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Perspectives in Chromosome Manipulation

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Perspectives in chromosome manipulation

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Three categories of chromosome manipulation are discussed, with examples from hexaploid wheat. First, uncontrolled events, such as intergeneric translocations induced by mutagenic agents, have been frequently isolated but have been infrequently incorporated into widely grown varieties. A method proposed by K. W. Shepherd is designed to select an accommodating genetic background for these interchanges and thereby overcome this deficiency. This relies on selection for yield among many selections that are homozygous for a translocation but segregating for many other genetic components.

The second category involves manipulations associated with the distinctive genetic activity of chromosome 5B. *ph* mutants are expected to play an important role in the transfer of genetic material from other genera to wheat. A method described by E. R. Sears is designed to isolate a small intercalated alien segment. This relies on crossing-over in an alien segment that is common to two distinct types of homoeologous-exchange chromosomes.

The third category involves manipulations associated with a male-sterility mutation on chromosome 4A and the distinctive genetic activities of this chromosome. A method, described by the author, is designed to produce hybrid wheat with an induced male-sterility mutation on this chromosome. Fertility restoration is being attempted with four chromosomes, a restriction on which is that they must not pair with the other chromosomes of the wheat complement. These are a modified 4A–2R translocation chromosome, part of the cereal rye genome, chromosome 4 of barley, and chromosome 4 of diploid wheat. It appears that chromosome 4A originated elsewhere than from diploid wheat; thus the A genome of common wheat arose from at least two species. As detected by M. A. Hossain and the author, part of the genetic material for male fertility on 4A has a counterpart on rye chromosome 2R. An exchange between the chromosomes of homoeologous groups 2 and 4 appears to have occurred, perhaps at the diploid level.

The manipulation of chromosomes or chromosome segments is assuming an increasingly important role in plant breeding. These manipulations, which have been used rather extensively in the improvement of common wheat (*Triticum aestivum* L.), include uncontrolled events induced by mutagenic agents and events that are under genetic control. The latter may be divided into two categories, depending on whether they are based upon the unusual genetic characteristics of chromosomes 5B or 4A. The impact of these three categories of manipulation on plant improvement is considered, as they collectively illustrate the developments in this general area of research.

Uncontrolled events induced by mutagenic agents

Considerable emphasis has been placed on the introduction of 'alien' genetic material, i.e. material from other than the host species, in the improvement of hexaploid wheat. Wide crossing has mostly involved species from the subtribe Triticinae Melderis; however, successful

crosses have been made beyond this boundary, e.g. those involving wheat and various species of *Hordeum* L., including *H. vulgare* L. (Islam *et al.* 1975).

The resultant amphiploids, with one exception, have not been directly used in agriculture. The exception, triticale (× *Triticosecale* Wittmack), resulting from crosses of wheat and rye (*Secale* L.), is being used in agriculture on an increasing scale.

Single-chromosome addition lines and substitution lines have not played a significant role in agriculture in that the alien genetic material added or substituted is too great and causes problems in production characters. The notable exception to this is the series of varieties that include the addition of rye (*Secale cereale* L.) chromosome 1R or the substitution of it for chromosome 1B (Mettin *et al.* 1973; Zeller 1973).

Transfer of less than a full chromosome to the recipient species has been accomplished by the use of ionizing radiation. The first transfer of this type involved transfer of a segment of an *Aegilops umbellulata* Zhuk. chromosome, bearing resistance to wheat leaf rust (*Puccinia recondita* Rob. ex Desm.), to chromosome 6B of wheat (Sears 1956).

The most successful transfer of this type involves the translocation of a segment of an *Agropyron elongatum* (Host) Beauv. chromosome that carries resistance to wheat stem rust (*Puccinia graminis tritici* Erikss.) to chromosome 6A of wheat (Knott 1961). This resistance has been incorporated into Australian wheat varieties Eagle (Martin 1971), Kite (Fisher & Martin 1974), Jabiru (Fisher & Syme 1977) and Avocet (R. H. Martin, personal communication), which in recent years have collectively constituted approximately 700 kha or 7 % of the Australian wheat crop.

However, most of these translocations (see Driscoll (1975*a*, 1976*a*) for listings) have not been successfully incorporated into widely grown varieties of wheat. The reasons for this are loss of important wheat genetic material and/or gain of deleterious alien genetic material in the exchange. For example, the Transec translocation involves transfer of a segment of rye chromosome 2R, bearing resistance to wheat leaf rust and to wheat powdery mildew (*Erysiphe graminis tritici* March.), to chromosome 4A of wheat (Driscoll & Jensen 1964). This translocation has not been incorporated into a released variety of wheat despite the fact that it has been available to plant breeders for over 15 years. The translocation is usually associated with lowered yield. It has been proposed by Shepherd (1977) that if selection is imposed on this type of material under specific conditions it may be possible to break this association. This proposal includes selection of large numbers of F_3, or equivalent, plants that are homozygous for the alien translocation and subjecting derived F_5, or equivalent, progenies to yield tests. The rationale is that many pairs of alleles will assort between F_3 and F_5, and considering that all lines are homozygous for the alien segment, selection for high yield will involve selection for a genetic background that accommodates the alien segment. K. W. Shepherd (personal communication) is examining this concept with spontaneously arising transfers of segments of rye chromosome 1R carrying wheat stem rust resistance and I am attempting to select an accommodating genetic background for the Transec translocation by this method. In the latter study 950 F_5 (or F_7) selections are being subjected to replicated yield testing in the 1980 season.

If these experiments prove to be successful they will be of some significance, as this may define the special treatment that can or must be applied to alien transfers to ensure their use in agriculture. It will be particularly important if high-yielding types are found that on further crossing provide high-yielding segregants in reasonably high frequencies. Special handling of that particular translocation may then not be necessary in further crosses. This would be more likely to arise if the improvement were due to an infrequent change in the alien segment itself or a

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simply inherited, but major, change in the genetic background, such as on a homoeologue of the translocation chromosome.

Alien transfers of the type being discussed may also come into greater prominence if hybrid wheat becomes significant. If an alien segment is present in only one parent, it is present in single rather than in double dose in the hybrid, and the replaced wheat segment is reintroduced (in single dose) by the other parent.

Despite these reasons why translocations induced by ionizing radiation may be more successfully used in the future, production of further transfers will be predominantly, if not entirely, engineered by way of *ph* mutants.

Manipulations involving chromosome 5B

The important role of chromosome 5B in control of recombination is discussed elsewhere (Riley *et al.*, this symposium); thus only one aspect will be considered here, namely the manipulations associated with the use of the *ph* mutants isolated by Riley (1968) and Sears (1977). It is expected that use of a *ph* mutant will result in more precise exchanges in terms of being homoeologous exchanges.

The extent of homoeologous pairing of these mutants has been examined mathematically (Driscoll *et al.* 1979, 1980; Driscoll 1979) and in one case (Sears 1977) the degree of pairing in an intergeneric hybrid exceeds that of the same hybrid involving nullisomic 5B.

Sears (1980) has outlined a method in which the exchange is also more precise in the length and positioning of the alien segment in terms of being short and intercalary. The method involves crossing a monosomic 5B, ditelosomic alien substitution line with a homozygous *ph* line. Offspring with 41 chromosomes involve homoeologous pairing, and the alien telochromosome and its wheat homoeologue that it had replaced are devoid of homologues and are therefore available for homoeologous exchange. There are two types of exchange chromosomes that bear the gene being transferred: an alien telocentric chromosome with a wheat segment distal to the gene, and a two-armed wheat chromosome bearing a terminal alien segment that carries the gene. These two types are specifically identified, after crossing, by the appropriate wheat ditelocentric stock, namely that involving the wheat arm involved in the exchanges. Furthermore, the extent of telocentric pairing in this generation serves as a guide to the length of translocated segment. Intercrossing lines that carry the two types of exchange brings together two chromosomes with a short alien segment in common (see figure 1). Exchange in this segment produces a chromosome with an intercalated alien segment bearing the gene being transferred.

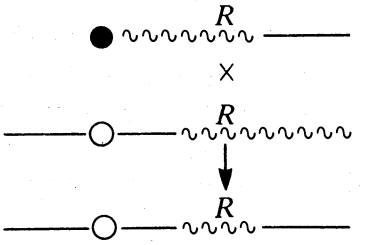

Figure 1. Crossing over between the two types of exchange chromosomes to produce the intercalated chromosome. *R* is the gene being transferred (after Sears 1980).

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Manipulations involving chromosome 4A

Chromosome 4A of *Triticum aestivum* and *Triticum durum* Desf. has a number of interesting features. Sears (1966) observed that all three nullisomics of homoeologous group 4 in hexaploid wheat are male sterile and generally non-vigorous. Further, nullisomic 4A tetrasomic 4B (or 4D) is quite normal in all respects except that it is male sterile, whereas the reciprocal, nullisomic 4B (or 4D) tetrasomic 4A, is normal in all respects including male fertility. Hence 4A has the gene(s) for male fertility that both 4B and 4D have and in addition it has a gene(s) for male fertility that neither 4B nor 4D have.

Secondly, 4A of hexaploid wheat is unusual in its pairing relationship with diploid *Triticum* species. Chapman *et al.* (1976) observed no pairing of the telocentric chromosomes in hybrids of double ditelocentric 4A of hexaploid wheat × *T. urartu* Thum. Telocentrics of the other six A-genome chromosomes of hexaploid wheat paired with chromosomes of *T. urartu*. Similar observations were made by Dvořák (1976), who employed ditelocentric 4Aα. Earlier, Chapman & Riley (1966) reported 48 % pairing of telocentric 4Aα in crosses to *T. thaoudar* Reut. However, K. W. Shepherd (personal communication) observed no pairing of telocentrics 4Aα and 4Aβ in crosses with *T. thaoudar*, *T. boeticum* Boiss., *T. aegilopoides* Bal. and *T. monococcum* L. Further, M. D. Hossain (personal communication) observed no pairing of the entire 4A of hexaploid wheat in crosses to *T. monococcum*. In this case, 4A of hexaploid wheat was recognized by means of N-banding (Gerlach 1977; Jewell 1979). From the above observations it can be concluded that 4A of hexaploid wheat originated elsewhere than from diploid wheat or it has been modified so dramatically it no longer pairs with, or is seen paired at metaphase I with, a chromosome of diploid wheat (presumably this can be referred to as chromosome 4 of diploid wheat as the other six chromosomes pair with the other six chromosomes of the A genome of hexaploid wheat).

Thirdly, the N-banding pattern of chromosome 4A makes it conspicuously different from the other six chromosomes of the A genome. Gerlach (1977) demonstrated that in hexaploid wheat all seven chromosomes of the B genome are heavily and distinctly banded, chromosome 7A has faint banding and chromosome 4A is heavily banded. Chromosome 4A has heavy banding on each side of the centromere and a subterminal band in the β arm. The same pattern of 4A is seen with polypyrimidine–polypurine satellite *in situ* hybridization (Dennis *et al.* 1980). Chromosome 4 of diploid wheat is not banded (Gerlach 1977). This evidence, in conjunction with that presented earlier, leads one to conclude that it is most likely that chromosome 4A of hexaploid wheat was derived elsewhere than from diploid wheat.

A number of manipulations of chromosome 4A have been carried out with interest in male sterility for plant breeding purposes. The male sterility of nullisomic 4A is due to loss of a gene or genes on the α arm, since ditelocentric 4Aα is fertile and ditelocentric 4Aβ is male sterile. There are five chromosomes of hexaploid wheat, namely 4A, 4B, 5A, 5B and 5D, of which the nullisomic is male sterile, one telocentric is male sterile and the other telocentric is male fertile (E. R. Sears, personal communication). These five were chosen for an induced-mutation study to isolate a recessive mutation for male sterility.

Euploid pollen was subjected to 250 or 500 R† of γ-rays or X-rays, at about the time of pollen shedding, and applied to emasculated monosomics 4A, 4B, 5A, 5B or 5D (Driscoll & Barlow 1976). Approximately three-quarters of the F_1s will not receive the monosome from the

† $1\ \mathrm{R} = 2.58 \times 10^{-4}\ \mathrm{C\ kg^{-1}}$.

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female parent, and if in these cases a recessive mutation is induced in the corresponding chromosome of the pollen, the recessive mutation will be expressed in the F_1. Of 795 F_1 plants (62 of which involved monosomic 4A as female parent) produced in this way, one male-sterility mutant was isolated, confirmed and maintained (Driscoll 1976*b*). This mutant has been registered as 'Cornerstone' (Driscoll 1977*a*) and has been comparatively analysed with two other recessive male-sterility mutants, referred to as 'Pugsley's' male sterile (Pugsley & Oram 1959) and the 'Probus' male sterile (Fossati & Ingold 1970). The three recessive mutants are allelic and are located on chromosome arm 4Aα (Driscoll 1975*b*; Barlow & Driscoll 1980). The Probus and the Cornerstone mutants are located at least 50 map units from the centromere. Each of these two mutant chromosomes when paired with the telocentric 4Aα undergo considerable desynapsis (Barlow & Driscoll 1980).

One other aspect of these studies is important. Progenies resulting from crossing monosomic 4B with the Probus (or Cornerstone) heterozygote contain individuals with an intermediate phenotype, in which many anthers fail to dehisce but others liberate functional pollen. These plants are regarded as being monosomic for chromosome 4B and heterozygous for the 4A mutant. The fact that the intermediate type was observed with the two mutants induced with ionizing radiation (Probus and Cornerstone) and not with the spontaneous mutant (Pugsley's) is interpreted as indicating that 4Aα bears two genes for male fertility, one of which is duplicated on 4B, and that both genes are deleted in the Probus and Cornerstone mutants but only the duplicated 4A gene is changed in the case of the spontaneous Pugsley's mutant. This is consistent with the observations on the nullisomic–tetrasomics of homoeologous group 4 (Sears 1966) mentioned above.

It is significant that the first three male-sterility mutants studied in hexaploid wheat fail to complement one another. The unduplicated 4A gene for male fertility is more likely to give rise to detectable mutations. However, it should be noted that a large number of male-sterility mutants have been induced with ethyl methanesulphonate by Franckowiak *et al.* (1976). These involved four non-allelic recessive mutants (Sasakuma *et al.* 1978). These mutants may be modified alleles that actively compete with their homoeoalleles. A similar situation has been experienced with chlorophyll mutants in hexaploid wheat (Sears & Sears 1968; Pettigrew & Driscoll 1970). In this case, almost no chlorophyll mutants arise with ionizing irradiation, which may predominantly induce deletions; however, chemical mutagens induce mutants, such as chlorina mutants, that involve intragenic changes.

The Cornerstone mutant is being used in composite crosses to render the wheat plant open-pollinating for a series of generations (Driscoll 1977*b*, 1980).

What may prove to be of greater significance are the attempts that are being made to use Cornerstone in production of hybrid wheat. Hybrid wheat production by using cytoplasmic male sterility, specifically *Triticum timopheevi* Zhuk., as described by Schmidt *et al.* (1962), has essentially been discontinued because of difficulties in fertility restoration. An alternative method of producing hybrid wheat, described by Driscoll (1972), involves a chromosomal male-sterility mutant and an additional chromosome that bears a gene(s) for fertility restoration and, secondly, does not pair at meiosis with the other chromosomes.

Cornerstone is suitable for this system; however, isolation of the additional chromosome with the two characteristics mentioned above is proving to be difficult. Similar to the nullisomic–tetrasomic situation with chromosome 4A, as mentioned above, substitution of an alien chromosome for chromosome 4A can give rise to a vigorous but male-sterile line. For example,

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substitution of chromosome 4E of diploid *Agropyron elongatum* for 4A results in this type of individual (Dvořák 1980). Thus it is assumed that an alien chromosome of this type will not restore fertility to the Cornerstone mutant, which is regarded as being a terminal deletion of chromosome arm 4Aα (Barlow & Driscoll 1980). There is one *Aegilops* chromosome that substitutes successfully for chromosome 4A in that it results in a male-fertile substitution line. This is chromosome $4S^1$ of *A. longissima* S. & M. as demonstrated by Maan (1975, 1976, 1980); however, this chromosome has a most peculiar characteristic: when present monosomically, male and female gametes not bearing the *Ae. longissima* chromosome are precluded from functioning. This behaviour has also been observed with a chromosome of *Ae. sharonensis* Eig by Maan (1975) and Chapman & Miller (1978); however, this may be the same chromosome, namely $4S^1$, as both *Ae. longissima* and *Ae. sharonensis* are regarded as having the same genome, i.e. S^1.

This gametocidal behaviour is not, however, unique to group 4, since a group 3 chromosome of *Ae. triuncialis* L. var. *triuncialis* (genomes CCC^uC^u), of *Ae. caudata* L. (CC) and of a synthetic *Ae. triuncialis* obtained by crossing *Ae. caudata* × *Ae. umbellulata* (C^uC^u) has a similar property (Endo & Tsunewaki 1974; Endo & Katayama 1978; Endo 1978). This chromosome, which is most probably chromosome 3C of *Ae. caudata* and substitutes well for 3A, 3B and 3D, but unsuccessfully for 4A, is different from the previous case in that its gametocidal action is partial rather than absolute (Endo 1978).

Endo (1980) also reports a gametocidal action of a chromosome of *Ae. cylindrica* Host (CCDD). It is also reported that although this chromosome is probably a C-genome chromosome it may not be the same chromosome as the one described in *Ae. triuncialis*.

As (partial) gametocidal action of chromosomes other than group 4 chromosomes have been recognized, the gametocidal action and the fertility restoration of $4S^1$ probably result from two distinct genetic activities. Therefore the two activities may result from two genes (or two groups of genes) that are separable. Isolation of a $4S^1$ chromosome that restores the fertility but does not have the gametocidal activity would provide an alien chromosome useful for the production of hybrid wheat by the method mentioned above. Isolation of a $4S^1$ chromosome with these properties could be attempted by an appropriately designed irradiation experiment; alternatively, it may occur naturally, and perhaps further accessions of *Ae. longissima* and *Ae. sharonensis* should be screened for this purpose.

If a fertility-restoring, non-gametocidal $4S^1$ chromosome (I shall refer to it as chromosome β) is isolated, the isolation of the reciprocal type, i.e. a gametocidal, non-restoring $4S^1$ chromosome (chromosome α) would allow the examination of an alternative method of producing hybrid wheat, which even though no abnormal cytoplasm is involved, is quite similar to the cytoplasmic system of producing hybrids. A further requirement would be the absence of crossing-over between the fertility-restoring and the gametocidal genes. A line with a pair of α chromosomes would be sterile and equivalent to an A line of the cytoplasmic system (Jones & Davis 1944). A line with one α and one β chromosome would be fertile and equivalent to a B line. The αα and αβ lines could be grown in mixture and only α-bearing male and female gametes would function and the mixture could be harvested in bulk to give only αα types. This would have advantages in pollen distribution. Maintenance of the αβ line, incidentally, would require pollination of an αα line with a ββ line, the latter being a self-reproducing type. The unusual character about the αα type, which could be produced in bulk, is that it carries two doses of a gametocidal chromosome. Hence the male parent of the hybrid production would also have to be αα type or else the hybrid crop will be an α type with the majority of eggs non-functional. Hence the hybrid

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crop would have to include a pair of α chromosomes, and this may negate any potential hybrid vigour. Thus, perhaps, isolation of an α chromosome and use in the XYZ system (Driscoll 1972) would be preferable.

Four other possibilities are being examined for use in the XYZ system. The first is the Transec chromosome. It involves a segment of rye chromosome 2R translocated to the β arm of 4A and located one map unit from the centromere (Driscoll & Bielig 1968). In crosses to Cornerstone the two 4A chromosomes are paired at metaphase I of the F_1 in only 3% of pollen mother cells (Barlow & Driscoll 1980). A line was produced that possessed two doses of Cornerstone 4A and one dose of Transec 4A, and this line involved trivalent formation at metaphase I in only 1% of pollen mother cells. This seemed low enough for this to be a successful Y line. However, from a linkage study between the Cornerstone and Transec 4A chromosomes, considerable crossing-over occurs between these chromosomes. In fact the rye segment on the β arm and the male-sterility deletion map 20 crossover units apart (Barlow & Driscoll 1980). Obviously, considerable desynapsis as well as asynapsis is involved in this case and these have been estimated at 37% and 60% respectively, from a formula derived by Driscoll (1978*b*). The desynapsis of this bivalent is due to the abnormality of both pairs of ends of this bivalent: the α end is heterozygous for the Cornerstone mutant, which is presumably a terminal deletion, and the β end is heterozygous for 2R and 4A segments. Considering that the 2R segment maps one crossover unit from the centromere, the 20% crossing-over in this bivalent presumably almost all occurs in the α arm.

Attempts are being made with the use of X-rays to reduce this pairing in the α-arm. The Transec chromosome has been irradiated (250 R at the time of pollen shed) and applied to ditelocentric 4Aα. The irradiated Transec 4A chromosomes are being scored for their abilities to remain paired until metaphase I with telocentric 4Aα, rather than with the Cornerstone 4A, because the non-irradiated Transec 4A seldom remains paired with the Cornerstone 4A and any reduction in zygotene pairing would not be detected in such a test. Of 36 irradiated Transec 4A chromosomes, 34 were observed at metaphase I to be paired with telocentric 4Aα with normal frequency (i.e. 78% paired) and 2 paired less frequently, namely 6/23 (26%) and 6/18 (33%). The last two have been selfed to produce lines that possess a pair of irradiated-Transec 4A chromosomes, to test for retention of the male fertility genes. These lines are also being crossed to Cornerstone to determine if their modified chromosome pairs negligibly with Cornerstone 4A. Of course this will have to be determined by a linkage study of the wheat leaf rust resistance gene carried by the 2R segment and the male-sterility allele, since the non-irradiated Transec and Cornerstone 4A chromosomes are seen paired at metaphase I only rarely and observations on the earlier stages of meiosis are too difficult.

The second source of fertility restoration that is being examined is cereal rye (*S. cereale*). The Cornerstone mutant has been transferred to the tetraploid level by backcrossing to *T. durum* and the hexaploid and tetraploid sterile stocks incorporated into octoploid amphiploids and hexaploid amphiploids, respectively, with rye. Both amphiploids have normal anthers and liberate normal pollen (Hossain & Driscoll 1980). Thus the entire rye genome is capable of restoring male fertility. These amphiploids do not have full seed set; however, many amphiploids of these types that do not involve a male-sterility mutation do not have full seed set. The heptaploid containing a single dose of the rye genome added to the hexaploid, homozygous for the male-sterility mutation, is also male fertile; thus the rye genome in single dose is capable of restoring fertility. It is not surprising that this is so; presumably all higher plants, or at least

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those of a restricted taxonomic group, construct functional anthers and pollen grains in similar, if not identical, ways. The differences could involve the packaging of the genes, as to whether a given pair or group of genes are linked in one species but not so in another species. The packaging in rye with respect to the genes on 4Aα, that have been deleted in Cornerstone, is being analysed by backcrossing the heptaploid to hexaploid Cornerstone and analysing the BC_2 progeny for rye-chromosome content and for male fertility or sterility. The identity of the rye chromosomes is being ascertained by C-banding (Gill & Kimber 1974) and the electrophoretic analysis of esterases and alcohol dehydrogenases, which are used as markers for chromosomes 3R and 4R, respectively (Barber *et al.* 1968; Tang & Hart 1975).

Although analysis of this BC_2 family is incomplete, a number of findings have already emerged (M. D. Hossain & C. J. Driscoll, unpublished). The possibility that 5R may carry the restorer gene(s) for the Cornerstone 4A mutation had its basis in the observation that the spontaneous wheat line W70a86 (Blaukorn) is male fertile and involves substitution of rye chromosome 5R for wheat chromosome 4A (Zeller & Baier 1973). However, isolation of a BC_2 plant with 5R added to Cornerstone is male sterile; thus W70a86 (Blaukorn) may be male fertile for a reason other than the presence of 5R, or the Blaukorn 5R is a distinctive one.

On the positive side, it is significant that rye chromosome 2R added to Cornerstone results in partial fertility (51 % seed set in primary and secondary florets). It was proposed earlier that Cornerstone involves the deletion of two genes for male fertility, only one of which is duplicated on the 4B and 4D homoeologues. Perhaps a gene comparable with the unduplicated gene on 4A is present on rye chromosome 2R. This chromosome is also related to chromosomes 2A, 2B and 2D of hexaploid wheat (Sears 1968). Interchange of chromosomes 2A, 4A and 6B was postulated by Sears (1966) on the basis of incomplete compensation in particular nullisomic–tetrasomic lines. This strongly suggests that 4A of hexaploid (and tetraploid) wheat arose from a chromosome that also has a relation with homoeologous group 2 of hexaploid wheat. This, and the fact that chromosome 4A of hexaploid wheat N-bands in a manner similar to a B-genome chromosome, suggests that the A genome of hexaploid wheat has a complex origin that includes diploid *Triticum* and at least one other genus, with chromosomes that heavily N-band.

Perhaps a second rye chromosome that will partly restore fertility will occur in the BC_2 population referred to above. This seems particularly likely when one considers that Koller & Zeller (1976) reported 4 and 19 % fertility restoration in monosomic 4R substitution for 4A and ditelosomic 4RS substitution for 4A, respectively. If two rye chromosomes are involved in restoring fertility it may be possible to combine their partial restoring activities into one chromosome by centric fusion (Sears 1972). The homoeology of a segment of 2R and a segment of 4A allows the possibility that the exchange between these two chromosomes in the Transec chromosome is a segmental homoeologous one.

It would, of course, be easier to find a chromosome 'off the peg'. One possibility of this is the third chromosome that is being examined in this context, namely chromosome 4 of barley (*H. vulgare*). Crosses of common wheat with cultivated barley and production of six of the possible seven addition lines have been achieved by Islam *et al.* (1981). Using N-banding of Gerlach (1977), Islam (1980) identified his addition line A as involving barley chromosome 4, as numbered in the trisomic series by Tsuchiya *et al.* (1960). Barley chromosome 4 was found to produce an alcohol dehydrogenase that is electrophoretically different to those produced by the group 4 chromosomes of wheat (Hart *et al.* 1980).

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An addition line with a pair of barley chromosome 4, including one chromosome with a markedly different arm ratio, was isolated and crossed to monosomic 4A. In a subsequent generation, monitoring of alcohol dehydrogenases led to the isolation of a disomic substitution line in which 4A is replaced by a pair of barley 4 with the modified arm ratio. This disomic substitution line is male fertile (A. K. M. R. Islam and K. W. Shepherd, personal communication). Work is under way to determine whether a normal barley 4 will also produce a male-fertile 4A substitution line. Thus barley chromosome 4, or perhaps more specifically the structurally modified barley chromosome 4, may prove to be a useful chromosome for the XYZ system. Development of X and Y lines are now in progress.

The fourth chromosome that is being examined for fertility restoration came into consideration because of a happy accident. In attempting to transfer the Cornerstone mutant for male sterility from tetraploid wheat to diploid wheat, male-sterile tetraploid was pollinated with *Triticum monococcum*. The F_1 was treated with colchicine in order to increase the mitotic index in root tips; however, it also had the additional, unplanned effect of producing a doubled sector consisting of two entire spikes. These contained genomes **AA**AABB, where the bold **A** is from *T. monococcum*. This doubled sector was fully fertile (M. D. Hossian & C. J. Driscoll, unpublished). The point of interest is that in the undoubled F_1 (i.e. **AA**B) only six of the seven **A**-genome chromosomes pair with A-genome homologues, N-banding these meiotic figures reveals that chromosome 4A of the A genome is unpaired. Thus chromosome 4 of the **A** genome does not pair with the chromosomes of hexaploid wheat. If full fertility is restored by genes on chromosome 4 of the **A** genome, it will be a useful chromosome for this system. *T. monococcum* is being crossed to hexaploid Cornerstone with the purpose of producing a chromosome 4 addition line on a male-sterile background.

In time, a suitable fertility-restoring chromosome will be isolated, and it may well come from one of the four mentioned above. This research is being persevered with because of two special features of producing hybrids in this way. First, the normal cytoplasm would be involved in the hybrid crop and, secondly, no alteration, in terms of sterility–fertility, is made with the male parent of the hybrid. Once X, Y and Z lines are produced in a given genotype, that genotype can be readily tested with a large number of other genotypes to search for combinations that have sufficient heterosis for yield.

I have discussed chromosome manipulations in wheat and a number of its relatives with the aim of introducing alien genes to wheat or to produce hybrid wheat. Many other examples with different species and different objectives could have been assembled. The examples chosen do, however, illustrate one very important concept, and that is that advanced manipulation of chromosomes depends heavily on a basic knowledge of the relations between chromosomes. This knowledge is well advanced with wheat, which reflects the fact that many aneuploids are available within this species. The examples given reflect in a general way the extent of development of the manipulation of chromosomes of higher plants for purposes of plant breeding.

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Discussion

T. E. Miller (*Plant Breeding Institute, Cambridge, U.K.*). Professor Driscoll has shown that chromosome 4A in hexaploid wheat may not be an A genome chromosome from the diploid wheats, and recent evidence at the Plant Breeding Institute would support this. Does Professor

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Driscoll consider that we should therefore question the integrity of genomes and hence their evolutionary origin in the polyploid cereals, particularly the B genome of wheat?

C. J. Driscoll. From the data discussed, it appears that the origin of the A genome of hexaploid wheat involved more than one diploid species. Diploid wheat apparently contributed five or, more likely, six chromosomes; however, chromosome 4A seems to have been derived from another species. Thus both the A genome and the B genome of hexaploid wheat appear to have had a complex origin.

Postscript. Prospects for hybrid wheat may be greater than is commonly envisaged. Most studies on heterosis in wheat have involved the *T. timopheevi* cytoplasm, which may negatively contribute to yield. Hybrid wheat based on a chromosomal male sterile will involve *T. aestivum* cytoplasm. Another advantage of the chromosomal system is that the genetic modifications for both the sterility and the fertility restoration are in the female parent. As the male parent is normal in all respects, one female parent can easily be tested with many male parents for levels of heterosis. Greater flexibility of the male parent allows greater scope in disease resistance gene management, in that different genes can be incorporated in the male parent for different years. Even though it may be possible to isolate homozygotes with equal yield to the hybrid, this involves an uncertainty of detection and considerable time. However, the genetic components for a chromosomal system have not as yet been isolated.

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