the N-banding technique (see figure 5-19). In chromosome 3B of

FIGURE 5-19 An N-banded karyotype of the Aegilops variabilis disomic addition line P (2n = 44). The wheat complement was observed to exhibit the normal N-banding pattern for Chinese Spring wheat chromosomes with the exception of a subterminal band present in the long arm of chromosome 3B.


1B
2B
3B
4B
5B
6B
7B
4A
7A
Ae.v.+ "P"

Chinese Spring wheat, the most prominent N-banding region is the proximal band in the short arm. The other bands in the short arm are faint and no apparent sub-terminal band is present in the long arm. In chromosome 3B of the cultivar Poso, the proximal band in the short arm is faint and the bands in the middle of the short arm are more intense than in the Chinese Spring cultivar (see figures 5-15 and 5-17). Chromosome 3B of Poso also has an intense band proximal to the centromere in the long arm and a sub-terminal band in this arm. The faint interstitial band approximately a third of the way along the long arm from the centromere in chromosome 3B of Chinese Spring is not present in chromosome 3B of the cultivar Poso. The 3B chromosome present in the Ae. variabilis addition line P has the Chinese Spring N-banded pattern for this chromosome with the exception of the presence of the sub-terminal band in the long arm which is in the same position as the sub-terminal band present in the long arm of chromosome 3B in the cultivar Poso.

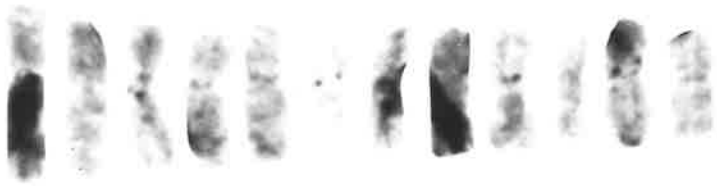
It is therefore proposed that the N-banded pattern of chromosome 3B in the Ae. variabilis addition line P is the result of an exchange of chromosome material between the Chinese Spring and Poso 3B chromosomes with the break point being distal to the interstitial band a third of the way down the long arm of the Chinese Spring chromosome. It would not be possible to cytologically detect this exchange of chromosome material without a chromosome banding technique and an understanding of the polymorphisms of banding patterns present in different cultivars.

Figure 5-20 illustrates the N-banded karyotype of the F_1 seed of a male sterile T. durum crossed with the diploid T.

FIGURE 5-20 An N-banded karyotype of the F_1 seed of a male sterile Triticum durum crossed with the diploid Triticum monococcum. The normally median chromosome 4A was observed to be structurally altered by a terminal deletion in the α arm resulting in an arm ratio of approximately 3:4. Only one dose of chromosome 4A and 7A could be identified.


1B
2B
3B
4B
5B
6B
7B


4A
7A



monococcum (Hossain, unpublished data). The N-banded chromosomes of this plant can be identified as a result of the polymorphisms of the banding not affecting chromosome identification. The bands in the nucleolar regions of 1B and 6B also aid in the identification of these chromosomes. It is of interest that in this plant, only one chromosome 4A and one 7A can be identified. If T. monococcum is the donor of the A genome then a pair of chromosome 4A and a pair of chromosome 7A should have been detected. The absence of the second 7A can be explained by banding polymorphism (see discussion above). However the absence of the second 4A pattern is difficult to explain on the basis of banding pattern variation as it is one of the most heavily N-banded chromosomes. Gerlach (1977) used N-banding to analyse two different lines of T. monococcum and found no N-banding. As no hexaploid wheat so far analysed has been observed to contain a 4A chromosome with greatly reduced N-banding, it is likely that at least the chromosome 4A did not originate from T. monococcum. The N-banded 4A chromosome in figure 5-20 is assumed to have originated from a male sterile plant of the hexaploid cultivar Cornerstone in which the sterility is postulated to be the result of a terminal deletion of the α arm (Driscoll, 1977). This is supported by the non-median appearance of this normally median chromosome. The N-banding analysis of this plant again demonstrates the power of this technique for cytological analysis with a high level of precision.

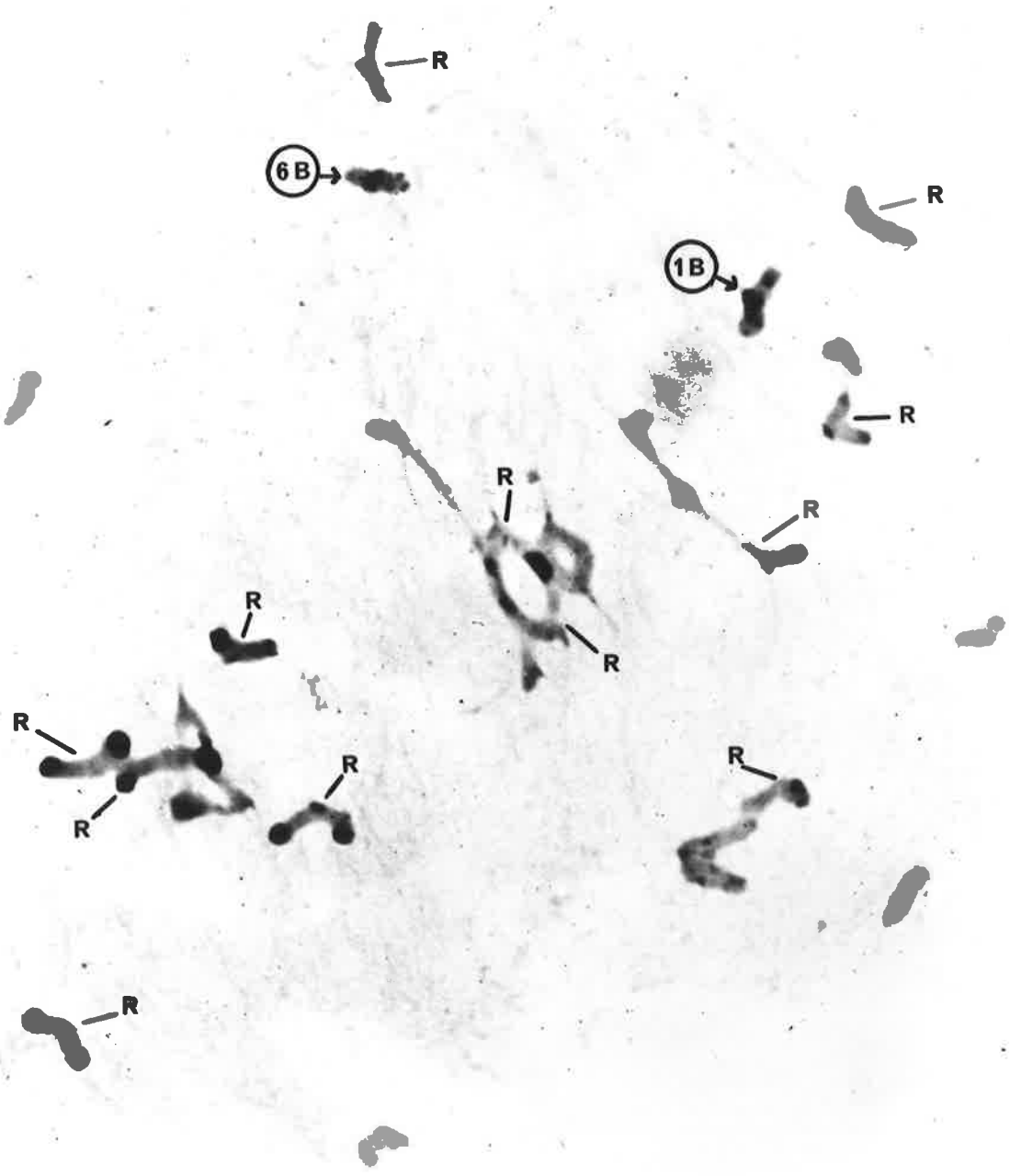
5.5 The identification of meiotic wheat chromosomes

Generally meiotic chromosomes have not been examined by banding techniques. A banding technique that can be applied to meiotic preparations has important advantages for the recognition of chromosomes, particularly alien univalents and univalents resulting from asynapsis or desynapsis. Meiotic preparations also offer a high proportion of cells for study, as the pollen mother cells divide synchronously.

C-banding has been used for the identification of meiotic rye chromosomes in both rye and triticale (see section 2). However, some difficulty has been experienced in that only the terminal bands and the nucleolar band on 1R can be stained, thus making chromosomes, with the exception of chromosome 1R, difficult to identify. Figure 5-21 illustrates a C-banded meiotic metaphase preparation of an F_1 plant from a cross of T 507 by Imperial rye. The rye chromosomes can be distinguished from the wheat chromosomes on the basis of the large terminal bands. However, little chromosome identifications is possible. Some banding is present on the wheat chromosomes, but only 1B and 6B could be identified.

Wheat chromosomes have not previously been identified at meiosis by chromosome banding studies. The N-banding technique with the treatment time in 1M NaH_2PO_4 reduced to 1-1.5 min. was observed to produce distinctive banding on 9 of the 21 bivalents of the hexaploid wheat cultivar Chinese Spring. The N-banded meiotic chromosomes were observed to exhibit identical banding

FIGURE 5-21 A C-banded meiotic metaphase preparation of an F_1 plant from a cross of the hexaploid triticale cultivar Coorong by the diploid rye cultivar Imperial. The rye chromosomes exhibit prominent terminal banding and are indicated by an 'R'. Chromosomes 1B and 6B are labelled and are the only two wheat chromosomes that could be identified.

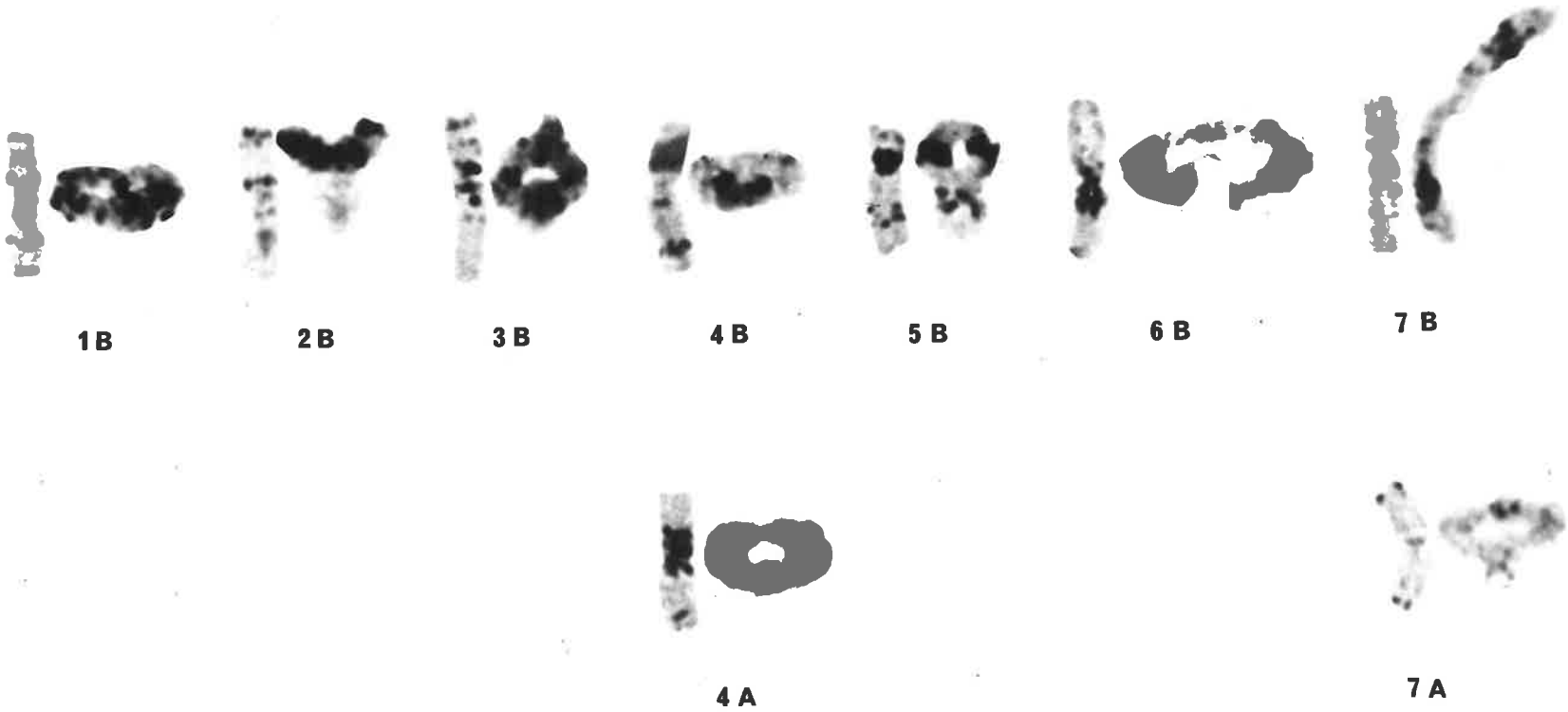

R
6B
R
1B
R
R
R
R
R
R
R
R
R
R
R

patterns to the mitotic chromosomes. Hence, chromosomes 1B to 7B, 4A and 7A can be identified at meiosis (see figure 5-22). The fact that these meiotic chromosomes could be N-banded so clearly is a definite advantage of the N-banding technique when compared to C-banding (see figure 5-21).

The N-bands appeared to be present in all stages of meiosis, thus supporting the well documented hypothesis that the chromosome bands can be detected at all stages of the cell cycle (see section 2).

The reason that the treatment time in the hot acid buffer needs to be reduced to produce N-bands on meiotic chromosomes is not clear.

FIGURE 5-22 A comparative karyotype of the mitotic and meiotic N-banded chromosomes of the hexaploid wheat cultivar Chinese Spring. The bivalents (the right-hand chromosome in each pair) are all from the same Metaphase I preparation.


1B
2B
3B
4B
5B
6B
7B
4A
7A

5.6 Verification of the nomenclature of the 9 N-banded wheat chromosomes

Gerlach (1977) demonstrated that N-banding could be used to identify the 9 chromosomes that exhibit banding in the hexaploid wheat cultivar Chinese Spring. Gerlach assigned these 9 chromosomes numbers based on the chromosomes genetic nomenclature (correlated with the homoeologous relationships) and used the ditelocentric tester stocks to identify these chromosomes. As an indirect result of N-banding analysis of some of the cultivars used in this thesis, some of this nomenclature could be verified. The following aneuploids in a Chinese Spring background were also used to verify the nomenclature used by Gerlach (1977): Nulli tetra 2D/2B and Tetra 2B were used to identify the banding pattern of chromosome 2B; Nulli tetra 3A/3B and Tetra 3B were used to identify the banding pattern of chromosome 3B; Tetra 4B was used to identify the banding pattern of chromosome 4B; Nulli 7B tetra 7A was used to identify the banding pattern of both 7B and 7A; and Tetra 4A was used to identify the banding pattern of chromosome 4A. The double ditelocentric aneuploid of chromosome 4A was used as a control, as Gerlach had also used this line.

Gill and Kimber (1974c) identified the chromosomes of the cultivar Chinese Spring using C-banding, and a comparison of the C-banded karyotype with the N-banded karyotype shows that although the N-banded and C-banded regions are different in a number of instances, there is a basic similarity in the two banding patterns of these chromosomes (see Gerlach, 1977). Gill and Kimber,

however, also used the double ditelocentric and telocentric tester stocks to assign the chromosomes their genetic nomenclature. Thus the use of different aneuploids enabled indirect verification of the results of Gerlach (1977) and Gill and Kimber (1974c) for the nomenclature of the 9 wheat chromosomes that exhibit N-banding.

The cultivar Orlando, for example, was observed to contain a 1R substitution for 1B, using the nomenclature assigned by Gerlach (1977). Thus, indirectly, the identification of the chromosome 1B banding pattern was confirmed. Further, the cultivar Poso was independently described as containing a reciprocal translocation involving chromosomes 5B and 7B (see Quinn and Driscoll, 1970) and after N-banding analysis, the chromosome banding patterns assigned to these chromosomes by Gerlach (1977) were indeed observed to be involved in the reciprocal translocation present in this cultivar.

On the basis of N-banding analysis on the stocks used in this thesis, the genetic nomenclature assigned to the 9 N-banded chromosomes of hexaploid wheat was confirmed.

SECTION 6. GENERAL DISCUSSION

6.1 The reliability of and possible basis for N-band formation

In section 4 it was confirmed that the standard N-banding technique could be used to reliably identify 9 of the 21 chromosomes of hexaploid wheat. The banding patterns obtained for these 9 chromosomes corresponded almost exactly with the patterns observed by Gerlach (1977) in a different laboratory. Subsequently, Islam (1980) was also able to reproduce the same N-banding patterns for these chromosomes. In section 5-6 the chromosome identification made by Gerlach (1977) for the 9 N-banded chromosomes of wheat was independently confirmed. The N-banding technique can therefore be regarded as a reliable technique for the identification of 9 of the chromosomes of Chinese Spring wheat.

The reliability of the N-banding technique differs from the apparent unreliability of the C-banding technique as evidenced by the different observations of Gill and Kimber (1974c) and Iordansky et al. (1978a) for the C-banding patterns of Chinese Spring wheat chromosomes.

Careful manipulation of the two critical procedures in the

N-banding technique (see section 4) was observed to reliably produce positive staining in the nucleolar regions of chromosomes 1B and 6B of wheat and chromosome 1R of rye. It was also possible to stain positively other regions of rye chromosomes that are unreliably stained with the standard N-banding technique (see section 3-4 and section 4-3).

The less stringent conditions required to produce banding in rye chromosomes and the nucleolar regions of chromosomes 1B and 6B of wheat do not, however, result in banding on the 12 pairs of unbanded wheat chromosomes. As the heterochromatin in the nucleolar regions is generally accepted as being quite specialised, and as all 21 wheat chromosomes exhibit a C-banding pattern which does not include all the N-banding sites (Gerlach, 1977), it is probable that at least five different types of heterochromatin or heterochromatin - protein complexes can be cytologically stained in rye and hexaploid wheat as follows:

1) the standard N-banded regions that overlap with the C-banding regions
2) the standard N-banded regions of wheat and rye chromosomes that do not overlap with the C-banded regions
3) the C-banded regions of wheat and rye chromosomes that do not overlap with N-banded regions
4) the non-standard N-banded regions of rye chromosomes (these regions only stain positively when the stringency of the technique is relaxed)
5) the nucleolar regions - known to contain highly specialised DNA.

Greilhuber (1975) proposed that chromosome banding studies on Vicia faba revealed a basic banding pattern more or less completely dependent upon whether more or less optimum procedures had been chosen. As the N-banding technique does not differentiate all the C-banding regions of wheat, the question arises as to whether the N-banding technique involves less optimum conditions than the C-banding technique, or in fact is more specific than the C-banding technique.

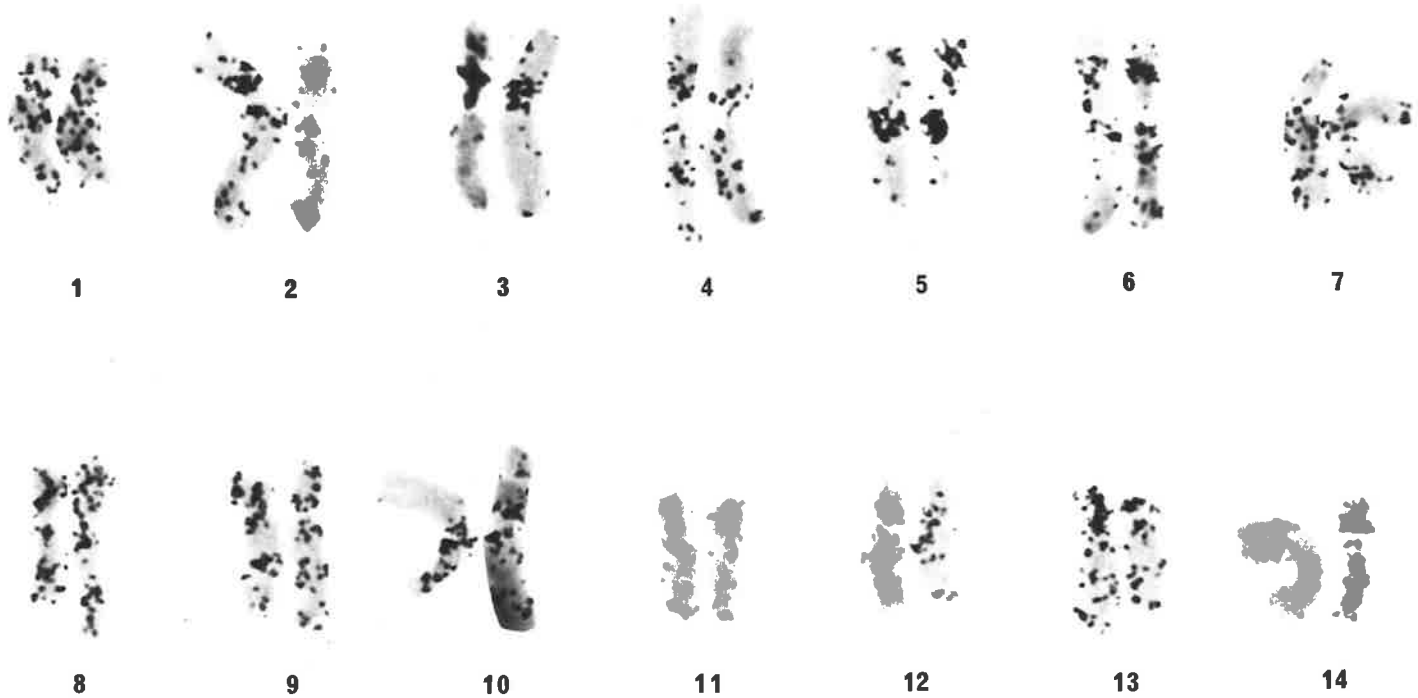
Gerlach, Appels, Dennis and Peacock (1978) demonstrated that a particular highly repeated DNA satellite sequence was located on 9 of the 21 chromosomes of Chinese Spring wheat. The chromosomal locations of this particular satellite were examined by in situ cytological hybridisation using an H^3 labelled cRNA (cRNA = an RNA prepared in an in vitro reaction using Escherichia coli RNA polymerase and the satellite DNA sequence as a template). The chromosomal locations of the satellite sequence in Chinese Spring wheat, as assayed by autoradiography, very closely resemble the N-banding patterns in this cultivar. It is notable that no labelling was observed in the nucleolar regions of chromosomes 1B and 6B of wheat. Recent results (Gerlach and Peacock, in press, and Dennis et al. in press) have noted the correspondence of N-bands and the satellite locations. However, more than one sequence appears to be involved. The general formulation of the sequence is thought to involve $(GAA)_m$ $(GAG)_n$, so that the N-banding may not necessarily be the consequence solely of one particular sequence. It is evident, however, that the N-banding

technique may be very specific in its staining specificity.

A small amount of the same cRNA as used by Gerlach et al. (1978) was kindly supplied by Dr. Gerlach and Dr. Peacock for cytological hybridisation to Ae. variabilis chromosomes in order to further test the relationship between this satellite location and the N-banding regions. The method of Gerlach and Peacock (in press) was followed exactly and the cRNA was observed to be located on all 14 chromosomes of Ae. variabilis. Following autoradiography, the chromosomes of Ae. variabilis could be identified (see figure 6-1) by comparison with the N-banded karyotype (figure 5-1). It was evident that the cRNA was not located on the diffuse banding sites in the short arms of chromosomes 4, 10 and 11 of Ae. variabilis (see figures 5-1 and 5-3).

As a result of the procedures in the N-banding technique, the involvement of non-histone proteins in the staining reaction is strongly indicated (see Funaki et al. 1975). It is therefore postulated that the biochemical basis for the formation of N-bands is a highly specific relationship between specific sequences of DNA (see above discussion) and protective, relatively acid stable, non-histone proteins. The possibility that chromosome coiling may play a minor role in determining the N-banding patterns by limiting access of the stain to the possible banding sites cannot, however, be ruled out. The second type of N-banding material stained after less stringent treatment may involve either a less acid stable class of non-histone proteins or the same class of

FIGURE 6-1 The chromosomal locations of a hexaploid wheat satellite DNA sequence in Aegilops variabilis as assayed by in situ autoradiography. The chromosomes were identified by comparing the satellite labelling pattern with the N-banding patterns of the Aegilops variabilis chromosomes. The cRNA used to label the chromosomes was provided by Dr. W.J. Peacock and Dr. W.L. Gerlach.


1
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non-histone proteins that are less acid stable due to the differences in the DNA sequences to which they are bound.

The standard N-banding technique can be conceived as staining a specific subset of constitutive heterochromatin as redefined by Greilhuber (1977) (see section 2-2-1).

6.2 The relative benefits of N-banding compared to C-banding

Brown's (1966) definition of constitutive heterochromatin redefined by Greilhuber (1977) as representing "unconvertible chromosome segments which do not decondense during telophase, so forming chromocenters during interphase, including all such very small bodies (heterochromomeres) which are consubstantial with chromocenters as revealed by staining behaviour or cytochemical tests" represents a definition with which the observations presented in this thesis are in accordance. If this definition is accepted then the bands produced by the N-banding technique and the C-banding technique may be regarded as representing two overlapping subsets of the total constitutive heterochromatin as proposed in section 6-1. The bands that can be obtained in the nucleolar regions of chromosomes 1B and 6B of wheat and in the rye complement by manipulating the critical procedures of the N-banding technique can be regarded as a further overlapping subset of constitutive heterochromatin.

The simplified C-banding technique (see sections 3-2 and 3-5) developed in this laboratory was observed to be satisfactory for the identification of rye chromosomes in mitotic metaphase preparations of the cultivar Imperial. The rye complement of some triticales could also be identified, although some difficulty was experienced in differentiating chromosome 3R from chromosome 7R, due to polymorphisms present in the banding patterns of these chromosomes. Good quality C-banding of wheat chromosomes was,

however, very difficult to achieve as chromosomes in a complete cell were seldom all clearly banded. Variations in the quality of C-banded wheat chromosomes also occurred between cells on the same slide and between slides in the same batch.

In comparison to C-banding, the N-banding technique produced high quality banding of wheat chromosomes in most slides with very little variation between and within cells on the same slide. It was, however, difficult to manipulate the critical procedures of the N-banding technique in order to achieve bands on rye chromosomes and in the nucleolar regions of chromosomes 1B and 6B of wheat. Thus from my observations, it would appear that the N-banding technique is easier to apply to wheat chromosomes than to rye chromosomes, and that the C-banding technique is easier to use for the identification of rye chromosomes than of wheat chromosomes. In C-banding studies of triticale (Merker, 1973a, 1973b, 1975), triticale - wheat hybrids (Merker, 1975) and also wheats containing rye substitutions and wheat-rye translocation chromosomes (Bennett and Smith, 1975; Munzer, 1977), there was insufficient linear differentiation for the wheat chromosomes to be identified, thus again demonstrating the difficulty of obtaining good quality C-banding of wheat chromosomes even in the presence of good quality C-bands on the rye chromosomes.

It is of interest that, when observed with phase contrast optics, the unstained interphase nuclei of rye are seen to contain large blocks of heterochromatin ("natural" heterochromatin), compared with the unstained interphase nuclei of wheat which

exhibit no large blocks of natural heterochromatin (see figure 4-8). On the basis of this observation, it would appear that the C-banding technique is more suited to the differentiation of natural heterochromatin than the N-banding technique. The unstained interphase cells of Ae. variabilis were observed to contain no large block of heterochromatin and the N-banding technique was found to give better linear chromosome differentiation than the C-banding technique. Islam (1980) also observed that N-banding produced more distinct bands on the chromosomes of barley than did C-banding. These observations are interpreted as further evidence that the C-banding technique is a technique of detecting several different types of heterochromatin in contrast to the standard N-banding technique which may offer an excellent method of detecting specific heterochromatin which is not readily apparent in unstained interphase nuclei.

The benefit of being able to detect both types of N-banding material is, however, illustrated by the identification of wheat-rye substitutions or translocations where the chromosomes involved exhibit N-bands (see sections 5-3-1 and 5-3-2).

Singh and Lelley (1975) and Lelley (1975) have demonstrated that the C-banding technique can be used to identify rye chromosomes at meiosis in both rye and triticale. It was only possible, however, to place the rye chromosomes in one of three groups of chromosomes, as only the major bands could be differentiated. The rye chromosomes were easily differentiated from wheat chromosomes due to the presence of large terminal

blocks of heterochromatin, whereas the wheat chromosomes were observed to exhibit little or no banding. A similar result was observed in this laboratory (see figure 5-21). In contrast, the N-banding technique was observed to produce good quality bands on 9 of the 21 bivalents of wheat chromosomes at metaphase I of meiosis, and, further, the banding patterns were observed to be identical to the mitotic patterns (see figure 5-22), thus allowing the identification of all 9 bivalents. This represents the first time that meiotic chromosomes of wheat have been identified.

A major advantage of the N-banding technique compared to the C-banding technique was observed with regard to the effect of band polymorphisms on chromosome identification in different cultivars of hexaploid wheat. Although polymorphisms in the N-banding pattern of all 9 banded chromosomes of wheat were observed, only the identification of chromosome 7A was affected. This is in marked contrast to the effect of polymorphisms in wheat detected with the C-banding technique, where only a few chromosomes could be reliably identified (see discussion in section 2-4). The fact that all the N-banded wheat chromosomes so far studied can be identified by comparison with the karyotype of the cultivar Chinese Spring is particularly valuable due to the proposed universal applicability of this technique to wheat chromosomes.

The polymorphisms observed in the N-banding patterns of wheat chromosomes from different cultivars can largely be explained if the suggestions of Bennett et al. (1977) are adopted.

Bennett et al. (1977) suggested that saltatory amplification events at the banding sites could be the reason for the initial increase in size of these regions and that subsequent unequal crossing-over between homologues may extend the range of variation. It is possible to explain the absence of the 7A pattern in some cultivars, and the absence of other bands including the nucleolar band on chromosome 5D (major nucleolar regions are known to be present on chromosomes 1B and 6B of wheat with a minor nucleolar region being present on chromosome 5D of wheat (Driscoll and Darvey, 1972; Viegas and Mello-Sampayo, 1975)), by assuming that the banding sites are too small to be optically detected.

6.3 The application of N-banding to wheat cytogenetic studies

It has been demonstrated in this thesis that the N-banding technique can be reliably used for the identification of chromosomes that exhibit banding patterns. Importantly, it has also been shown that in the wheat chromosomes examined so far, the polymorphisms present in the banding patterns have not affected the identification of the banded chromosomes. The N-banding patterns have also been observed to be stable throughout the cell cycle and to be independent of both alien cytoplasm and alien genetic material. For chromosomes that exhibit N-banding patterns, the N-banding technique therefore represents a powerful cytological tool for the identification of chromosome rearrangements and the presence of alien material in wheat at a level hitherto impossible to achieve. In contrast, identification of wheat chromosomes by their general morphology (arm ratios, length and secondary constrictions) has been shown to be inaccurate (see Schlegel and Mettin, 1978). Isozyme studies are also inaccurate for the identification of whole chromosomes, as distinct from modified chromosomes, as only a fragment of the chromosome that carries the relevant gene or genes need be present to provide the same isozyme pattern as the whole chromosome.

Pairing studies between proposed identical chromosomes from different origins may also be difficult to interpret. For example, in the cross between the Ae. variabilis addition line A and the Ae. umbellulata addition line B, the two alien

chromosomes were observed to pair in only 54% of the meiocytes (Driscoll, personal communication). The Ae. variabilis chromosome A and the Ae. umbellulata chromosome B can, however, be shown to be the same chromosome on the basis of their N-banding patterns. It is interesting to speculate that the polymorphic variations of banding pattern observed in these two chromosomes may reflect actual genetic differences between these two chromosomes. This difference in banding pattern may also partly account for the incomplete pairing of the two chromosomes, due to the different amounts of heterochromatin present physically obstructing complete pairing.

If polymorphic variations of banding patterns are taken into account, the N-banding technique can be applied to the problem of genome origin in polyploid species. The diploid Ae. umbellulata can be confirmed as the donor of the Cu genome in the tetraploid species Ae. variabilis on the basis of the N-banding patterns of these chromosomes. By comparing the four chromosomes identified in addition lines of the diploid Ae. longissimum with the Ae. variabilis karyotype, it is possible to conclude that the S^l genome of this diploid and the S^v genome of Ae. variabilis may, in fact, be the same genome, although the N-banding patterns of the remaining 3 chromosomes of Ae. longissimum have yet to be established. It is of interest that all 7 chromosomes of the B genome in hexaploid wheat and all 14 chromosomes of Ae. variabilis exhibit N-banding. However, it was not possible to detect any homology between the B genome chromosomes and the Ae. variabilis chromosomes. This is in agreement with the fact that

less than one chiasmata is formed in the F_1 between Ae. variabilis and Chinese Spring wheat (Driscoll and Quinn, 1968).

The observation that only the B genome and chromosomes 4A and 7A of hexaploid wheat exhibit N-bands is further evidence in support of the conclusion of Sears (1966) that the A and D genomes appeared to be more closely related than were the A and B or B and D genomes of hexaploid wheat. On this basis it is possible that the Cu, S^v and S^l genomes may be more closely related in an evolutionary sense to the B genome of hexaploid wheat than to either the A or D genomes.

The N-banding technique can also be used to cytologically observe the frequency of homologous chromosome cross-over products in the F_1 hybrids between wheat cultivars where the two parental cultivars exhibit diagnostic polymorphic differences in banding pattern (see section 5-4). N-banding analysis has also been demonstrated to allow the identification of some chromosomes in the abnormal BC1 plants of Chinese Spring wheat X Ae. variabilis. The cytogenetic analyses cited in the above two examples could not at present be performed without the aid of a technique such as the N-banding technique.

In this thesis, all structural modifications of wheat chromosomes have been detected relative to the basic N-banded karyotype of the cultivar Chinese Spring. The Chinese Spring karyotype was chosen for this purpose due to the wealth of genetic

knowledge available in this cultivar and because this cultivar is universally accepted as the standard. Due to the lack of a standard karyotype in some of the genera related to wheat, it is important, when identifying alien material in a wheat background, that the alien parent also be analysed. If the karyotpe of the alien parent is known, it is then possible to detect chromosomes that have undergone structural change during their incorporation in the wheat background. This may be obscured if different cultivars of the alien species already differ by chromosome structural rearrangements. For example, different cultivars of Ae. variabilis are thought to contain translocation differences (Feldman, personal communication). It is proposed that the karyotype of Ae. variabilis presented in this thesis be regarded as the standard karyotype by which the karyotpe differences in other cultivars of Ae. variabilis can be measured.

The fact that chromosomes exhibiting N-banding patterns can be identified in meiotic preparations paves the way for cytological analysis of these preparations at a previously impossible level. For example, pairing between non-homologous chromosomes can be observed, and the identity of the univalents exhibiting N-banding can be determined. Further, Riley (1960) proposed that association of homoeologous bivalents occurred at metaphase I of meiosis. N-banding of meiocytes of plants containing a heteromorphic bivalent of either chromosome 7D or 4D could be used to check this proposal (Driscoll, personal communication), as the other chromosomes of group 4 and 7 are banded.

The results presented in this thesis indicate that the N-banding technique is a powerful cytogenetic tool. Due to the reliability, universal applicability and the relative simplicity with which the N-banding technique can be performed, it is envisaged that, in the future, this technique will have many applications to cytogenetic analysis of wheat and its near relatives.

SECTION 7. APPENDICES

7.1 Appendix I

The homoeologous relationships of the chromosomes of wheat and related species

The chromosomes of T. aestivum (hexaploid wheat, 2n = 42) can be divided into seven homoeologous groups, each group consisting of three pairs of homologous chromosomes; the pairs being representatives from the genomes A, B and D (Sears, 1952, and Sears, 1954). The seven homoeologous groups of chromosomes are numbered one to seven and the chromosomes within each group are symbolised by A, B or D, depending upon the genome to which they belong. As an example, chromosomes 2A and 2A are homologous, but this pair of homologues is homoeologous to the 2B and 2D chromosomes. These three pairs of chromosomes from the A, B and D genomes comprise the group 2 homoeologues of hexaploid wheat.

Sears (1966) demonstrated that each member of a homoeologous group in T. aestivum (cultivar Chinese Spring) could compensate to varying extents for the loss of either of the other two members of the group if present in an additional dose. Some chromosomes from other species (alien species) within the TRITICINAE have been substituted for wheat chromosomes. If the substitutions are successful in that the alien chromosome

compensates, at least in part, for any or all of a particular group of wheat chromosomes, the alien chromosome can be designated in accordance with the homoeology involved. Such studies have been reviewed by Sears (1968, 1969) and Bielig and Driscoll (1971, 1973). For example, the tetraploid species Ae. variabilis is known to contain the genomes designated Cu and S^v (see Kihara, 1949) and some of these chromosomes have been successfully substituted for wheat chromosomes (see Driscoll, 1975). It is therefore possible, for example, to speak about chromosome 2 Cu and 2 S^v as being members of the same homoeologous group as the group 2 chromosomes of wheat once their relationship has been established.

The ability for homoeologous chromosomes to compensate is determined by comparative analysis of morphological and fertility characteristics.

7.2 Appendix II

Pedigrees of the Aegilops variabilis addition lines

ADDITION LINE:	PEDIGREE
A	Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 4* Chinese Spring
B	Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 5* Chinese Spring
C	Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 5* Chinese Spring
D	Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 5* Chinese Spring
E	Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 5* Chinese Spring
F	Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 5* Chinese Spring
G	Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 6* Chinese Spring
H	Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops

variabilis /3/ 4* Chinese Spring

I Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 4* Chinese Spring

J Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 5* Chinese Spring

L Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 5* Chinese Spring

M Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 4* Chinese Spring

N Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 4* Chinese Spring

O Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 4* Chinese Spring

P Poso-48(Cal.) / 3* Chinese Spring /2/ Aegilops variabilis /3/ 5* Chinese Spring

THE ABOVE PEDIGREES ARE WRITTEN AFTER THE METHOD PROPOSED BY L.H. PURDY ET AL. (1968).

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