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Identification of Rye Chromosomes: the Giemsa Banding Pattern and the Translocation Tester Set

J.M. de Vries and J. Sybenga

Dept. of Genetics, University of Agriculture, Wageningen (The Netherlands)

Summary. The Giemsa banding pattern is given for eleven reciprocal translocations of rye, *Secale cereale* L., together involving all chromosomes at least once, and one telocentric substitution. It is possible to correlate the identification system based on the Giemsa pattern with that based on the translocation tester set. The location of the translocation break points could be determined very exactly for a number of translocations, somewhat less exactly for others. The variations in the banding pattern, resulting from genetic, environmental and technical variation, make definite identification with the nomenclature system of the different rye additions to wheat difficult. An attempt is made, but some caution is necessary.

Introduction

The Giemsa banding technique has not been as successful in plants as in animals. This is probably mainly due to the structure of the cell boundary and the constitution of the cytoplasm and perhaps also to differences in the chemical composition of the chromosomes. Yet several instances have been reported of successful identification of chromosomes, and even short segments of chromosomes, on the basis of Giemsa banding. One example is rye, which takes an intermediate position in banding pattern: it does not have the detailed pattern of mouse and man, but it does have several characteristic large bands (predominantly at the chromosome ends) and a number of smaller and very small bands, some of which are also quite characteristic. Because of the great interest in rye cytogenetics, several authors have attempted to develop new, or to adapt existing, techniques of Giemsa banding to rye. There appears to be considerable variation in the results, which may be due to genetic differences between the materials used, differences in the biochemical or physiological condition of the tissues worked with and to slight, or occasionally considerable, differences between the techniques applied. It is usually difficult to decide which factor has operated in each case where a different outcome is observed. There is clearly a genetic factor, but even with the same material grown under apparently the same conditions and with, as far as can be judged, the same techniques, rather variable results may be obtained. Although the most characteristic terminal bands tend to be rather constant (with a few exceptions), as are a few large and small interstitial bands, many of the smaller bands are variable in expression. Proximal bands appear quite clearly in some instances, but often do not appear at all.

There are four main fields of interest in Giemsa banding of rye chromosomes for identification purposes:

a) Identification of all individual chromosomes in the normal rye complement (Sarma and Natarajan 1973; Verma and Rees 1974).

b) Identification of chromosomes, chromosome arms or even smaller segments in structural (translocation etc.) and numerical (trisomic) variants.

c) Identification of individual rye chromosomes and their possible structural rearrangement in addition lines of rye, primarily to wheat (Darvey and Gustafson 1975; Gill and Kimber 1974).

d) Tracing the fate of individual rye chromosomes in interspecific hybrids and amphidiploids, mainly Triticales (Darvey and Gustafson 1975; Merker 1973).

Analysis of the Giemsa banding pattern of rye is also interesting for comparisons with wild *Secale* species in studies of the evolution of the genus.

We have attempted first to describe again the normal genome of rye, secondly to locate the breakpoints of the translocations in the tester set, and thirdly to correlate the nomenclature of rye chromosomes as based on the translocation tester set of Sybenga and Wolters (1972) with that based on the additions to wheat. This is of special interest, since in the latter the naming of the rye chromosomes has been made to correspond with that of the wheat homoeologous groups.

Materials and Methods

In addition to the normal complement, eleven reciprocal translocations, all except a few described earlier as being part of the "tester set" of Sybenga and Wolters (1972), two telocentric substitutions and the standard B-chromosome were analysed. The material was derived from the combination of different inbred and outbred lines with a preponderance of Petkus origin, and was mainly of spring type. Of the rearrangements, homozygotes as well as heterozygotes were studied. The few normal types studied were derived from the same general genetic population as the translocations.

The Giemsa technique used is a variant of the techniques reported by several authors. Seedlings were germinated in petridishes for 36 hrs (adapted from Wolff and Luippold 1956). This was followed by a 3 hrs treatment in 0.5 % colchicine, fixation in 1:3 acetic alcohol for 5(-15) min., 45 % acetic acid for 30 min., and 5 % cellulase + 5 % pectinase for 2 - 4 hrs. Squash preparations were made in 45 % acetic acid, using egg-albumen-glycerine coated cover slips. The slides were made slightly greasy. After removing in absolute alcohol the coverslips were dried overnight in open air at room temperature. The next day a 4 1/2 min. $Ba(OH)_2$ (air-free, saturated) treatment at 23.5 °C was followed by thorough rinsing in running water.

Subsequently the coverslips were treated in 2×SSC at 60 °C (1 h.) and then cautiously rinsed in gently running water. After drying in air the preparations were stained in 1 % Giemsa (Gurr improved R66) for 15 min. at pH 6.8 with occasional stirring of the solution. Differentiation was accomplished by a 15 min. rinse in gently stirred buffered (pH 6.8) water. After drying, via a xylene series, permanent slides were made in Euparal.

This technique, although somewhat more discriminative, resembles the C-banding techniques of animal cytology, which are considered to stain principally the constitutive heterochromatin. This may be one reason why finer bands are less clearly stained than the large bands and why in plants, the banding patterns are in general not as detailed as those in animals produced with the G-banding techniques involving trypsin-SSC treatments. In plants, the trypsin treatment is not effective.

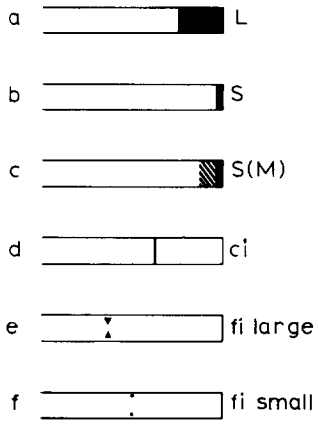

Fig. 1. Classification of bands (compare Figs. 2, 3, 4).
a: Constant terminal band, large size (L)
b: Constant terminal band, small (S)
c: Terminal band varying between small (S) and medium (M)
d: Constant interstitial band of small size (ci)
e: Facultative interstitial band, relatively large (fi)
f: Facultative interstitial band, small (fi)

Results

The Bands (Figs. 1,2)

a) Centromeric (proximal) bands appeared infrequently with our technique and, when they appeared, were similar for all chromosomes. As these bands did not contribute to the identification of particular chromosomes, no attempts were made to improve their intensity and/or the frequency of their appearance.

b) Distal bands were very characteristic and were the main means of recognizing chromosome arms. They varied from absent to large, and an arbitrary scale of ten units was introduced to describe them ($0, 0^+, S^-, S, S^+, M^-, M, M^+, L^-, L$). Some bands were much more constant than others, but in general only a few were really variable in size. In the drawings the area over which these varied has been shaded. Some very small bands could disappear in weakly stained cells.

c) "Constant" interstitial bands (ci). Except for a few, these bands tended to be small. Their frequency was low. They were quite characteristic for the segments in which they occurred.

d) "Facultative" interstitial bands (fi). Small, often somewhat indistinct bands appearing rather infrequently. In some cells their frequency could be relatively high, but generally there was little system and correlation in their appearance. A few were sufficiently characteristic to aid identification of specific chromosome segments.

The normal karyotype (Fig. 2)

The classification is that of Sybenga and Wolters (1972). Since, in fact, the definite classification has been made with the aid of the translocations and telocentric substitutions discussed in the next section, it would seem logical to consider the normal karyotype after the translocations. To simplify the discussions, however, it was decided to start with a description of the normal karyotype.

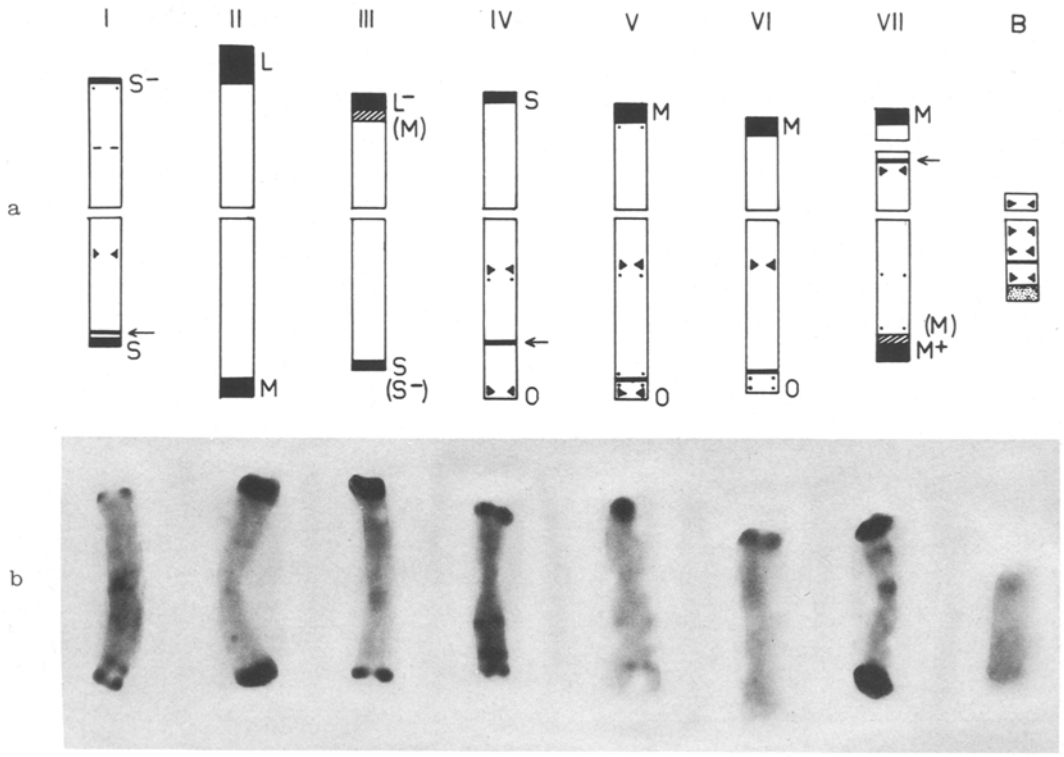

Fig.2. The Giemsa banding pattern of the rye genome
a: Diagrammatic
b: Photomicrographs of the actual chromosomes

Chromosome I: metacentric; arm length ratio 1.0 - 1.1. Small terminal band on both arms, one (S) sligthly larger than the other (S^-). Narrow, constant interstitial band (ci) close to larger terminal band. Single facultative narrow interstitial band (fi) in both arms; that in arm with smaller terminal band more proximal than the other.

Chromosome II: metacentric; arm length ratio 1.1 - 1.2. Large terminal band in one arm, medium (M) band in other. No further features ever observed.

Chromosome III: submetacentric; arm length ratio 1.2 - 1.3. Somewhat variable, medium to large ($M - L^-$) band in shorter arm, short ($S^- - S$) band in longer arm. No further clear discriminating features.

Chromosome IV: submetacentric, arm length ratio 1.4 - 1.6. Small terminal band (S) in shorter arm, no terminal band (0) in longer arm. Clear narrow, constant interstitial (ci) band in long arm. Facultative double interstitial (fi) bands subterminal and at 1/3 from centromere, again in long arm.

Chromosome V: subacrocentric, arm length ratio 1.6 - 1.8. Medium terminal band (M) in short arm, and very small terminal band (0^+) in long arm. Narrow, constant interstitial (ci) band near end of long arm. Facultative but often rather conspicuous (fi) band near middle of long arm. Small facultative (fi) often conspicuous bands distally almost terminally, especially in long arm, less frequent distally in short arm.

Chromosome VI: subacrocentric, arm length ratio 1.8 - 2.1. Banding pattern very similar to that of chromosome V, but with somewhat less frequent and less conspicuous facultative bands, particularly in distal portion of long arm. Terminal bands in long arm never observed.

Chromosome VII: (satellite chromosome): submedian, arm length ratio 1.2 - 1.3. The only chromosome consistently recognizable without the aid of banding. Medium terminal band (M) in short, satellited arm, somewhat variable ($M - M^+$) medium terminal (M) band in long arm. Substantial, constantly appearing but slightly variable interstitial band (ci) just proximal to secondary constriction. Facultative small interstitial bands (fi) in central and distal positions in long arm and in central position in short arm.

Standard B-chromosome: small, acrocentric, arm length ratio 3.5 - 5.0. Large but (in our material) only rather light staining terminal band in long arm. No terminal, but one ci band in short arm. One constant, (ci) and two facultative (fi) narrow, rather light staining interstitial bands in long arm. Small, rather constant, light staining centromeric bands. The banding pattern is similar to that of all literature reports, but the staining intensity is rather low in our material.

On the basis of these descriptions it should be possible to correlate this classification with those given elsewhere in the literature, but the correspondence between the different reports is somewhat discouraging. Possible reasons have been discussed in the introduction. An attempt to correlate the different nomenclature systems will be made in the discussion.

Translocations and telocentric substitution

Two complete telocentric substitutions were available, for chromosomes II and VII. Since chromosome VII can be recognized unequivocally, only chromosome II will be considered. Since it was of special importance for the identifications, the telocentric substitution was identified as chromosome II by testing against the translocation tester set. Very consistently one of the two telo's carried a large terminal band, the other a medium band. In the normal set only one chromosome with this constitution can be distinguished, which, therefore, must be chromosome II.

Translocation 240 (chromosomes II and VI, Fig. 3^a): exchanged segments very different in length, resulting in recognizable translocation chromosomes. The small translocation chromosome necessarily contains the complete short arm of VI and at the end of the other arm has a translocated segment of II. In that particular arm a large terminal band is present, indicating which arm of II is involved. The other arm has a M terminal band, which (therefore) characterizes the short arm of VI. The large translocation chromosome carries a M band in the shorter arm, corresponding in length with the M-carrying arm of II. The longer arm lacks a terminal band, and must correspond distally with the long arm of VI. In the stock used very little could be observed of smaller interstitial bands. The breakpoints can, therefore, only be located approximately on the basis of length comparisons. Homozygotes for translocation 240 lack normal chromosome VI, and thus permit a description of V, which is the only subacrocentric chromosome left. Occasionally, chromosome IV may resemble V in shape, and thus homozygotes for translocation 240 can only help to describe V when some distinctive characteristics of IV and V are known.

Translocation 242 (chromosomes III and V, Fig. 3^b). Again, the translocation chromosomes can be recognized. The short translocation chromosome has one complete arm derived from V. It carries a clear M band, as present in the short arms of V and VI. The other arm of this chromosome is completely free of terminal bands. This is peculiar, since both arms of III carry a clear terminal band. It would suggest a "simple" translocation, but it is known that at meiosis the heterozygote forms ring quadrivalents in 10 % of the PMC's in some material, requiring the presence of a considerable exchanged segment. It is possible that part of the terminal band of III is transferred to the remnant of the long arm of V where it is not expressed as a band. In the long translocation chromosome the variable L terminal band of the shorter arm of III appears as a terminal band, but the S terminal band of the longer arm of III now appears as an interstitial band in the long arm of the large translocation chromosome. The end of this arm does not carry a band recognizable in the material studied; it is derived from the long arm of V. The constant and facultative interstitial bands of V reappear. The proximal facultative band in the long arm of V now is located in the short arm of the small translocation chromosome, close to the end. This shows that the breakpoint must have been just distal of this band. For this translocation, therefore, the breakpoints can be determined rather accurately.

Translocation 248 (chromosomes V and VII, Fig. 3^c). The exchanged segments are not very different, but since the satellite is clearly increased in size and the short arm of V has become just enough shorter to be recognized, the translocation and the arms involved can be recognized even in normal preparations. The exchanged segments each carry a M terminal band. This translocation together with 242 permits a good characterization of chromosome V (compare Fig.2). The location of the breakpoint can be determined rather exactly even without banding, which in this material did not add to the accuracy.

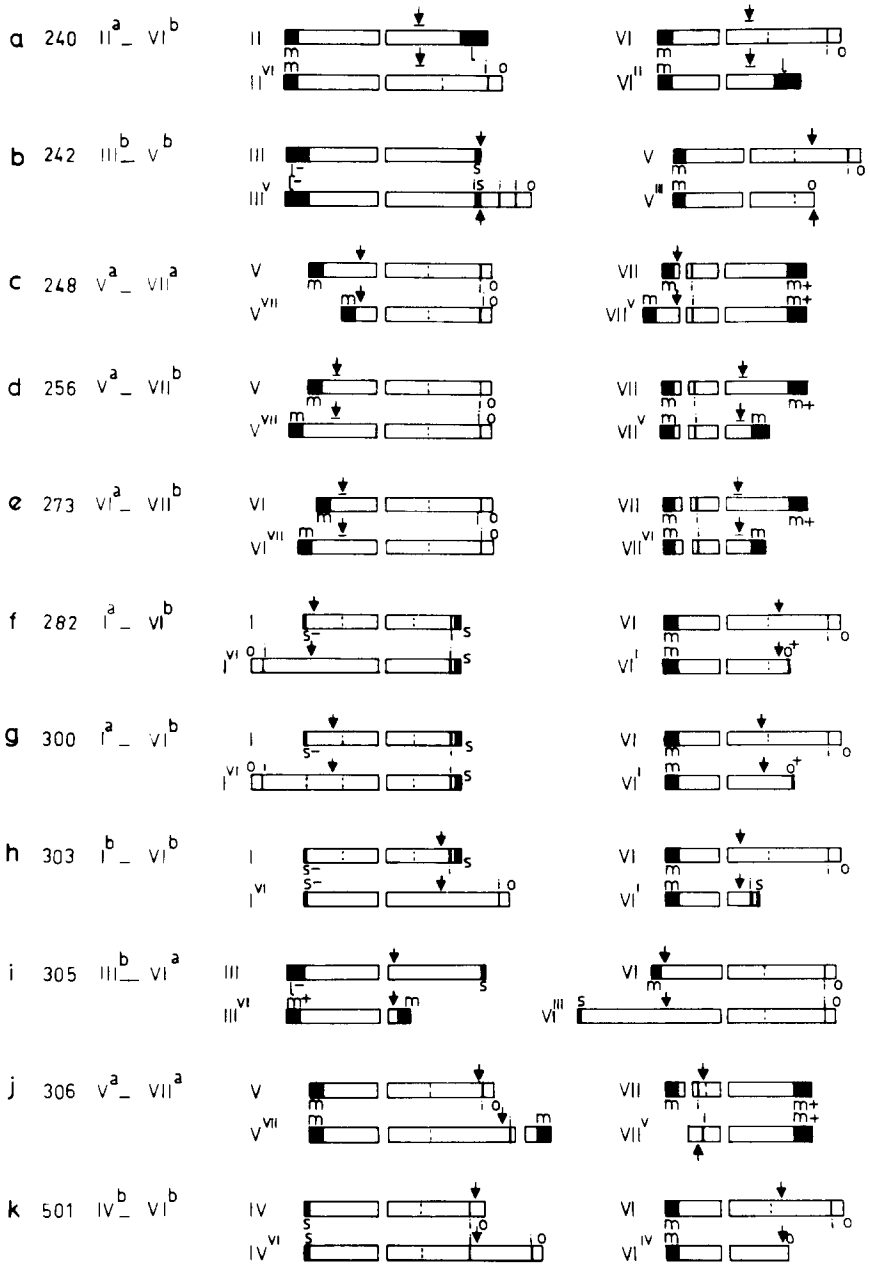

Fig. 3. Diagrams of the banding pattern for eleven translocations of rye, together with the normal chromosomes involved. The breakpoints are indicated by arrows

Translocation 256 (chromosomes V and VII, Fig. 3^{d}). The non-satellited, long arm of VII is involved and has become much shorter. The facultative small interstitial bands were too unclear in the material used to serve as a landmark for locating the breakpoint in this arm. The short arm of V is involved also, is considerably longer and now carries a M^{+} terminal band. This translocation also is useful for characterizing chromosome V. The breakpoint could not be located very accurately.

Translocation 273 (chromosomes VI and VII, Fig. 3^{e}) resembles 256, but now involves the short arm of VI in addition to the long arm of VII. It helps in characterizing VI, which closely resembles V, but does not yield much further information.

Translocations 282, 300, 303 (Figs. 3^{f,g,h}; 4^{a}) are an interesting set, since all involve chromosomes I and VI (the long arm of which is shortened). In particular, 282 and 300 are very similar in appearance, both involving the same arm of I. Translocation 303

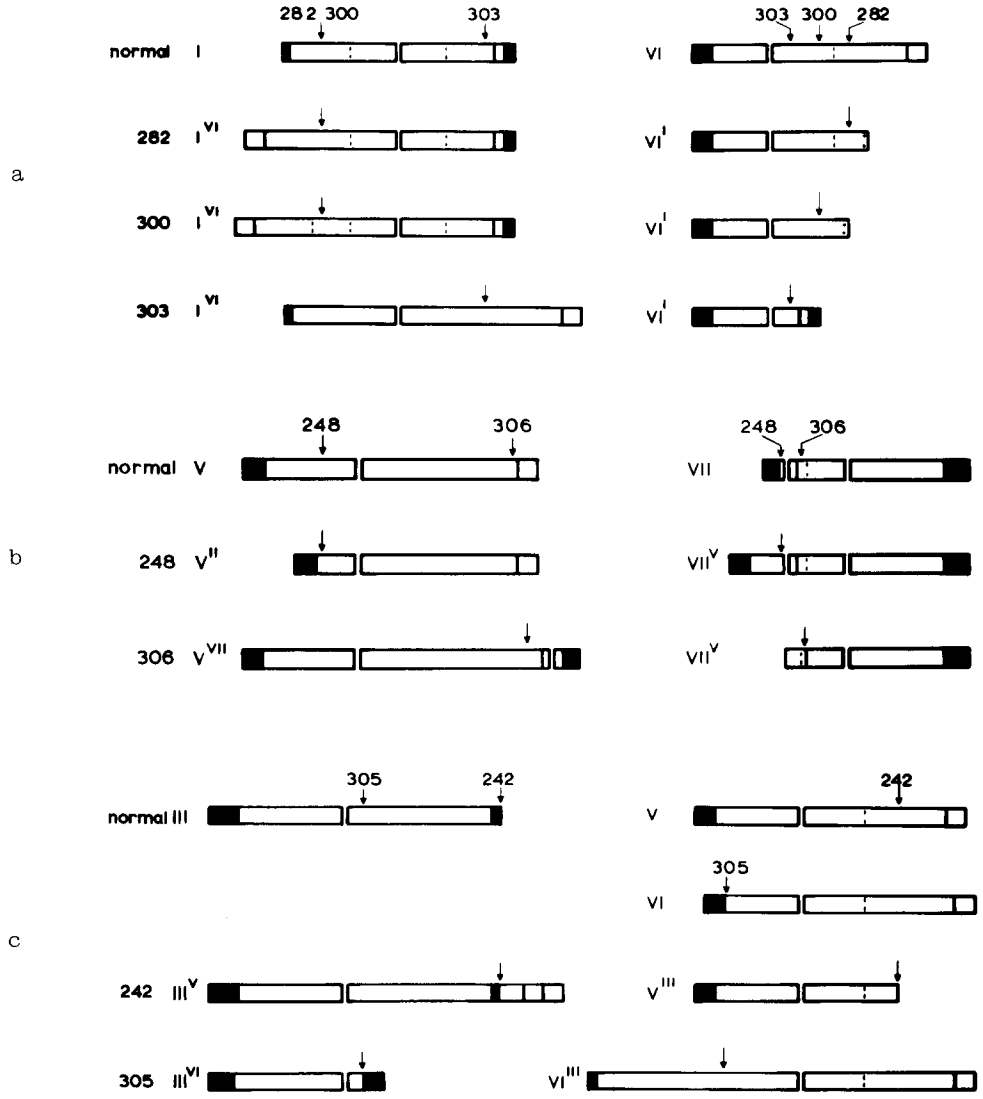

Fig.4. Comparisons of a number of translocations with breakpoints at different locations in the same chromosomes.
a: 282, 300 and 303
b: 242 and 305
c: 248

involves the other arm and also has a different appearance as the long arm of VI is even more reduced than in the other two. In 282 and 300 the translocation transfers the S terminal band of I to the (originally) long arm of VI. The segment lost from I must be small as VI^{I} is small. In both cases the S^{-} expression of this arm seems to be reduced to 0^{+}, but how consistent this is remains unclear. The segment removed from VI to I contains the interstitial band of VI in the case of 300, but not so for 282. Since the small VI^{I} chromosomes of both translocations are very similar, the I segment transferred to VI must be slightly larger for 300 than for 282. This leaves little room for variation, and thus the breakpoints are fairly exactly determined. In translocation 303, the breakpoint in the long arm of VI must be quite proximal, as this arm is very small after translocation, and it can be seen to contain the ci and S segments of the arm derived from I. The breakpoint in I must be rather distal. Although in the material used the interstitial bands in I and VI could not be clearly observed, because of the shortness of the translocation arm of the VI^{I} chromosome, the breakpoints can be fairly exactly located.

Translocation 305 (chromosomes III and VI, Fig. 3^{i}, 4^{b}). Unlike most translocations involving the subacrocentric chromosomes V and VI, in this case the short

arm of VI is involved. As in 242, the long arm of III is involved, but the breakpoint must be close to the centromere, as the translocated III^{VI} arm is quite small and still carries the entire M terminal band of the short arm of VI. For the same reason the breakpoint in the short arm of VI must be quite distal, just inside the terminal band. In the nontranslocated arm of VI^{III}, the normal ci band of the long arm of VI was clearly recognized.

Translocation 306 (chromosomes V and VII, Fig.3^{j}) involves the same chromosomes as 248 (Fig.3^{c},4^{c}) but differs from it conspicuously in shape. One breakpoint is in the short arm of VII, proximal to the satellite, transferring the satellite to V, where the breakpoint is in the long arm. The location of the breakpoints, curiously, presents some problems. An interstitial band is observed in the translocated V^{VII} arm, close to the satellite and too far out in this arm to be the constant interstitial band of V. It is smaller, however, than the band normally appearing near the secondary constriction in the satellite chromosome. A rather narrow interstitial band also appears in the translocated arm of VII^{V}, which is somewhat less distal than is normal for the ci band in the long arm of V, and also more proximal than the ci band in VII. The most probable conclusion is that the breakpoint is rather proximal in VII and that the i band of VII is transferred to V, where its expression is reduced. The terminal segment transferred from V to VII must then show an unusual extension.

Translocation 501 (chromosomes IV and VI, Fig.3^{k}) in appearance resembles 240, 242, 282 and 300. A large segment of the long arm of VI, including the subterminal ci band, is transferred to the long arm of IV. In IV, the breakpoint must be distal of the ci band, since this band appears in the large translocation chromosome. Where exactly in the long arm of VI the other breakpoint is located is not clear, as the facultative i band in VI did not show up in the translocation material used. From length comparisons, however, the location can be determined quite accurately.

Discussion

One of the purposes of this analysis was to correlate our rye chromosome nomenclature (based on arm length ratio and used in designating the chromosomes involved in the translocation tester set) with the nomenclature used in rye-wheat cytogenetics. In the latter case, the rye chromosomes are present as additions to wheat, and it is reasonably well established with which wheat chromosomes they are homoeologous. Thus, rye chromosome 1 is designated as R1 and is homoeologous to wheat chromosomes A1, B1, D1. Comparison of our results with those of Gill and Kimber (1974) and Darvey and Gustafson (1975) might thus make it possible to correlate the two systems of nomenclature. We will also consider the analysis by Verma and Rees (1974) of a *S. cereale* × *S. vavilovii* derivative, closely related to *S. cereale*.

It is clear that there are considerable differences. These may be due to variations in the techniques, differences in the condition of the material, differing expression of certain bands resulting from genotypic differences in the rye chromosomes, differing expression of certain bands in wheat vs. rye genetic background and, perhaps, chromosomal rearrangements. Nevertheless, a few chromosomes can be recognized beyond doubt.

Chromosome 1R, for instance, clearly corresponds with our VII, the satellite chromosome. In wheat, the secondary constriction (as is the nucleolus at interphase) of this chromosome is suppressed.

Chromosome 2R is variable in shape in the different sources of Darvey and Gustafson (1975) but metacentric in Gill and Kimber's (1974) photograph. We believe it to correspond to our II, although the two heavy terminal bands we observed do not both occur in Gill and Kimber's (1974) 2R. Our II fits in the range of Darvey and Gustafson's (1975) 2R, but our main argument that 2R is II is that there is no other convincing candidate.

Chromosome 3R also might be II, but we are more inclined to believe that it is I. Darvey and Gustafson (1975) observe only occasional doubleness of one of the two bands, and they, as well as Kimber, report occasional small interstitial bands, as in our I. In our material I is median, like 3R in the literature reports, but I is not usually one of the largest chromosomes of the set as Darvey and Gustafson (1975) report for 3R. One complication with I is that it is involved in the translocations which have occurred in the evolution of *S. cereale* (Van Heemert and Sybenga 1972). This should have made substitution comparisons inconclusive for this chromosome but this does not appear from the literature.

Since, in those old translocations, only one segment of this particular chromosome was exchanged, perhaps its effect was too small to be detected in substitutions. For these three chromosomes (VII, II and I), correspondence with Verma and Rees (1974) was good.

Chromosome 4R/7R apparently is involved in a translocation of old origin and, for this reason, the other two chromosomes involved in the complex just mentioned are the first candidates: III and V (Van Heemert and Sybenga 1972). It would have been more satisfactory if it had been possible first to establish convincing correspondence between Darvey and Gustafson's (1975) and our classification and subsequently to conclude that our III and V correspond with 4R/7R and 7R/4R. In view of the many uncertainties encountered, however, we feel that the present approach is the best. According to Darvey and Gustafson (1975), 4R/7R (probably corresponding with 7R of Gill and Kimber 1974) has a characteristic interstitial band in the long arm and a few small approximately terminal bands in the same arm, combined with a rather heavy band in the short arm, so it is more probably identical to V than to III. The submedian location of the centromere and the often pronounced terminal band in the long arm are not sufficient reason to reject this conclusion, as there is enough variation in the material to cover this descrepancy satisfactorily. Verma and Rees' (1974) 5, however, has a large band in the long arm which is definitely deviant from ours, but corresponds with the long arm of 4R/7R of Darvey and Gustafson (1975). Their 4 and 6 correspond well with our IV and VI.

Chromosome 5R obviously corresponds with our VI, chromosome 6R with our IV and chromosome 7R/4R (Gill and Kimber 1974, chromosome 4R) with our III. The 3 of Verma and Rees (1974) has somewhat different bands compared with our III, but not seriously so.

Although with some reservations (descrepancies may have been ironed out too easily), the following table can now be constructed:

Darvey and Gustafson (1975)	Sybenga and Wolters (1972) Gill and Kimber (1974)
1R	VII
2R	II
3R	I
4R/7R	V
5R	VI
6R	IV
7R/4R	III

In view of the variation observed in the rye chromosomes of different origin, and even the same origin under different conditions, it is necessary to have some more certainty. This is rather difficult to obtain. In an attempt to combine the translocations of the tester set with the addition lines received from Drs. Sears and Driscoll (the same source as used by Darvey and Gustafson 1975), we found seriously reduced association between homologous rye chromosomes and some increased association between homoeologous wheat chromosomes. This resulted in trivalent formation involving wheat chromosomes, and insufficient trivalent formation between rye chromosomes in the cases where a rye addition chromosome should have associated with two partly homologous translocation chromosomes of rye. This made the analysis inconclusive. In a few instances, however, rather convincing indications were observed. One was that Sears' addition line D(4R/7R according to Darvey 1973) would correspond with our I rather than with V. This is not entirely impossible, but rather improbable, especially since it would be hard to consider 3R equivalent to our V, which would be necessary if the two chromosomes had to be interchanged. More disturbing is the indication that Sears' F (6R according to Darvey 1973) would be either III or V. It closely resembles IV. The conclusion that 2R would be II is confirmed.

Our checks on the correspondence between Sears' and Riley's series also did not agree with Darvey's on all points. For instance, 4R/7R and 7R/4R seemed to be interchanged. We must consider the possibility that in the widely distributed material of these additions and related material some errors have slipped in, which one must be aware of when drawing conclusions as to which chromosome is involved in each particular case.

Literature

Darvey, N.L.: Genetics of seed shrivelling in wheat and Triticale. Proc. 4th Int. Wheat Genet. Symp., Mo Agric. Exp. Sta., Columbia Mo., USA; 155-160 (1973)

Darvey, N.L.; Gustafson, J.P.: Identification of rye chromosomes in wheat-rye addition lines and Triticale by heterochromatin bands. Crop Science 15, 239-243 (1975)

Gill, B.S.; Kimber, G.: The Giemsa c-banded karyotype of rye. Proc. Nat. Acad. Sci. USA 71, 1247-1249 (1974)

Heemert, C. van; Sybenga, J.: Identification of the three chromosomes involved in the translocations which structurally differentiate the genome of *Secale cereale* L. from those of *Secale montanum* Guss. and *Secale vavilovii* Grossh. Genetica 43, 387-393 (1972)

Merker, A.: A Giemsa technique for rapid identification of chromosomes in Triticale. Hereditas 75, 280-282 (1973)

Sarma, N.P.; Natarajan, A.T.: Identification of heterochromatic regions in the chromosomes of rye. Hereditas 74, 233-238 (1973)

Sybenga, J.; Wolters, A.H.G.: The classification of the chromosome of rye (*Secale cereale* L.): a translocation tester set. Genetica 43, 453-464 (1972)

Verma, S.C.; Rees, H.: Giemsa staining and the distribution of heterochromatin in rye chromosomes. Heredity 32, 118-121 (1974)

Wolff, S.; Luippold, H.E.: Obtaining large numbers of metaphases in barley root tips. Stain Technology 31, 201-205 (1956)

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J.M. de Vries
Dr. J. Sybenga
Department of Genetics
University of Agriculture
53 Generaal Foulkesweg
Wageningen (The Netherlands)