THE IDENTIFICATION OF MONOSOMIC LINES OF AVENA SATIVA L. AND THEIR ASSOCIATION WITH DISTINCT MARKERS

BY

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ABSTRACT

Five monosomic lines from the variety Garry and four from the variety Rodney, were studied to identify the univalent chromosome in relation to the standard karyotype of Rajhathy, and to associate phenotypic markers with each deficiency. Six new monosomics were thus described.

Line 4-27 was monosomic for chromosome 20. It exhibited a malformation of the panicle neck, termed kinky, which was more pronounced in nullisomics. Plants monotelosomic for the short arm also exhibited the extreme kinkiness of the nullisomic suggesting that the long arm carries a gene for normal neck.

Line R 742 was monosomic for the longest sub-terminal chromosome 7. It also exhibited the neck kinkiness, quite similar to that of monosomic 20. The meiotic behaviour of F_1 40-chromosome plants from these two lines indicated that the missing chromosomes were different. Line R 742 tended to have abaxial curling of leaves and geniculate awns. The absence of chromosome 7 seemed to cause a reduction of chiasmata, in at least six chromosome pairs. Another line R 751 also was found deficient for chromosome 7 but its meiotic behaviour showed evidence of a reciprocal translocation involving the critical chromosome.

Line R 32 displayed the fatuoid characteristics, being heterozygous in expression in monosomics, but true in nullisomics, monotelosomics and ditelosomics for the short arm. Nullisomic plants were asynaptic and sterile as expected on the basis of previous work. This line also exhibited some degree of kinkiness. Chromosome 13 was tentatively suggested as the missing chromosome.

Line S 214 was deficient for chromosome 12. No nullisomic plant could be obtained. In monosomic plants, flowers showed a lack of synchronism in maturing reproductive organs, anthers being very slow to mature.

Line M 724 was deficient for the satellited chromosome 8. No nullisomics were obtained. Monosomic plants were associated with the presence of large necrotic spots which developed well after heading, and started on older leaves.

Line R 810 was monosomic for chromosome 14. It was characterized by abaxial turning of leaves, and in nullisomics, by a semi-abscission separation of the spikelets and their peculiar appearance before anthesis.

Line R 824, deficient for the shortest chromosome, number 21, was associated with chlorophyll production, in accordance with previous reports. Line A 411, which was deficient for chromosome 10, did not exhibit any distinctive marker.

Frequency of the univalent transmission was calculated for each line by counting micronuclei in PMC's. This was found to be very low and varied little with lines (4 to 11%). A higher frequency in two lines (R 751 and S 214) was attributed to the occurrence of reciprocal translocations involving the univalent. Backcrosses to the parent variety are recommended to eliminate such difficulties.

With most lines the observed frequency of disomics, monosomics and nullisomics in progenies derived from selfed monosomics differed markedly from the calculated values. These differences were explained by a certation effect in favor of the 21-chromosome pollen, and the lethality of nullisomic zygotes.

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INTRODUCTION

The recent concerted attempts to produce a complete series of monosomics in the cultivated oat Avena sativa L. (2n = 6x = 42) leave little doubt about the feasibility of assembling and maintaining such a series. McGinnis (15), Hacker and Riley (8), Chang and Sadanaga (2), Schulenburg (28), Sun (32), and others described several aneuploid lines, and in many instances, identified them in accordance with the standard karyotype of Rajhathy (25).

These aneuploid lines were obtained by screening either large non-treated populations to detect naturally-occurring monosomics, or populations which were treated with various mutagens to increase the frequency of monosomic lines. Sears (29) was more fortunate with wheat, obtaining the complete series of monosomics from a haploid plant and a partially asynaptic line. With oats, the lack of such sources of monosomics might prolong the period of search. Yet, it is quite possible that the complete set of 21 monosomics might already be available but distributed among several research centers. This is difficult to verify since each worker deals with his own variety.

A large number of monosomics was obtained at this institution from their spontaneous occurrence in natural oat populations and from artificial induction. The purpose of this work was to identify some of these, according to the standard karyotype (25), and associate the univalent chromosome with a distinctive marker. The latter objective is of primary importance for future work in view of the maintenance of the various monosomic lines.

LITERATURE REVIEW

Several methods have been used to identify the deficient chromosomes involved in aneuploid lines of Avena sativa L. Many years ago, the phenotypic expression of the plant was associated with the deficiency of a pair of homologous chromosomes. More recently, the proposal of a standard karyotype has stimulated the use of cytological techniques to identify the critical chromosome. However, since several chromosomes have a similar appearance, interline crosses were used to determine with assurance the homology or non-homology of deficient chromosomes. Much attention was also paid to the meiotic behaviour of aneuploids.

Phenotypic expression (Morphology)

Huskins (9) was the first one to associate in oats a phenotypic expression with a deficient chromosome, that is fatuoidy with the "C" chromosome, later studied in detail by Nishiyama (19, 20). Philp (23, 24) demonstrated that a chlorophyll deficiency and a narrow leaf width were caused by the nullisomic condition of homologous chromosomes. Recently, McGinnis and Taylor (18), McGinnis and Andrews (16) showed that chromosomes 21 and 15 have genes for chlorophyll production, albinism being produced by the nullisomic condition. Chang and Sadanaga (2) described the morphological features of six monosomics but could not identify any specific chromosome. Sun (32)

had more success, identifying three monosomics and associating distinct markers with them. McGinnis and Lin (17) showed that both the monosomy and nullisomy for chromosome 15 produced a unilateral panicle.

Hacker and Riley (7,8) isolated 40 monosomic lines from untreated populations, classified them into thirteen categories on the basis of their phenotypes, and those of the derived nullisomics. This classification was checked by cytological observations. Equivalence between similar phenotypes, and non-equivalence between different phenotypes were confirmed in 28 monosomic lines.

In summary, very few cases were reported where a distinct marker could be associated with the deficiency of one chromosome, that is an ineffective gene in the hemizygous condition. The deficiency of one chromosome often produced a series of similar phenotype variations in different lines, i.e. low fertility, shorter straw, lack of vigour, smaller panicles, retarded growth, etc.

Even with nullisomics, McGinnis (15) and Hacker and Riley (8) pointed out the limitations of the use of morphological features for identification purposes considering the phenotypic similarity of distinct nullisomics (i.e. albinism in monosomics 15 and 21), and the phenotypic dissimilarities resulting from the deficiency of the same homologous chromosomes (i.e. unilateral panicle and albinism with chromosome 15).

This situation is not unexpected since Avena sativa L. is an hexaploid species. It is probable that many characters are controlled

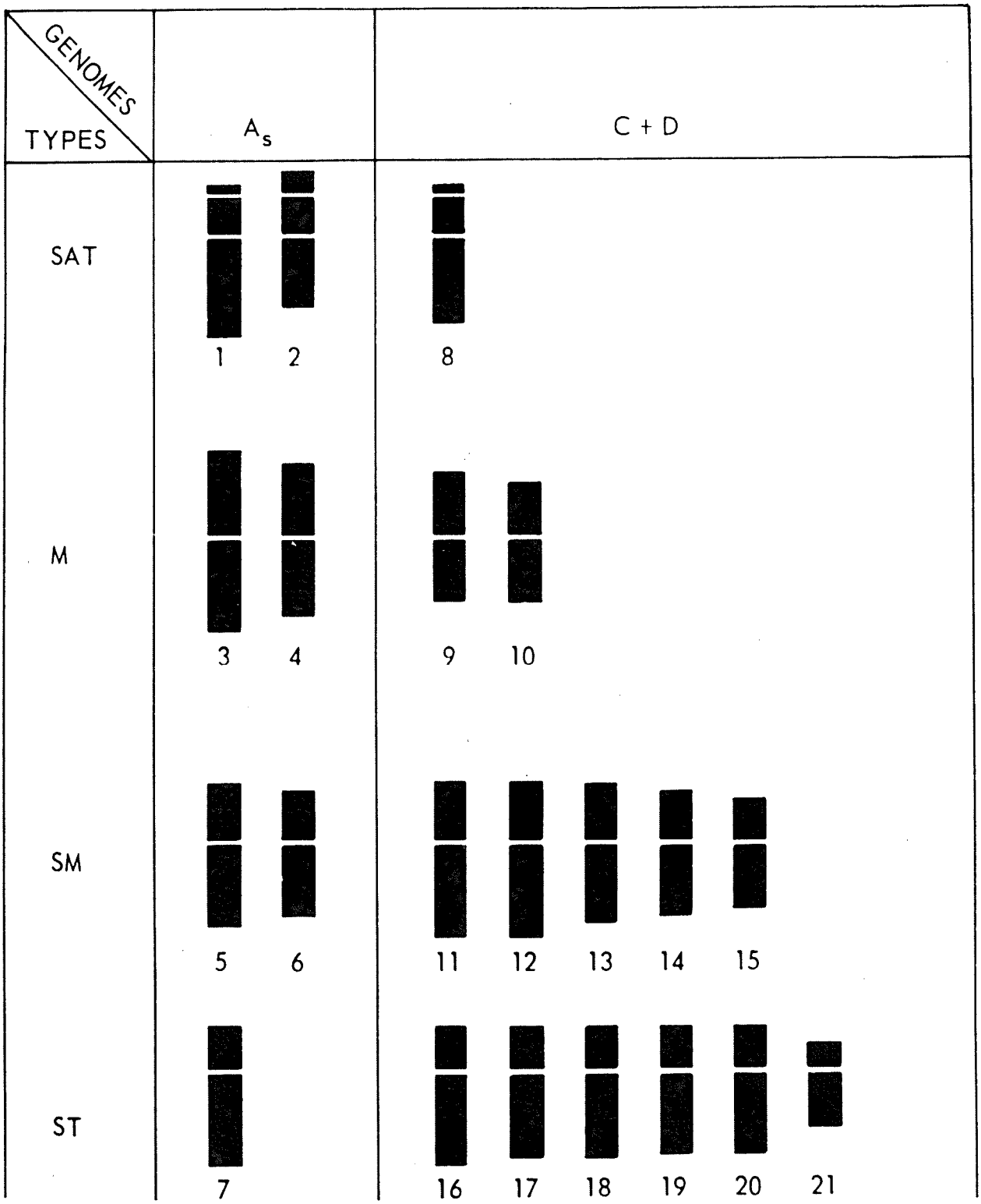
by more than one gene located in different genomes. It may also be an indication of evolutionary divergence of homologous chromosomes of the varieties (8). The heterogeneity of the variety expressed by the segregation into different phenotypes in the same nullisomic lines (8, 17) is an other explanation. The possibility of translocations must not be ignored, especially when different varieties are used. The reciprocal translocations in That cher wheat relative to Chinese Spring, are a classical example (31).

It seems therefore, that the phenotypic expressions should be used with caution for identification purposes. They are quite useful for screening purposes, and in the initial stage of a programme. However, their usefulness will be of utmost importance in the latter part of the programme for the maintenance of pure stocks of a complete series of aneuploids.

Karyotype

Idiograms of oat species, including Avena sativa L., were first published by Rajhathy and Morrison (26). McGinnis (14) proposed the numbering of these chromosomes. However, Rajhathy (25) presented a refined idiogram, and on the basis of their structure and size, divided the oat chromosomes into four groups. He concluded that nine of them can be distinguished conclusively by means of karyotype studies, and four more by two interline crosses as a check for homology (Plate I).

PLATE I - Idiogram of *Avena sativa* L. after Rajhathy (25).


GENOMES
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C + D
SAT
M
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Chromosome 21 and 15 had already been identified by McGinnis and Taylor (18), and McGinnis and Andrews (16) but the proposal of the new karyotype stimulated the identification of deficient chromosomes: chromosomes 4, 6, 14 and 18 by Sun (32), chromosome 7 in Avena byzantina by Singh and Wallace (31), chromosome 1 and 2 by Hacker and Riley (7) and several others (McGinnis, 14).

Chang and Sadanaga (2) and Schulenburg (28) described several aneuploid lines but did not attempt to identify critically the missing chromosome; they merely ascribed them to one of the four subgroups. Hacker and Riley (8) identified satellites 1 and 2 missing in some of their lines. They did not attempt to identify other deficient chromosomes "owing to the morphological similarity of most of the chromosomes of the hexaploid oat karyotype". This is in contradiction with Rajhathy (25) and McGinnis (14).

Interline crosses

Rajhathy (25) and McGinnis (15) suggested the use of interline crosses as a means of determining with assurance the homology of two different lines whose univalent chromosomes appear similar from critical karyotype studies.

Hacker and Riley (8) utilized with partial success interline crosses as a means to assess the similarity of the lines within their 13 groups, and dissimilarity of lines between groups. Sun (32) also

utilized interline crosses to distinguish chromosomes 6 and 14 but failed with other crosses.

To attain success with interline crosses it is essential to have functional 20-chromosome gametes from both parents. These are produced with a fairly high frequency on the female side. On the male side, the situation is more complicated: 20-chromosome gametes must be functional in at least one parent, either in male-fertile nullisomics, or in monosomics giving a high frequency of nullisomics. This is often not the case since several authors (2, 6, 15, 17, 28) reported male sterility in aneuploid lines, and a definite certation effect favoring the 21-chromosome pollen grains. Moreover, the lethality of nullisomic zygotes was observed frequently.

For this reason, it might be impossible to use this method fully until mono-, or di-telocentric lines are available (8).

Meiotic behaviour

The pairing behaviour in aneuploid lines is usually normal. However, several gross abnormalities were observed and associated with specific chromosomes: asynapsis in two genomes with the fatuoid chromosome (8, 19, 20, 31), complete asynapsis with satellite 1 (8), desynapsis with chromosome 7 of Avena byzantina (31). These gross aberrations are easily noticed, and useful for identification purposes.

The frequency of the univalent transmission through the microgametophytes and megagametophytes was shown to vary a great deal with different monosomic lines. Micronuclei counts at the quartet stage

of PMC's were utilized to evaluate this frequency on the assumption that a quartet with no micronuclei produces two 21-chromosome gametes, and a quartet with one micronucleus, one 21-chromosome gamete.

A close relationship was observed in the eggs between these calculations and the actual univalent transmission. With pollen cells, the situation was different in most cases, there being little agreement between the expected frequency, and that observed in crosses involving disomics as female, and monosomics as the male parent. This was explained by the certation effect in favor of 21-chromosome gametes (6, 32). Zygotic lethality was also suggested to explain in monosomic progenies the discrepancy between the expected and actual number of nullisomics, monosomics and disomics (4, 19).

Obviously, the peculiarities of the univalent transmission of specific monosomic lines represent useful information, and are an essential requirement for future genetic studies. But as a tool for identification purposes, they have a limited value, being quite variable depending on the origin of the line, and the environmental conditions (13).

MATERIALS AND METHODS

McGinnis (14) found 24 spontaneous aneuploids out of 4,023 seedlings in the variety Garry. Later on, Andrews and McGinnis (1) studied the artificial induction of aneuploids in the varieties Garry and Rodney by using X-irradiation (75 r to 600 r), ultra-sonic vibrations (5 to 40 minutes), myleran (10^{-6} to 10^{-3}), and acetone (.002). A total of 130 aneuploids were obtained by these methods.

Lines from these stocks were chosen for this study in both Garry and Rodney varieties. Garry was developed at the Research Station of the Canada Department of Agriculture, Winnipeg, Manitoba from the cross Victoria X (Hajira X Banner) X Victory. Rodney was developed at the same station from the cross R.L. 1574 X Roxton.

A description of the monosomic lines utilized in this study follows:

Monosomic line	Variety	Origin
4-27	Garry	Spontaneous
R 32	"	X-irradiation - 300 r
M 724	"	myleran - 10^{-3}; 2 hours
S 214	"	ultrasonic vibrations - 20 minutes
A 411	"	acetone - .002 conc.
R 742	Rodney	X-irradiation - 150 r
R 751	"	" "
R 810	"	" "
R 824	"	" "

These monosomics were grown during three consecutive years, in 1964, 1965 and 1966, in the greenhouse and in the field. Seeds of the monosomic lines were treated with a fungicide, Arasan, and germinated in plastic containers filled with vermiculite saturated with water.

Seeds were cold treated at 0 - 2°C for two days to break dormancy, and then kept at room temperature until the rootlets were 2 - 3 cm in length. Root tips were excised, pre-treated in ice water for 24 hours at 0 - 2°C and fixed in 3: 1 Farmer's fluid. After three days, the root tips were hydrolyzed in 1 N HCL at 60°C for 10 - 12 minutes, stained with Feulgen and examined by the aceto-carmine squash technique. Chromosome counts were made at mitotic metaphase. Monosomics, nullisomics and disomics were sorted out, and grown either in the greenhouse in 5-inch pots, or in the field as spaced plants. Panicles were collected and fixed in Carnoy's 6:3:1 solution in order to check the chromosome counts and observe the meiotic behaviour. Any observed morphological differences between the disomics, and nullisomics or monosomics were assumed to be the result of the deficiency of a particular chromosome. In several instances, however, the monosomic lines were backcrossed to the parent variety in order to eliminate possible mutations in chromosomes.

A critical analysis of the karyotype was performed on each monosomic line. Camera lucida drawings, micro-projection drawings and enlarged photomicrographs were utilized for this purpose. Enlarged photomicrographs of well-spread mitotic figures were found particularly efficient since chromosomes could be cut, matched and mounted on metric paper, and their identification concurrently checked by the visual examination of the cell, using a 90x oil immersion objective. Slides were made permanent by the

quick freezing method with carbon dioxide, a modification of the method outlined by Conger and Fairchild (3).

With these means, certain of the univalent chromosome could be readily identified. Those aneuploid lines that had a similar morphology were intercrossed, and the 40-chromosome plants analyzed at metaphase I of meiosis. Configurations of 20 bivalents in inter-line hybrids would indicate that the monosomic lines were deficient for the same chromosome but 19 bivalents plus two univalents would indicate that different chromosomes were involved.

RESULTS AND DISCUSSION

Results obtained with each monosomic line will be discussed separately, for purposes of clarity and brevity, with emphasis on morphology, karyotype and meiotic behaviour.

Line 4-27

a) Morphology

The nullisomic plants differed from the disomics in that they attained only three-quarters of the normal height, were a few days earlier, and with one exception were completely sterile in the greenhouse and produced very few seeds under field conditions. They lacked vigour as seedlings but tillered profusely and produced numerous

small panicles. Except for reduced anther size, floral development appeared normal, and both eggs and pollen were functional in crosses with disomics. The monosomics were vigorous and similar in appearance to the disomics. They showed a low fertility in the greenhouse but were about 35 per cent fertile under field conditions. Some spaced plants produced over 300 seeds.

These morphological differences from the disomics, although significant, are not specific for this line since they are also observed in certain other aneuploid lines. However, a distinctive marker was detected which appeared to be characteristic for this line and similar to that described by Sun (32) and Hacker and Riley (8). It consists in a malformation of the neck (i.e. the section just below the basal node of the panicle main axis) that becomes crooked or wavy (Plate II). For this reason, the line was termed "kinky" neck. Although the expression of this character is influenced by the environment it is fairly stable and can always be observed in the nullisomic. Its expression is somewhat intermediate in the monosomic, and in the greenhouse is not obvious on all tillers. Monosomic plants from backcrosses to Garry and Rodney oats also exhibited the "kinky" neck phenotype.

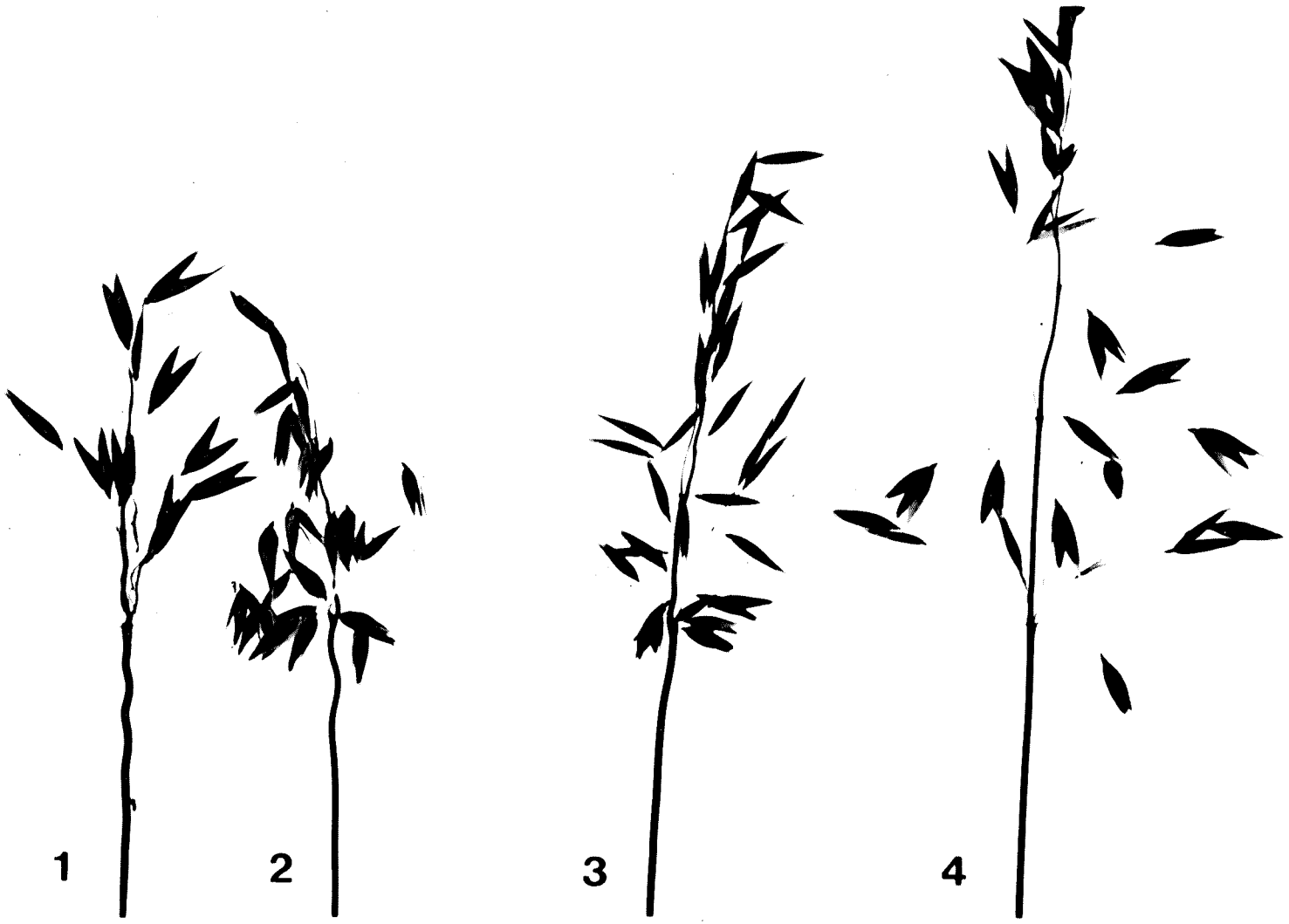
To determine with certainty the homology or non-homology of the critical chromosome in the two kinky lines 4-27 and R 364 of Sun (32) intercrosses were attempted in four different seasons.

PLATE II - Morphology of monosomic-20 (line 4-27)

Figures 1 and 2. Typical kinky neck expression in nullisomic plants.

Figure 3. A mild kinky neck expression in monosomic plants.

Figure 4. A disomic plant.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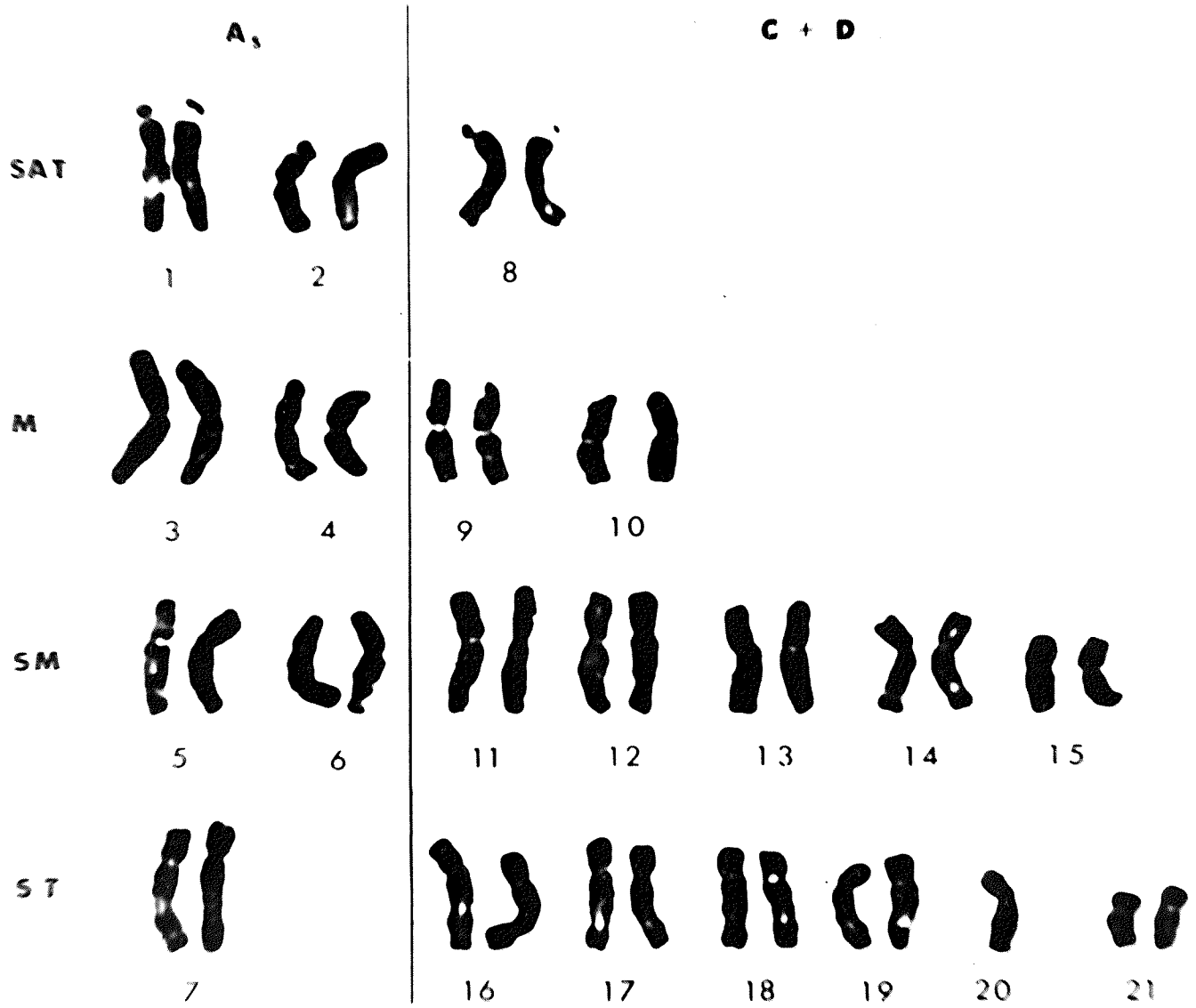
They failed even though nullisomics are viable in both lines. This failure could be an indication that the missing chromosome is the same (refer to more detailed discussion on page 61). Another attempt is presently being made.

b) Karyotype

Preliminary observations of numerous cells indicated that the missing chromosome belongs to the subterminal group of chromosomes 17 to 20 according to the standard karyotype proposed by Rajhathy (25). Seven excellent mitotic figures from different monosomic plants were analysed critically using the methods mentioned earlier. From these analyses it appears that the critical chromosome is the shortest of this group of four, namely chromosome 20 (Plate III). In two plants monotelosomic for the short arm, the expression of the "kinky" condition was as intensive as in nullisomics. This suggests that the long arm of the critical chromosome carries a gene for the normal neck expression or a gene that inhibits the "kinky" condition.

The karyotype obtained from this line resembled closely the standard karyotype of Rajhathy (25), with minor exceptions. For instance, it was obvious here that chromosome 7 was longer than chromosome 16 and could be identified easily. It was also noted that the relative lengths of chromosomes 10 and 15 varied from cell to cell, i.e. sometimes chromosome 15 was as long as 10, or even longer. However, they could still be identified by the position of the centromere.

PLATE III - Karyotype of monosomic-20 (line 4-27)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c) Meiotic behaviour

Chromosome counts were made on root tips of germinated seeds, derived from monosomic plants grown in the field. The distribution of aneuploids and disomics in 278 seedlings is presented in Table I.

TABLE I

Frequency of 42-, 41-, 40+ telocentric, and 40-chromosome oat seedlings in monosomic-20 progenies.

Chromosome number	Number of seedlings	Percentage of total
42	14	5.0
41	229	82.4
40 + telocentric	7	2.5
40	28	10.1
Total	278	100.0

The small percentage of disomics (5.0) indicates that the univalent transmission frequency was relatively low, at least in the eggs. This is in accordance with previous observations of Philp (24), McGinnis and Taylor (18), Schulenburg (28), McGinnis and Andrews (16),

Chang (2), Lin (13) and Lafever and Patterson (12, Sun (32). The nullisomics, however, also occurred at a low frequency (10.1%), which is slightly higher than the frequency of disomics. Such a combination could be produced through a certation effect in favour of the 21-chromosome pollen grains, or by some lethality in one class.

The transmission rate was checked in the PMC's of six monosomic plants grown in the field by counting the micronuclei at the quartet stage. These results are presented in Table 2. It can be assumed that quartets without micronuclei produce two gametes with 21 chromosomes and two with 20 chromosomes; those with one micronucleus, one gamete with 21 chromosomes and three with 20 chromosomes while those with two or more micronuclei probably produce only deficient gametes. On this basis, the transmission rate of the univalent was calculated to be only 9.0%. On the assumption of a similar meiotic behaviour in the egg mother cells as in PMC's the low transmission frequency of the univalent can explain the actual transmission frequency of disomic in seedlings provided there is some certation effect in favour of the 21-chromosome pollen grains.

Reciprocal crosses between monosomic 20 and disomic were made to check this assumption and to determine the transmission rate in the pollen. Unfortunately, the seed set was very low. Twenty

seeds were obtained from crosses with mono-20 as female, giving two disomics and 18 monosomics. The transmission rate of the univalent was therefore 10.0%, which is in agreement with that calculated from micronuclei counts. Fifteen seeds were obtained when the monosomic was the male parent, and these produced five disomics and ten monosomics giving a transmission rate in the pollen of 33.3%.

TABLE 2

Number of micronuclei in quartets of monosomic-20 plants and calculation of univalent transmission frequency.

Number of micronuclei per quartet	Number of quartets	Number of gametes	Gametes with n = 21	Per cent gametes with n = 21
0	174	696	348	50
1	612	2448	612	25
2	1388	5552	0	0
3 or more	500	2000	0	0
Total	2674	10696	960	9.0

Although these data are insufficient to draw definite conclusions, they suggest strongly that certation played a major role

in the pollen by increasing the transmission rate threefold. However, this factor alone cannot explain the low frequency of nullisomics in the progenies of selfed monosomics. With a 9.0% transmission of the gametes with 21 chromosomes in the egg, and 33.3% in the pollen, the frequency of 42, 41 and 40-chromosome plants should be respectively, 3.0, 35.7 and 57.3% as compared to the observed distribution of 5.0, 84.9 (82.4 + 2.5) and 10.1% (Table 1). The deficiency of nullisomics can therefore only be explained by a marked lethality of 40-chromosome zygotes. Such a probability was suggested much earlier by Nishiyama (19), and more recently by Costa-Rodrigues (4). On the other hand, Hacker (6) concluded that nullisomic zygote inviability was not important in three monosomics studied, and that dissimilarities in the frequencies with which different monosomics segregated nullisomics were found to be due to differences in the frequencies that 20-chromosome pollen functioned.

The micronuclei counts reported in Table 2 show that 18.7% of quartets contained three or more micronuclei. This frequency is somewhat higher than that observed by McGinnis and Taylor (9), Lin (13) and Sun (32). This could result from more misdivision of daughter univalents at anaphase II, and/or from a slight increase in asynapsis of other chromosomes.

The plants monotelosomic for the short arm of chromosome 20 (Plate IV) were also analysed at meiosis. At anaphase I the telocentric always divided as observed for whole univalents by McGinnis and Taylor (18). Micronuclei counts in 464 quartets gave a transmission rate of 8.7% for the fragment which was quite similar to the transmission of the whole univalent. The telocentric produced minute micronuclei that could be identified readily, and as a consequence, the frequency of large micronuclei resulting from univalents could be recorded with accuracy. Two or more large micronuclei occurred in 16.5% of the quartets studied. This relatively high percentage is most probably the result of asynapsis in other chromosome pairs and is in agreement with the above observation of the relatively high frequency of three or more micronuclei (18.7%) observed in the monosomic quartets. Seven monotelosomics, or 2.5%, were observed in the progenies of monosomic-20 (Table 1), six of them being for the short arm. It is possible moreover that some telocentrics for the long arm might have been missed since they are less obvious.

Line R 742

a) Morphology

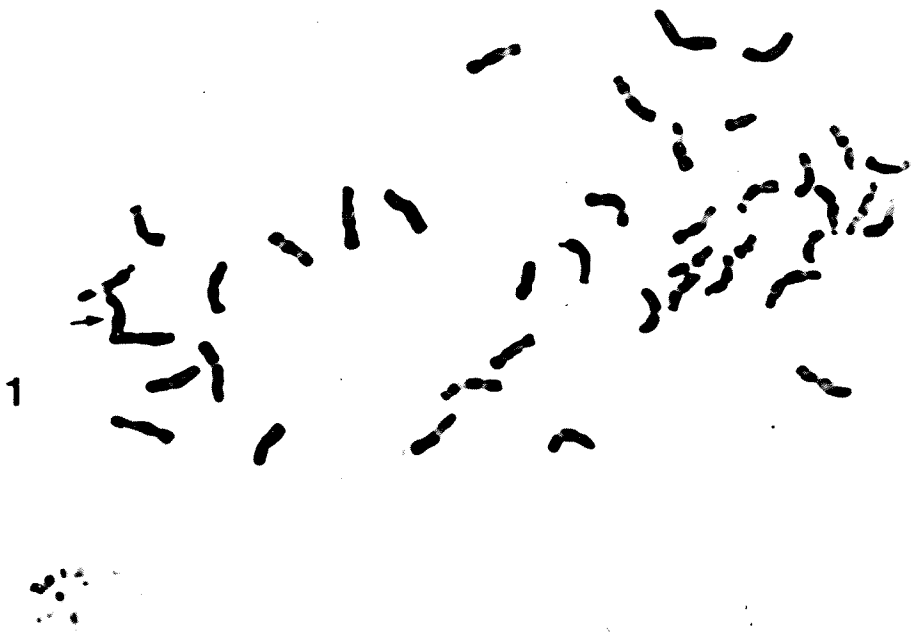
This monosomic line was vigorous and seemed almost normal under greenhouse as well as field conditions. Monosomics had a slightly shorter straw than disomics, and the seed set averaged about 50%, being variable from one plant to another. They tended to have curled leaves and a kinky neck.

PLATE IV. - Metaphases of monosomic-20 (line 4-27)

Figure 1. Mitotic configuration from which the karyotype was prepared (see arrow).

Figure 2. Mitotic metaphase of plant monotelosomic for short arm (see arrow).

Figure 3. At 1st anaphase, a monotelosomic for the short arm divides and produces two equal-size minute dots (see arrows).



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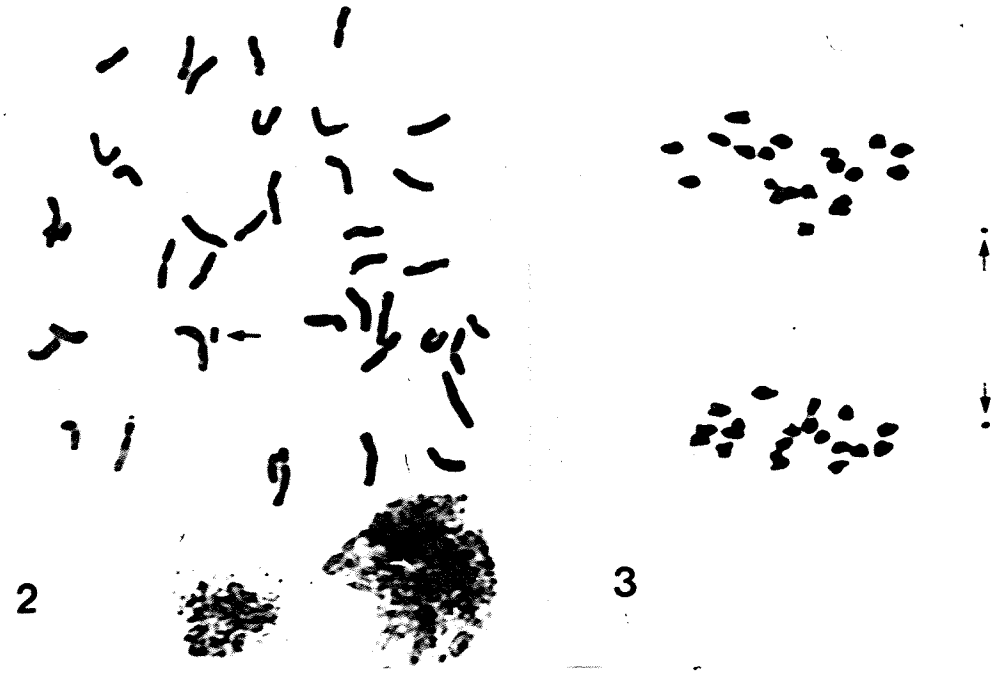

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In the earlier stages of this study, no nullisomic plant was obtained but after three generations and also a backcross to the parent variety Rodney, a few nullisomics were obtained. These had a much shorter stem, and tended to tiller profusely and were completely sterile. They exhibited a malformation of the neck termed kinky (Plate V) quite similar to that of line 4-27 described earlier, of monosomic 18 by Sun (33), and of monosomic 2 by Hacker and Riley (8). They were also characterized by a strong awn on the primary lemma, and abaxial turning of leaves. This description corresponds exactly to that of Class II of Hacker and Riley (8). Because only a few telocentrics were obtained, it was impossible to associate either arm with any of these marker genes.

To check if an identical chromosome was involved in these three lines, namely monosomic-20 (line 4-27), line R 742 and monosomic-18 (R 364), interline crosses were performed using monosomic plants as female parents, and nullisomics as males. A few 40-chromosome plants were obtained, and their meiotic behaviour confirmed that the critical chromosome in line R 742 differed from that of 4-27 and R 364 (Plate VI, figures 1 and 2).

b) Karyotype

Numerous cells from different plants were analysed for the identification of the critical chromosome, and 14 well-spread mitotic figures were critically analysed on enlarged photographs. It was

PLATE V - Morphology of monosomic-7 (line R 742)

Figure 1. Typical kinky necks in nullisomic and monosomic plants.

Figure 2. Abaxial turning of leaves in monosomic plants.

40

41

42

41

PLATE VI. - Meiotic behaviour of F1 hybrid 40-chromosome plants.

Figure 1. Cross R 742 (mono-7) X 4-27 (mono-20) showing 19 bivalents plus 2 univalents (see arrows).

Figure 2. Cross R 742 (mono-7) X R 364 (mono-18) showing 19 bivalents plus 2 univalents (see arrows).

Figure 3. Cross R 32 (mono-13) X 4-27 (mono-20) showing 19 bivalents plus 2 univalents (see arrows).

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2

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concluded from these analyses that the longest chromosome of the sub-terminal group, namely 7, was missing (Plate VII). Several cells did not show any difference in size between chromosomes 7 and 16. However six good preparations showed conclusively that chromosome 7 was longer and missing. It is assumed here that chromosome 7 belongs to the "A" genome since Rajhathy (25) stated that chromosomes 7 and 16 were similar in size and could not be distinguished. Earlier, it was pointed out that these two chromosomes differed in size and could be distinguished.

c) Meiotic behaviour

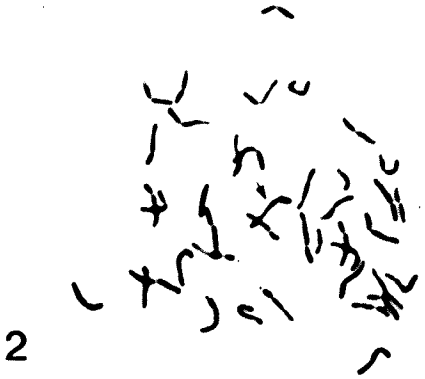
The meiotic behaviour in monosomic 7 was normal. At metaphase I, the univalent could be observed outside the equatorial plate, and only occasional opened bivalents could be seen among the twenty bivalents. The meiotic behaviour in the nullisomic plants was quite different and abnormal. In early diakinesis, the frequency of loose pairs was obvious, and in metaphase I, at least six opened bivalents were constantly present (Plate VIII) in all nullisomics examined cytologically. Joshi and Howard (11) reported some irregularities in the meiotic behaviour of Avena sativa L. but not to such an extent. It would seem, therefore, that chromosome

PLATE VII. - Monosomic -7 (line R 742)

Figures 1, 2 and 3. Mitotic metaphases (see arrows).

Figure 4. Karyotype prepared from the mitotic configuration shown in figure 1.

1



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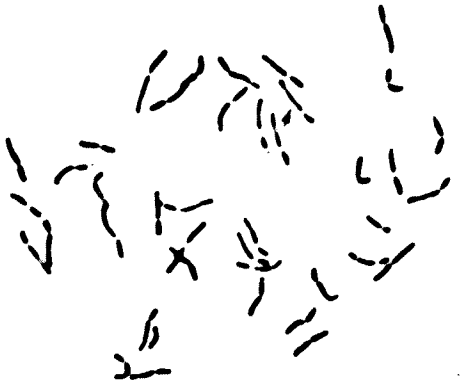

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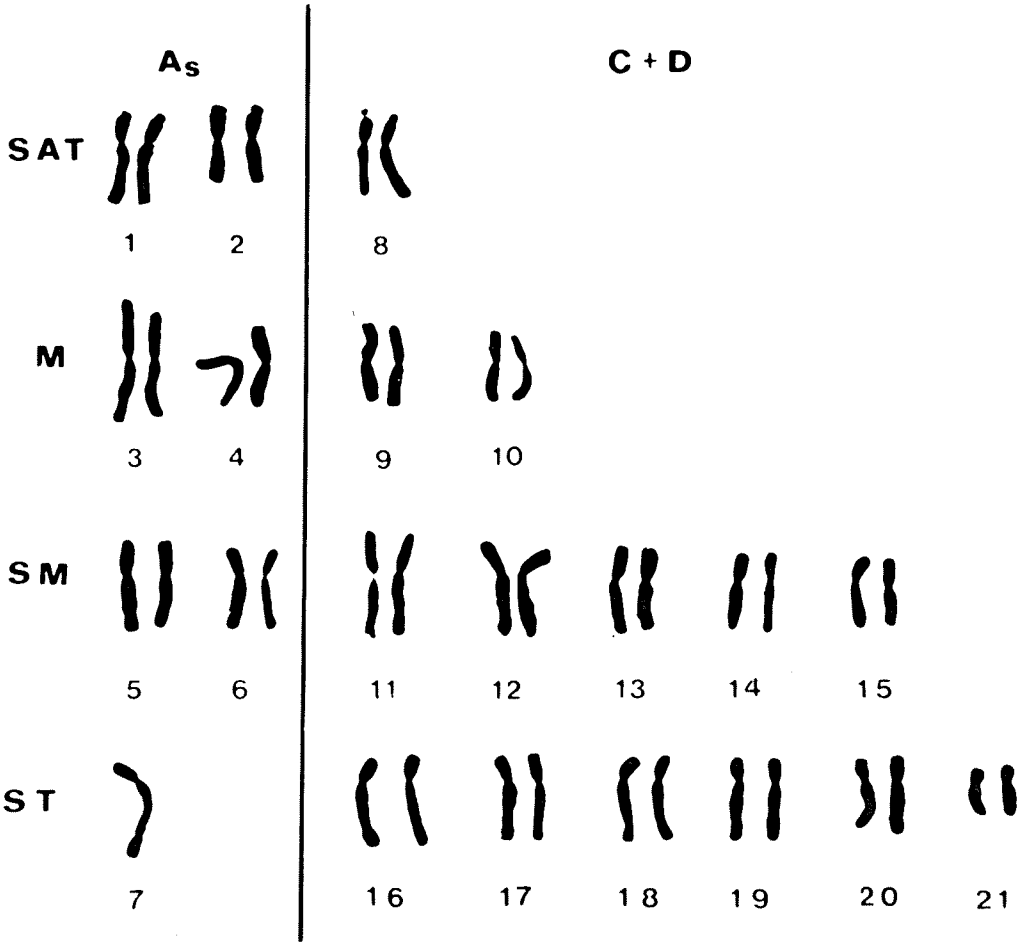


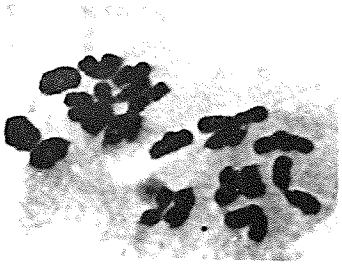
4

PLATE VIII. - Meiotic behaviour of nullisomic-7 (line R 742) showing at least six opened bivalents.

Figures 1, 2 and 3. Metaphase I.

Figures 4, 5 and 6. Early diakinesis.

1



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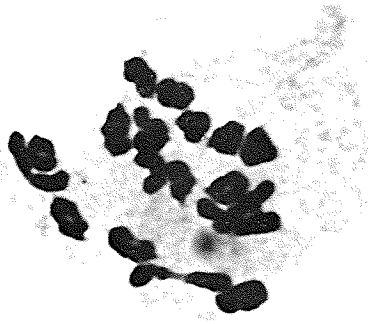

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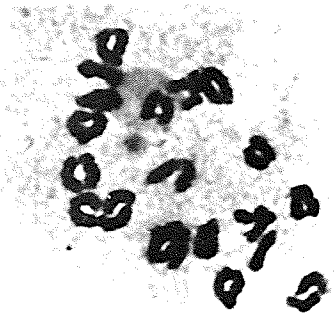

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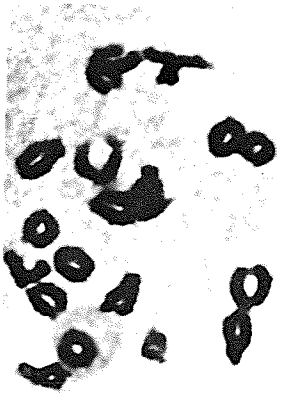

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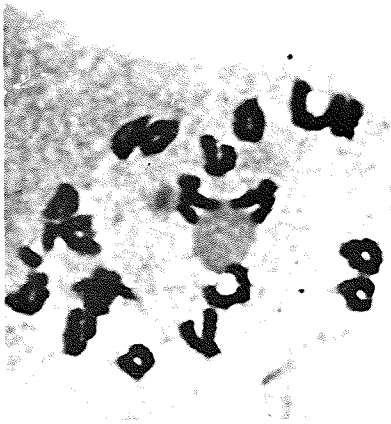


6

7 controls the extent of pairing in one genome. The abnormality was not drastic enough to cause asynapsis or desynapsis; it simply reduced the number of chiasmata in at least six chromosomes. Actually, only occasional quartets could be noticed with several micronuclei, as an evidence of gross aberration. Singh and Wallace (31), working with A. byzantina, observed the desynaptic effect associated with the absence of chromosome 7, which caused the pairing failure of all chromosomes in the early prophase and affected the movement of 12 univalents at anaphase I. Recently, Thomas and Rajhathy (33) described a desynaptic mutant in tetraploid oats (A. barbata X A. Abyssinica) characterized by a high incidence of univalents at metaphase I; these were attributed either to a reduction in chiasma formation or premature terminalization. Although the meiotic aberrations in R 742 nullisomics were not as extreme as those reported above, they could be part of the same phenomenon if it is assumed that in the variety Rodney, modifying factors exist on other chromosomes and control the full manifestation of this aberration. More simply, it could be another manifestation of the genotypic control of chiasma frequency, well established by Rees (27).

Singh and Wallace (31) also suggested that chromosome 7 belongs to the A_S genome, comparing the effect of its absence with that of the asynaptic "C" chromosome, considered by Nishiyama (22)

to belong to the C genome. This belief is further substantiated by the detection of a desynaptic mutant in Avena strigosa by Dyck and Rajhathy (5), and in tetraploid oats by Thomas and Rajhathy (33). These findings are in contradiction with Nishiyama (21) who concluded "that the present hexaploids have lost physically or functionally the synaptic factors in the A and B genomes and are dependent for their normal chromosome pairing upon the synaptic factor in the C genome".

To determine with assurance if the deficient chromosome in monosomic-7 belongs to the A_S genome, a cross is underway between a primary trisomic for chromosome 7 (Rajhathy, personal communication), and nullisomic-7, as the male parent.

The abnormal meiotic behaviour in nullisomics did not seem to increase the number of micronuclei in PMC, or to modify the transmission rate of the univalent. Micronuclei were counted at the quartet stage in the PMC's of several monosomic plants (Table 3).

On the usual assumptions, the transmission was calculated to be 11.6%. This is in agreement with previous reports (2, 12, 16, 18, 24, 28, 32).

If a similar meiotic behaviour is assumed in the eggs, the progenies of selfed monosomics should consist of 1.4% disomics, 20.5% monosomics and 78.1% nullisomics. A careful cytological check on 278 plants showed that the observed frequencies were not related to the

number of micronuclei in quartets since the progenies consisted of 85% monosomics (Table 4). This phenomenon was observed by several authors and explained on the basis of certation effect in favor of 21-chromosome pollen (6, 7, 26, 32), and zygotic lethality (4, 19).

TABLE 3

Number of micronuclei in quartets of monosomic-7 plants and calculation of univalent transmission frequency.

Number of micronuclei per quartet	Number of quartets	Number of gametes	Gametes with n = 21	Per cent gametes with n = 21
0	59	236	118	50
1	197	788	197	25
2	334	1336	0	0
3 or more	67	268	0	0
Total	657	2628	315	11.6

TABLE 4

Frequency of 42-, 41-, 40+ telocentric, and 40-chromosome oat seedlings in selfed monosomic-7 progenies.

Chromosome number	Number of seedlings	Percentage of total
42	14	5.3
41	228	85.0
40+ telocentric	2	0.8
40	24	8.9
Total	278	100.0

Actually, from 10 seeds obtained in crosses between disomics as female, and monosomics as male, only two produced monosomic plants, thus confirming a definite certation effect. Besides a reduced seed set, an abnormal number of non-germinated seeds was also observed. Most of these could be nullisomics.

Line R 751

a) Morphology

The general appearance of monosomics in line R 751 was quite similar to those of R 742 with their widely spread panicle, good fertility and vigour, and awning habit. However, they showed little tendency to develop kinky necks and curled leaves.

No nullisomic was obtained and therefore, its morphology could not be compared adequately with that of R 742.

b) Karyotype

Several good mitotic metaphases were analysed, and indicated that chromosome 7 was deficient. This monosomic line was crossed to monosomic-7 (line R 742). Forty-chromosome plants produced at metaphase I either 20 bivalents, or 18 bivalents plus a chain of 4, or 18 bivalents plus a chain of three and one univalent. This indicated that the univalents were similar but that one of them was involved in a translocation.

c) Meiotic behaviour

Earlier studies indicated an unusual breeding behaviour of these monosomics since their progenies included a large number of disomics. The frequency of the univalent transmission calculated by means of micronucleus counts in 897 PMC of several plants was evaluated at

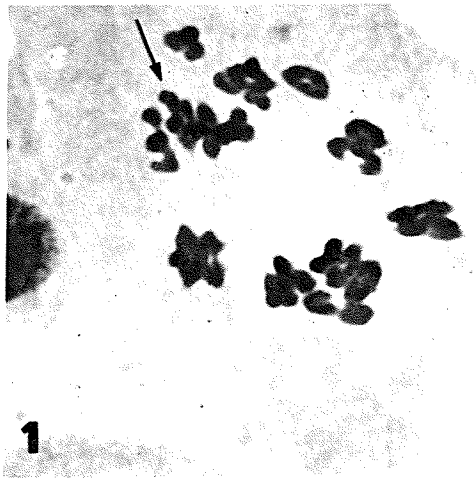
50.6%. At metaphase I, sometimes the univalent could be seen outside the metaphase plate; more often, a chain of three involving the univalent could be observed (Plate IX), and occasionally three univalents about the plate. Recent calculations on monosomics derived from backcrosses to Rodney indicated that the frequency of the univalent transmission was about 5%.

This behaviour indicated that the critical chromosome involved a major translocation that affected the univalent transmission frequency, and could change significantly its morphology.

PLATE IX. - Meiotic behaviour of line R 751.

Figures 1 and 2. At metaphase I, 19 bivalents and a chain of 3 (see arrows).

Figures 3 and 4. At anaphase I, one cell with 21 chromosomes, and the other with 20 chromosomes.



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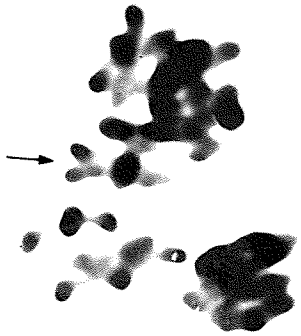

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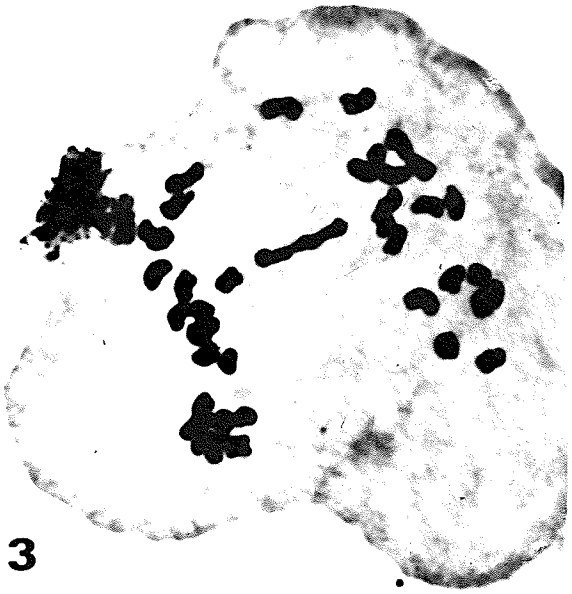

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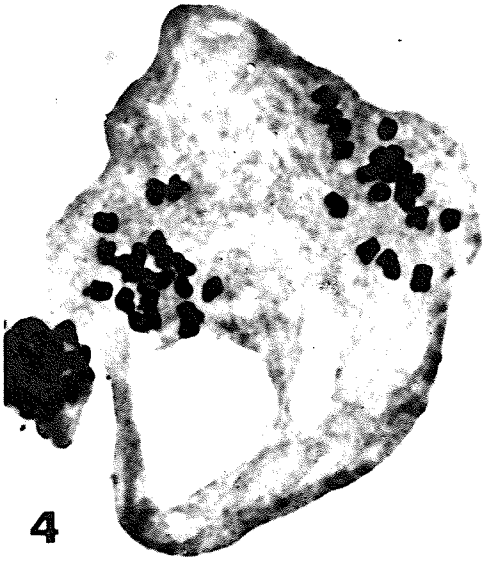


4

Line R 32

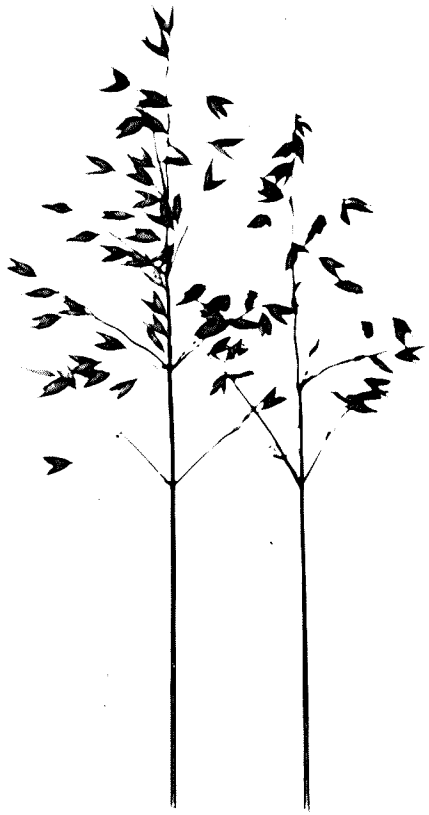
a) Morphology

Monosomic plants from the R 32 line, induced by X-irradiation in the variety Garry, were vigorous with thick culms. The seed set was good, and spikelets exhibited constantly the heterozygous expression of the fatuoid characteristics, i.e. a long twisted geniculate awn on the primary grain, and a few basal hairs. Grain hulls were coarse and thick.

Nullisomics were rare, only two were found among several hundred plants. Nullisomic plants were weak with sparse tillering and short culms, and completely sterile. They expressed the fatuoid characteristics, having a pubescent sucker mouth disarticulation on each grain and a twisted geniculate awn on each lemma (Plate X).

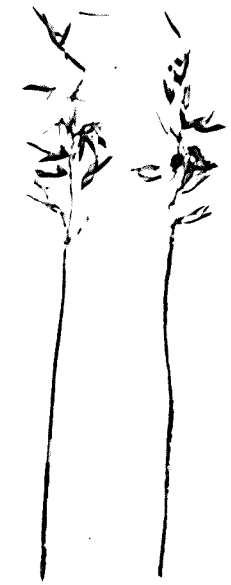
Plants monotelosomic, ditelosomic or with one iso-chromosome for the short arm of the critical chromosome were observed frequently (Plate XI). Their phenotype was quite similar to that of nullisomics but they were more vigorous, tillered profusely and produced a few seeds. The culms, however, were much shorter than that of monosomics. This behaviour indicates that genes for the cultivated oats, or genes epistatic to those controlling the expression of fatuoidy, are located on the long arm of the critical chromosome.

PLATE X. - Characteristic morphology of monosomic-13 (line R 32) showing a fair expression of kinkiness in the nullisomic, monotelosomic for the short arm and monosomic plants; the presence of a geniculate, twisted awn on each grain of the nullisomic and monotelosomic plants, but only on the primary grain of the monosomic plants. The sucker mouth abscission is present on each grain of nullisomic and monotelosomic plants but not too obvious in this photograph.



42

41



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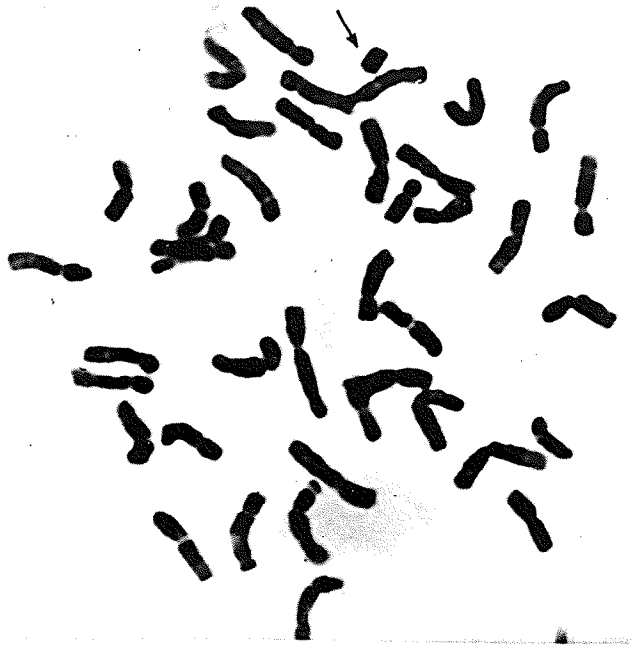
40

PLATE XI. - Metaphase of monosomic-13 (line R 32).

Figure 1. Mitotic metaphase of a monotelosomic for the short arm (see arrow).

Figure 2. Mitotic metaphase of a ditelosomic for the short arm (see arrow).

Figure 3. Metaphase I showing 20 bivalents plus an isochromosome for the short arm forming a ring (see arrows).



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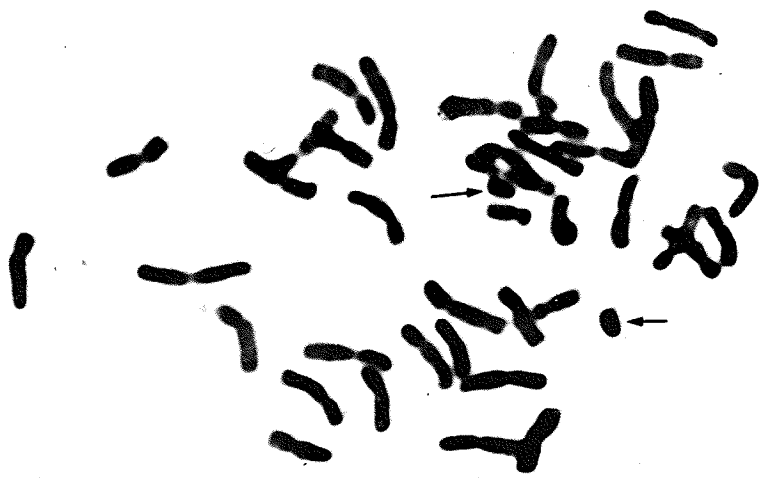

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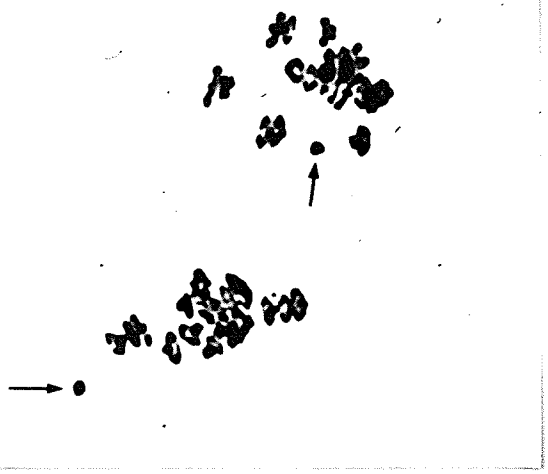


3

This description corresponds exactly with that of the "C" chromosome by Huskins (9), Nishiyama (20) and others. However, it differs somewhat from that of Sun (32) who observed the full fatuoid expression in monosomic-14 of line R 256 and the sub-intermediate fatuoid expression in nullisomic-14 of line R 176.

As mentioned previously, monosomics from line R 32 tended to express the kinky malformation (Plate X). However, the kinkiness was not as obvious as that associated with chromosomes 2, 7, 18 and 20, except in rare cases. Actually, F_1 40-chromosome hybrids between R 32 and monosomic-20 (line 4-27) produced 19 bivalents plus two univalents (Plate VI). Kinkiness was quite variable in R 32, thus confirming that the expression of this characteristic is influenced by the environment.

b) Karyotype

Several good mitotic preparations were analysed critically with the methods mentioned previously, and led to the conclusion that the critical chromosome belong to the sub-median group, being either 6, 13 or 14. Chromosome 6 can be eliminated on the ground that the C chromosome was attributed to the C genome (22). Chromosomes 13 and 14 are quite similar in appearance, and hard to distinguish (17, 25). Nevertheless, after a critical study of many cells, Sun (32)

concluded that chromosome 14 was missing in his lines R 176 and R 252. Although these lines produced fatuoids, their behaviour appears quite different from that of R 32. For this reason, and from the evidence obtained from the analysis of many cells (Plate XII), it is proposed tentatively to associate fatuoidy and line R 32 with chromosome 13. Further decisive evidence should be obtained through interline crosses. These were attempted but failed so far; they are being repeated.

c) Meiotic behaviour

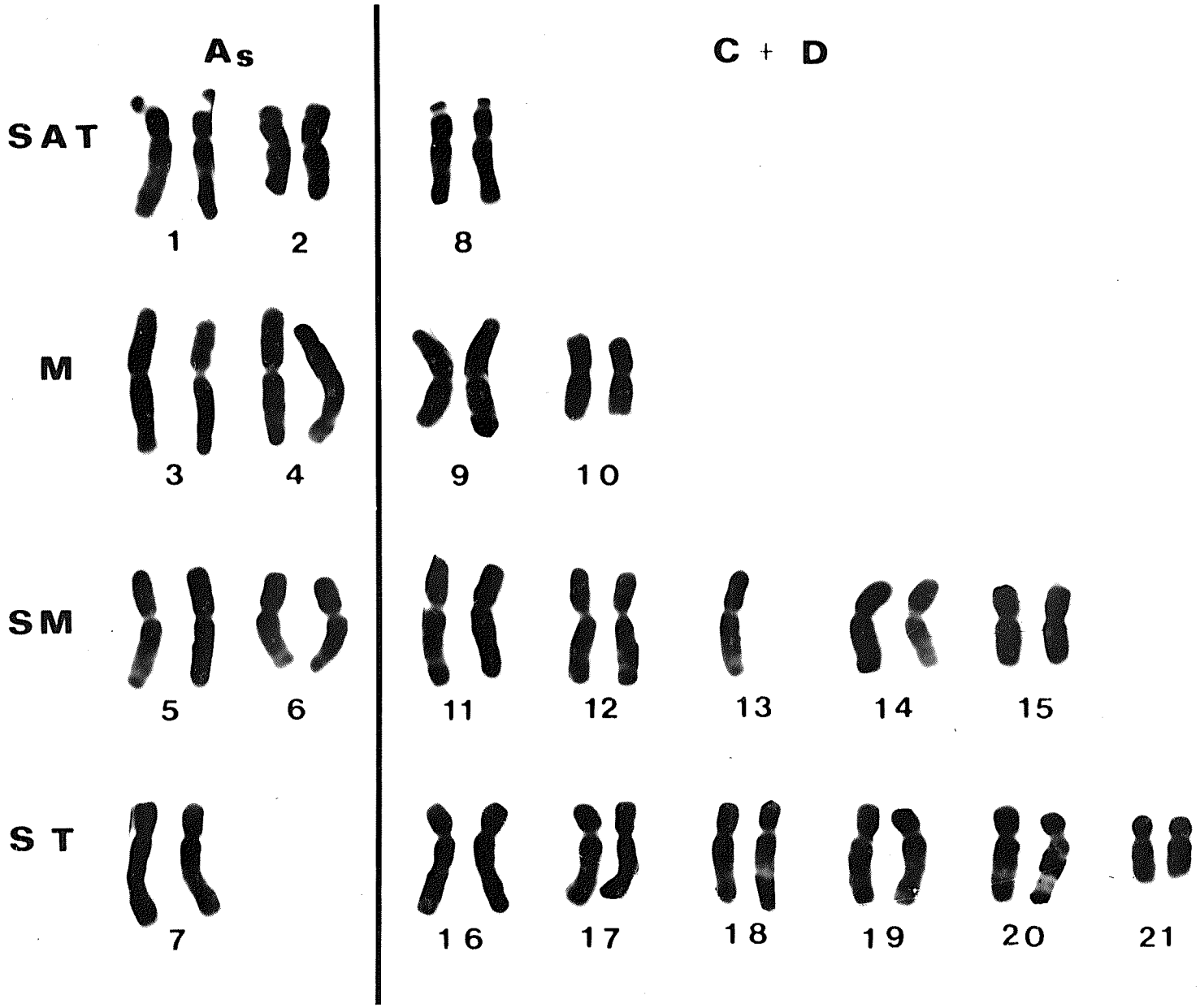
The meiotic behaviour of monosomics-13 was practically normal. Micronuclei were counted in PMC's of several monosomic plants. On the usual assumptions, the transmission frequency was calculated to be 3.7% (Table 5).

TABLE 5

Number of micronuclei in quartets of monosomic-13 plants and calculation of univalent transmission frequency.

Number of micronuclei per quartet	number of quartets	number of gametes	Gametes with n = 21	Per cent gametes with n =21
0	13	52	26	50
1	147	588	147	25
2	733	2932	0	0
3 or more	265	1060	0	0
Total	1158	4632	173	3.7%

PLATE XII. - Karyotype of monosomic-13 (line R 32).


As
C + D
SAT
M
SM
ST
1
2
8
3
4
9
10
5
6
11
12
13
14
15
7
16
17
18
19
20
21

This is somewhat lower than most previous observations. It is possible that the tendency of this chromosome to misdivide at the second division, as evidenced by the high proportion of 3-micronucleus quartets, might have exaggerated the number of 2-micronucleus quartets.

The observed frequency of nullisomics, monosomics and disomics in selfed monosomic progenies was not related to the micronuclei counts in PMC's as observed earlier. The same explanations are valid to explain the high frequency of monosomics which amounted to 84.3% of the population (Table 6). In line R 32, the high proportion of non-germinating seeds was obvious especially in progenies of monotelosomics, ditelosomics and isochromosomes.

TABLE 6

Frequency of 42-, 41-, 40+ telocentric, 40+ isochromosome and 40-chromosome oat seedlings in monosomic-13 progenies.

Chromosome number	Number of seedlings	Percentage of total
42	8	2.9
41	236	84.3
40+ telocentric or isochromosome	32	11.4
40	4	1.4
Total	280	100.0

A relatively high percentage (11.4%) of telocentrics and isochromosomes for the short arm was observed. Telocentrics could be identified in routine mitotic counts, and confirmed later by the fatuoid appearance of the plant. Plants with an isochromosome were classified as 41 in routine root-tip cytological analyses but the fatuoid expression threw some doubts on the classification. Meiotic checks confirmed the presence of a ring-shape univalent at metaphase I (Plate XI, figure 3).

Nullisomics showed evidence of meiotic asynapsis, in accordance with previous descriptions by Nishiyama (19), Huskins and Hearns (10), Chang and Sadanaga (2), Hacker and Riley (8), and Singh and Wallace (31). At metaphase I, only 0 to 9 bivalents, often rods, could be observed, the rest being univalents. This behaviour could also be observed at anaphase I; sometimes, univalents only could be seen. As a result numerous micronuclei of all sizes were formed in quartets (Plate XIII).

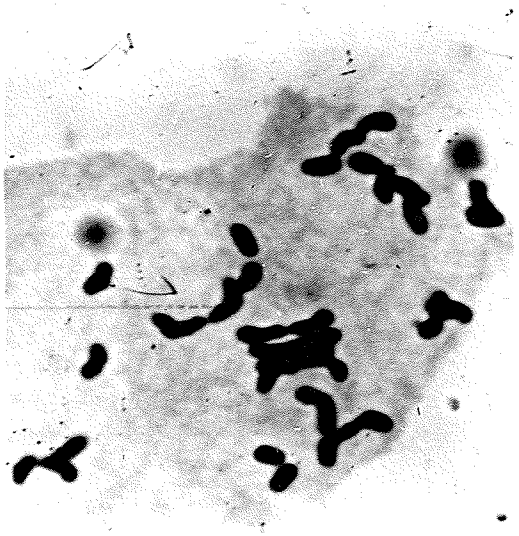
PLATE XIII. - Asynaptic meiotic behaviour of nullisomic-13 (line R 32).

Figure 1. Late metaphase I showing 6 rod bivalents.

Figure 2. Anaphase showing 6 chromosomes that moved to the poles, and 3 rod bivalents dividing.

Figure 3. Late anaphase showing 7 chromosomes that moved to the poles, and 26 chromosomes remaining in the central plate.

Figure 4. Quartet stage showing a great number of micronuclei of all size.



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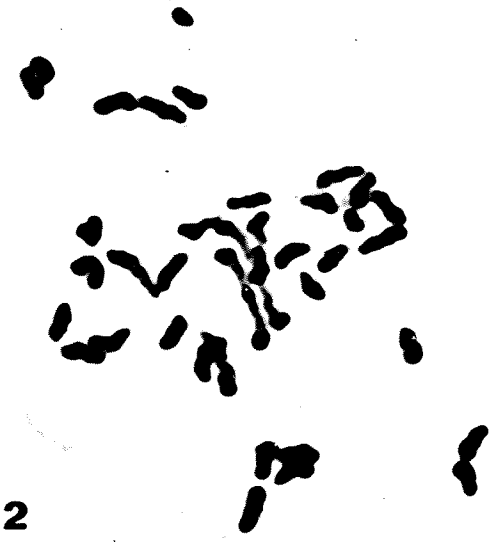

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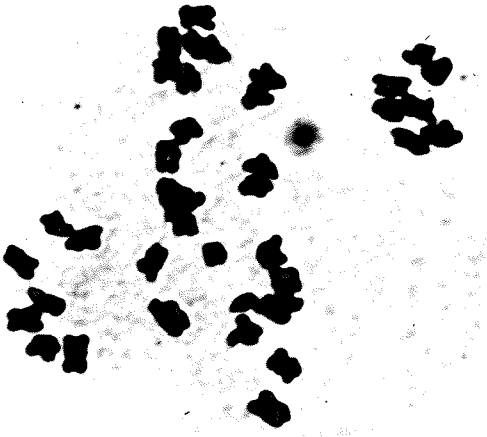

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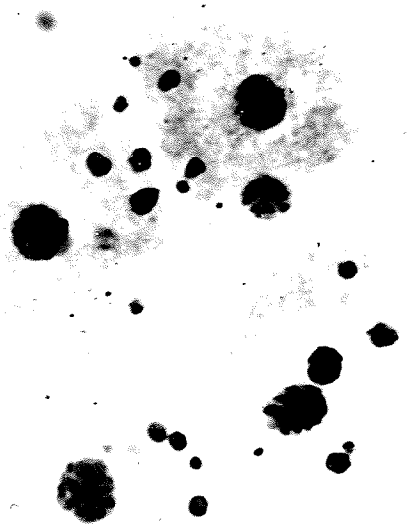


4

Line R 810

a) Morphology

Line R 810, an induced mutation in the variety Rodney, produced vigorous healthy monosomic plants with a good seed set. Nullisomics were healthy and tillered profusely although they had a short culm and a very low seed set. Leaves had a tendency to be dark green.

Both monosomic and nullisomic plants displayed abaxial turning of leaves, that is, curled backward away from the plant (Plate XIV). Spikelets at an early stage had a distinctive appearance. Before anthesis, they were small, oppressed, and the secondary grains were well enclosed by the primary grain lemmas. This morphology made the emasculation a delicate operation. The base of the primary grain was pubescent, and the spikelet separation looked like a semi-abscission.

b) Karyotype

A critical analysis of several enlarged photomicrographs revealed that a small chromosome of the sub-median group, either 6 or 14, was missing. The distinction between these two is not an easy one although Sun (32) succeeded in distinguishing them. Interline crosses were made with his monosomic-14 but yielded only monosomic plants. Other attempts are being made to cross R 810 with monosomics 6 and 14.

PLATE XIV. - Morphology of monosomic-14 showing the abaxial turning of leaves in monosomic and nullisomic plants as compared to the disomic plant.


42
41
40

Tentatively, line R 810 is classified as monosomic-14 (Plate XV) on account of some morphological resemblances to Sun's monosomic-14 (32), and in spite of the absence of white striations and fatuoid characters.

c) Meiotic behaviour

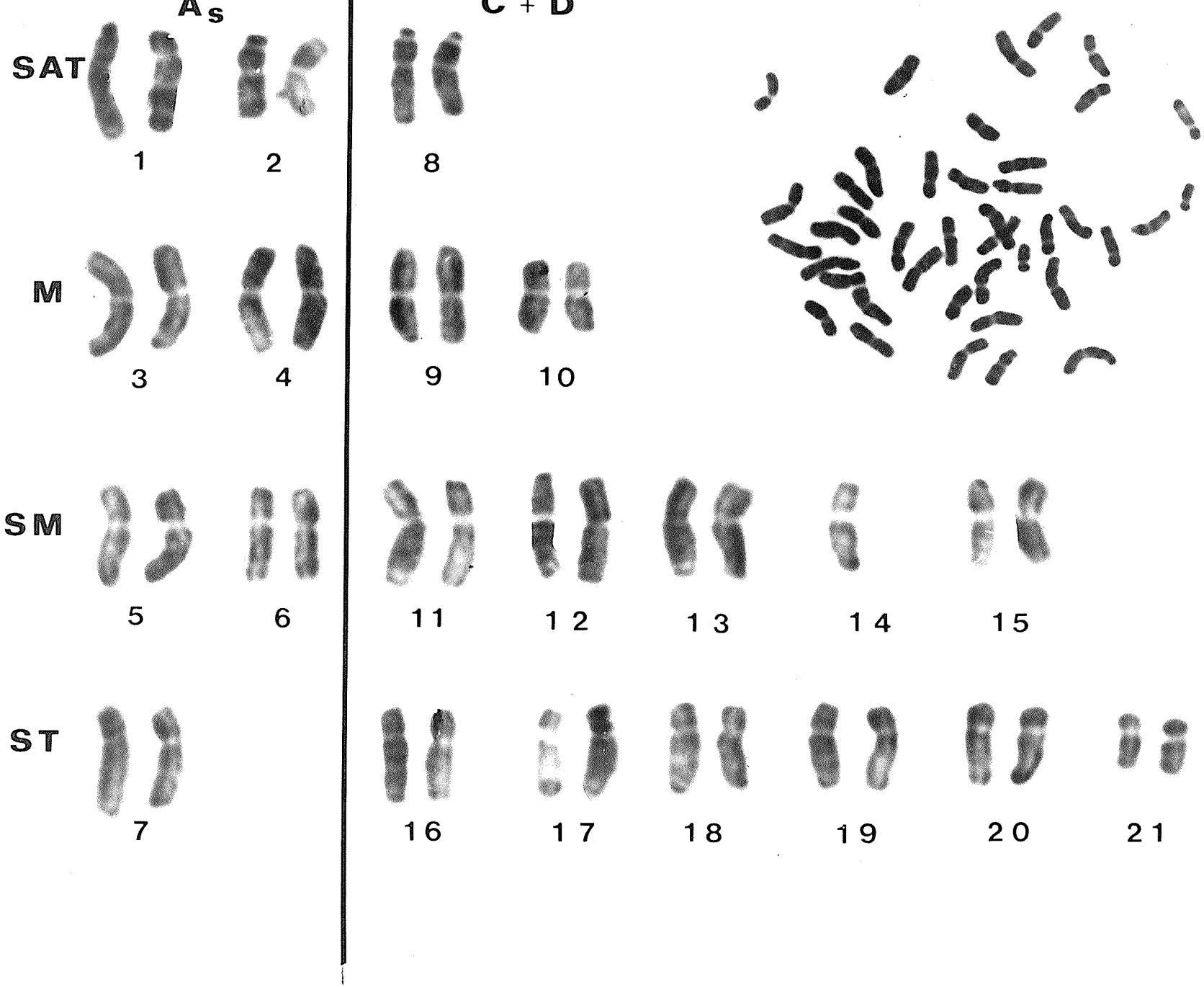
Meiosis was normal in nullisomics and monosomics. The univalent transmission frequency was evaluated at 9.2% through micronucleus counts in PMC's with the usual assumptions (Table 7). This is in agreement with previous calculations for other monosomic lines but less than the 16% frequency observed by Sun (32) in monosomic-14.

TABLE 7

Number of micronuclei in quartets of monosomic-14 plants and calculation of univalent transmission frequency.

Number of micronuclei per quartet	Number of quartets	Number of gametes	Gametes with n = 21	Per cent gametes with n = 21
0	102	408	204	50
1	356	1424	356	25
2	886	3544	0	0
3 or more	170	680	0	0
Total	1514	6056	560	9.2%

PLATE XV. - Karyotype of monosomic-14 (line R 810), and mitotic configuration from which the karyotype was prepared.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Here also, the observed frequency of disomics, monosomics and nullisomics in the progenies derived from monosomic plants differed markedly from the expected values calculated by micronucleus counts. Even though nullisomics were more frequent than in other lines, monosomics formed by far the bulk of the populations.

Line S 214

a) Morphology

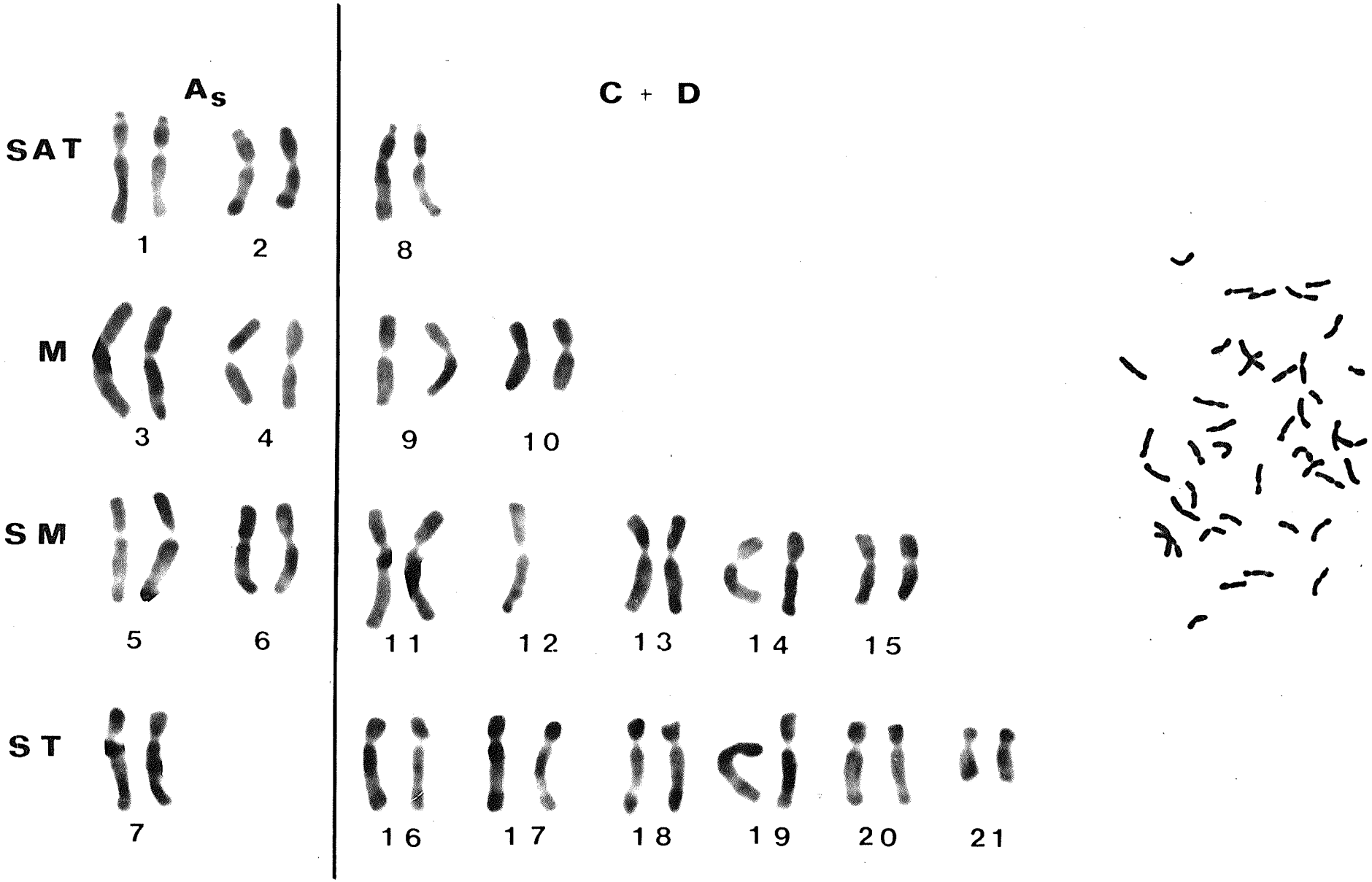
Line S 214, an induced mutation in the variety Garry, produced fairly vigorous and healthy monosomic plants with a fair fertility under field conditions. They did not tiller much but were taller than most other monosomic lines.

A unique feature was the abnormal slowness of anthers to reach maturity. Anthers were still in the green stage and not mature when stigmas were well developed and receptive. For PMC cytological analyses, the right stage came late, that is when anthers were nice and large, much larger than usual. A lack of synchronism seemed to exist in the maturing process of the male and female organs. This might explain the high incidence of ergot in this line.

b) Karyotype

A critical analysis of many enlarged photomicrographs indicated that a large sub-median chromosome was missing, namely chromosome 12 (Plate XVI). Chromosome 12 was preferred to chromosome 5 on the basis of a lower arm ratio (short/long arm) as compared to chromosome 5.

PLATE XVI. - Karyotype of monosomic-12 (line S 214), and mitotic configuration from which the karyotype was prepared.


A_S
C + D
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M
SM
ST
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2
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3
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21

c) Meiotic behaviour

The meiotic behaviour was examined in monosomic plants only. Micronucleus counts in 868 quartets of PMC's from several plants were made in the early stage of this study. The frequency of the univalent transmission was calculated to be 17.8%. A check of the metaphase I indicated the occasional presence of a chain of three chromosomes involving the univalent, which could explain the higher calculated frequency of the univalent transmission. More recent micronucleus counts yielded a univalent transmission frequency of 11.9% which would be more in line with previous reported date.

As observed for all other lines, the observed frequency of disomic, monosomic and nullisomic plants in progenies derived from monosomic-12 differed materially from the expected values.

Line M 724

a) Morphology

The line M 724, an induced mutation from the variety Garry, produced weak plants in early generations but gradually improved after three years. However, no nullisomic or telosomic plants could be observed.

Monosomics were less vigorous than disomics but grew and tillered well enough to produce a fair number of seeds, both under greenhouse and field conditions. Monosomic plants exhibited a very distinctive marker. It consisted in a series of necrotic spots enlarging progressively and similar in appearance to those attributed to mineral deficiencies or toxicities (Plate XVII). These spots appeared well after the heading stage, starting on the older leaves and extending on all leaves. This characteristic showed up both under greenhouse and field conditions. However, in the field, necrotic spots were less conspicuous being somewhat masked by other leaf diseases. The cause of such spotting could not be determined.

b) Karyotype

For some reason or other good mitotic metaphases were hard to obtain, chromosomes sticking together in a pile impossible to spread out. Nevertheless, a preliminary analysis of many cells showed that a satellite was missing, either satellite 1 or 8.

PLATE XVII. - Morphology of monosomic-8 (line M 724) showing large necrotic spots on leaves.

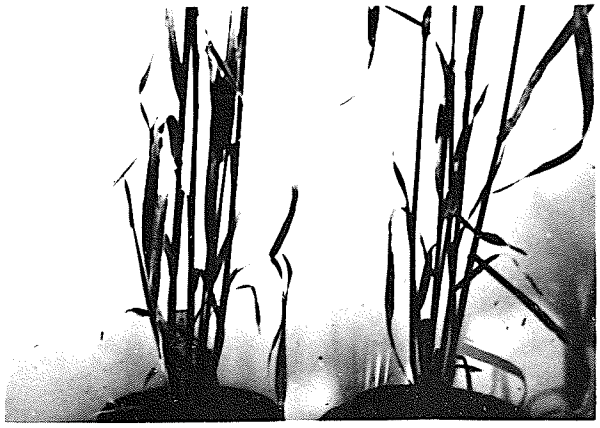
Figure 1. Disomic and monosomic plants.

Figure 2. Two monosomic plants.

Figure 3. A close-up of healthy leaves from a disomic, and necrotic leaves from a monosomic plant.

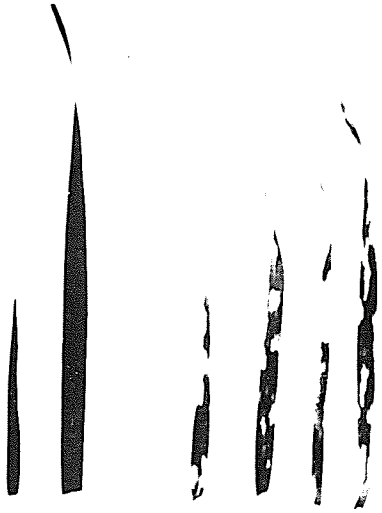
1

42 41



2

41 41



3

42 41

More critical analyses indicated that chromosome 8 was the deficient one (Plate XVIII).

c) Meiotic behaviour

As mentioned above, no nullisomic plant was obtained. It was hoped to check their meiotic behaviour since Hacker and Riley (8) have attributed a complete asynaptic effect to the absence of satellite 1, which is very similar in appearance to chromosome 8.

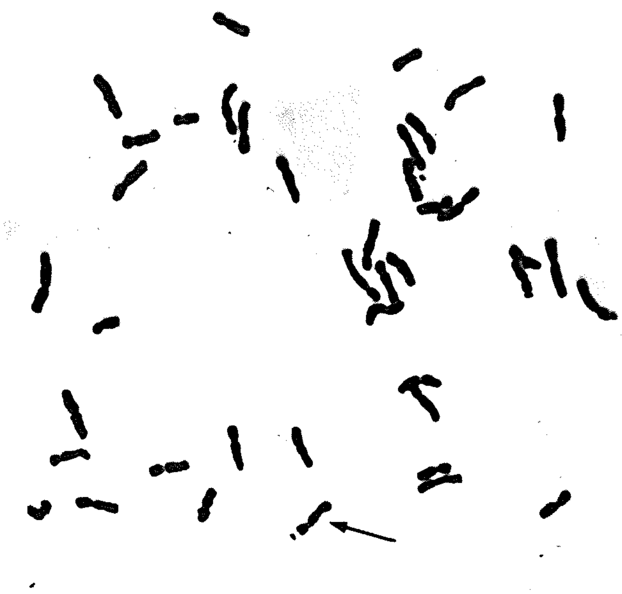
The meiotic behaviour of monosomics was in line with previous observations on other monosomic lines. Actually, from micronucleus counts in PMC's, the frequency of the univalent transmission was calculated to be 9.4% (Table 8).

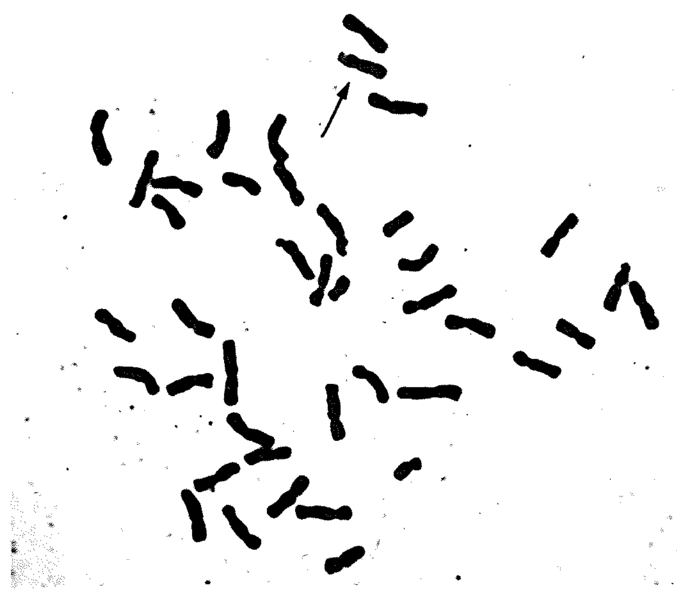
TABLE 8

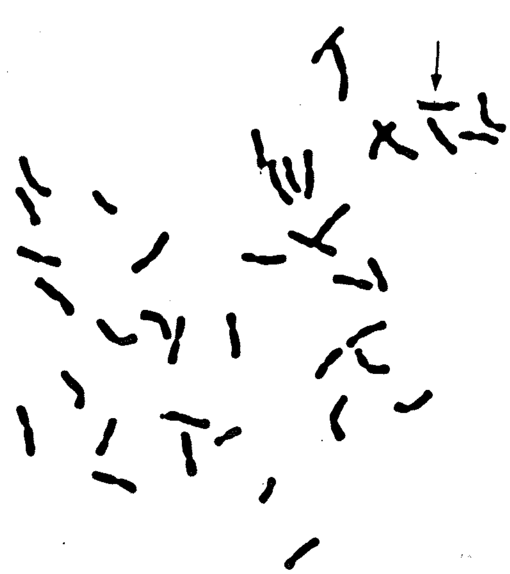
Number of micronuclei in quartets of monosomic-8 plants and calculation of univalent transmission frequency.

Number of micronuclei per quartet	Number of quartets	Number of gametes	Gametes with n = 21	Per cent gametes with n = 21
0	78	312	156	50
1	160	640	160	25
2	381	1524	0	0
3 or more	223	892	0	0
Total	842	3368	316	9.4

PLATE XVIII. - Mitotic metaphases of chromosome 8 (see arrows) Line M 724.







As with previous monosomics, the observed frequency of disomics, monosomics and nullisomics differed markedly from the expected values since no nullisomic was produced.

Line A 411

Line A 411, an induced monosomic in the variety Garry, was fairly vigorous and fertile. It was impossible to associate any distinctive marker with this monosomic line. The deficient chromosome was identified as being the shortest of the medium group, namely chromosome 10.

Line R 824

Line R 824, an induced monosomic in the variety Rodney, gave healthy and vigorous monosomic plants possessing a high fertility. Nullisomics were all albino plants, and attained a frequency of almost 60%.

The deficient chromosome was identified as the shortest of the complement, namely chromosome 21. This behaviour was in close agreement with the description given by McGinnis and Taylor (18) of monosomic 21 derived from the variety Garry.

GENERAL DISCUSSION AND CONCLUSIONS

The identification of univalent chromosomes in monosomic lines of Avena sativa L. is feasible through the use of various methods such as the karyotype analysis, the meiotic behaviour of nullisomic and monosomic plants, the phenotypic expression and the meiotic study of 40-chromosome F_1 hybrids. McGinnis (15) concluded that the only reliable method is by cytological analysis. Transmission data were shown to be quite variable, even with the same chromosome (13). The phenotypic expression, although found useful in the initial stages of a programme (8), can be misleading since the deficiency of a specific chromosome can cause a different phenotypic expression in different varieties (8), and furthermore, a specific phenotypic expression can be associated with different chromosomes, as shown for the chlorophyll production (16,18), and for kinky necks associated in the present study to chromosomes 7, 13, 20 and to chromosome 18 by Sun (32). The cytological analysis of oat chromosomes can be fruitful because they exhibit considerable variations, as illustrated by the standard idiogram (25). It was suggested that nine chromosomes could be readily identified through karyotype analysis, and the others distinguished with the aid of a few interline crosses (15,25).

Sun (32) showed that a careful examination of several good mitotic preparations can lead with a fair assurance, to the identification of the deficient chromosome. The present study confirmed this belief in the case of several monosomic lines. Yet the karyotype analysis is not exempt of pitfalls. The behaviour of two lines, R 751 and S 214, showed that reciprocal translocations involved the critical chromosome, and caused a change in the univalent transmission frequency. Such translocations, if involving unequal segments of non-homologous chromosomes, could provoke a significant change in the appearance of the chromosomes, i.e. the position of the centromere and the lengths of the chromosome. To avoid such errors and before karyotype studies are initiated monosomics should be backcrossed to the parent variety to check the meiotic behaviour, and if necessary, to restore the original karyotype. Even if the importance for such backcrosses appears more obvious when dealing with induced monosomics where gross exchanges could have occurred, reciprocal translocations can also give rise to spontaneous monosomics and must not be ignored.

The karyotype analysis, when properly performed, represents indeed, an essential tool to ascribe a number to the critical chromosome. However as a means of eliminating all doubts,

interline crosses represent the final and decisive step that reveals the complete independence of univalent chromosomes, and the absence of translocations.

In the present study considerable time was devoted to interline crosses in view of checking the equivalence or non-equivalence of similar deficient chromosomes, as utilized by Hacker and Riley (8) and Sun (32). Results were disappointing, nullisomic plants being seldom obtained. These negative results can be attributed in part, to the difficulty of securing good functional 20-chromosome pollen in many lines of Garry and Rodney, enhanced by the inherent low success in oat crossing.

An improvement must be sought to allow a full use of interline crosses. These will also be essential to compare the various monosomic series available, or to utilize a specific monosomic from a different variety to complete a series. It was mentioned earlier that with wheat, an ideal set up occurred with the development by Sears(30) of the complete monosomic series in the variety Chinese Spring, and its general use for the production of all other series. In oats each worker deals with a variety of his own choice, such as Garry, Rodney, Manod, Condor, (15), Sun II (7), Borreck (28), Cherokee (2), etc. Although it is hoped that most chromosomes in these are identical as to the standard karyotype, it is probable that differences do exist. The immediate

adoption of a common check variety would be most useful, and eliminate future inconsistent data from different scientists.

The association of specific chromosomes with a specific phenotype, or markers represented an important phase of this study. It proved a difficult task for several reasons. First, on account of the hexaploid nature of common oats, many factors are expected to be controlled by more than one pair of genes located on non-homologous chromosomes, and therefore, the absence of one pair of homologous chromosomes might not bring out a specific expression since other genes fulfill the same role. This phenomenon was well illustrated in the case of chlorophyll production where nullisomics 15 segregated 3 green: 1 albino (16). Luckily, in this case, the second locus controlling chlorophyll production was in heterozygous condition. Such a marker has a limited value since it can hardly be transferred into another variety without modifications to the genotype. Secondly, oats do not exhibit as wide a range of phenotypic variations as that found in other species, such as barley or wheat. Too often the absence of different chromosomes causes similar phenotypic variations. Thirdly, the rare occurrence of nullisomics in many lines of the Garry and Rodney varieties was a serious drawback. Fortunately, all varieties do not react the same way, as evidenced by other data (8).

Nevertheless, excellent markers were detected one of which is the neck kinkiness associated with chromosomes 7, 20 and 13 in the present study, and with 18 by Sun (32). This marker is of special interest because of its mild expression in monosomics. This expression varied somewhat in four different lines grown under identical conditions (Plate XIX). Monosomics 7 and 20 behaved in a similar fashion, kinkiness being slight in monosomics but quite exaggerated in nullisomic plants. On the other hand, the expression of kinkiness was more obvious in monosomics 18 of Sun (32) and 13 but did not increase significantly in their nullisomics. The abortion of the lower whorl of spikelets in nullisomic-18 sometimes gave the false impression of a high degree of kinkiness (Plate XIX). The control of kinkiness seems to act in one way in monosomics 7 and 20 but in a different manner in monosomics 13 and 18. Interline crosses proved the non-homology of monosomics 7 and 20, 7 and 18, and 13 and 20. A confirmation of non-homology between 18 and 20, and 13 and 18 remains to be done. Sun (32), considering the hexaploid nature of common oats, suggested that several genes could control this malformation. This is certainly logical but not sufficient to explain its occurrence in four different lines. The possibility of translocations, especially between chromosomes 7 and 20, 13 and 18, must be faced. Evolutionary divergence in the genetic activities of

PLATE XIX. - Various expressions of kinkiness in nullisomic and monosomic plants of lines R 32 (mono-13), R 364 (mono-18), 4-27 (mono-20) and R 742 (mono-7).

R742

4-27

R364

R32

41

40

homologous chromosomes (8) offers another explanation since monosomic 7 (R 742) was derived from Rodney whereas the three others, from Garry.

The frequency of univalent transmission offers little help in the identification of deficient chromosomes, being too variable (13). Yet, it represents an essential tool for future crossing and genetic work. It was computed for each line by counting micronuclei in PMC's. The frequency varied very little from line to line (4 to 11%), except in lines R 751 and S 214. In these the higher frequency was attributed to a reciprocal translocation involving the univalent, which resulted frequently in the formation of a chain of three chromosomes dividing normally, that is 2 chromosomes moving to one pole, and one chromosome to the other pole.

A wide discrepancy between the calculated and observed transmission of the univalent was observed. It was shown to be a consequence of a marked certation effect in favor of the 21-chromosome pollen and attributed partly to some lethality of nullisomic zygotes.

Aberrant meiotic pairing of some nullisomics constitutes also a useful marker even though it is detectable only in nullisomics. It is easily recognized, and can characterize a line. The asynaptic C chromosome which is well known was described here.

Hacker and Riley (8) described two other nullisomics in which different meiotic disturbances were seen. In this study nullisomic-7 (line R 742) was associated with a reduction in chiasma frequency in at least six bivalents which formed rods. This behaviour could be related to the desynaptic effect caused by the absence of chromosome 7 in Avena byzantina (31).

In the course of this study, several irregularities were detected in the reproduction. For instance, monosomic-20 (line 4-27) gave twin embryos one of which was a normal monosomic whereas the other was a mono-triploid (62 chromosomes); it also produced a 82-chromosome plant that died at an early stage. The R 751 line produced a mono-haploid plant which is presently growing in the greenhouse. In the progenies of monosomics 7 and 20 three 40-chromosome plants formed only 19 bivalents plus two univalents. These possibly resulted from a univalent shift (22) probably by the fusion of a 21-chromosome male gamete with a 19-chromosome egg. These aberrants were detected because they did not exhibit the typical nullisomic phenotype. This occurrence and detection stress the importance of markers in the maintenance of a monosomic series, and the necessity of continuous checks in such work.

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