DETERMINATION OF THE CHROMOSOMAL LOCATION OF A GLUTAMATE OXALOACETATE TRANSAMINASE STRUCTURAL GENE USING TRITICUM-AGROPYRON TRANSLOCATIONS

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ABSTRACT

The glutamate oxaloacetate transaminase (GOT) zymogram phenotypes of a series of 15 translocation lines, a chromosome addition line and a chromosome substitution line were determined. In each of the translocation lines a segment of the long arm of Triticum aestivum chromosome 3D has been replaced by a portion of an Agropyron elongatum homoeologue. Evidence was obtained that the products of the T. aestivum GOT-3 triplicate structural gene set randomly dimerize with the product of the homoeologous A. elongatum gene. Each translocation chromosome was found to carry either Got-D3 or Got-Ag3. By correlating the zymogram phenotype expressed by each translocation line with the observed frequency of meiotic pairing of each 3D/3Ag translocation chromosome with telocentric-3D1, it was shown that Got-D3 is located in the proximal portion of 3DL, slightly more than 4.3 crossover units from the centromere. The results of this genetic study confirm and extend earlier conclusions derived from cytogenetic studies as to the physical nature of the various 3D/3Ag chromosomes.

STRUCTURAL genes for a number of isozymes have been linked to specific chromosome arms of hexaploid wheat (Triticum aestivum L. em. Thell., 2n = 42, genomes A, B, and D) by zymogram analyses of appropriate aneuploid strains (BARBER et al. 1968, 1969; HART 1970, 1973, 1975; HART and LANGSTON 1975; NISHIKAWA and NOBUHARA 1971). The available aneuploid strains of the cultivar “Chinese Spring” allow an examination of the phenotypic effects of from zero to four doses of each chromosome and of most chromosome arms (SEARS 1954, 1966a, 1966b). The structural gene linkages have been obtained by the demonstration that the level of expression of a specific gene product varies concordantly with the dosage of a specific chromosome arm. This type of genetic analysis accordingly employs as genetic variation genetic differences between

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the A, B, and D genomes of the species rather than differences between allelic forms of genes.

This paper reports the determination of an approximate gene-centromere genetic distance for a glutamate oxaloacetate transaminase (GOT: E.C.2.6.1.1.) structural gene, Got-D3, located in the long arm of chromosome 3D. Hexaploid wheat expresses several genetically-independent GOT systems (Hart 1974, 1975). Got-D3 is one of three members of a homoeologous set of structural genes which encode the isozymes of the GOT-3 system (Hart 1975). The genetic distance between gene and centromere is readily determined by means of telocentric chromosomes (Sears 1962, 1966b), provided allelic variation is available. In the absence of a variant form of Got-D3, we have used as genetic variation a difference between Got-D3 and Got-Ag3, a homoeologous Agropyron elongatum gene. The genetic distance was determined by zymogram analysis of a series of 15 translocation lines in which segments of 3DL of substantially differing lengths have been replaced by portions of 3AgL, an A. elongatum homoeologue (Sears 1972a, 1973). The results of this study confirm and extend Sears' earlier conclusions (1973) as to the physical nature of the 3D/3Ag chromosomes.

This paper also reports recent cytological studies of five other translocation chromosomes of similar derivation and a zymogram analysis of lines containing these chromosomes.

Earlier studies of variation in proteins and enzymes associated with segmental interchanges between hexaploid wheat and related species have been reported by Bhatia and Smith (1966), Upadhya (1968), Barber et al. (1969), and Macdonald and Smith (1972).

MATERIALS AND METHODS

The pedigree and characteristics of the 15 homozygous 3D/3Ag translocation lines used in this study have been described (Sears 1972a, 1973). 3Ag, an Agropyron elongatum (2n=70, x=7) chromosome which conditions resistance to a leaf-rust fungus, Puccinia recondita, is substituted for wheat chromosome 3D in the line designated TAP 67. A substitution line derived from TAP 67 by a cross and three backcrosses to “Chinese Spring” was made monosomic for chromosome 5B, and was then used as the female parent in a cross with “Chinese Spring” nullisomic-5B, tetrasoniic-5D, in order to obtain nulli-5B, tri-5D, mono-3D, mono-3Ag plants. Meiotic pairing between 3D and 3Ag was made possible in these plants due to the absence of Ph, the chromosome 5B locus which prevents homoeologous pairing. Pairing of 3D with 3Ag occurred in about 30% of the meiocytes of the plants, as a consequence of which numerous 3D/3Ag translocation chromosomes carrying leaf-rust resistance were recovered. Fifteen disomic lines, each homozygous for a different 3D/3Ag translocation chromosome, were subsequently derived. Tests of these lines indicated that in each a segment of the long arm of the Agropyron chromosome 3Ag (3AgL, formerly designated 3Ag0) had replaced a segment of the long arm of wheat chromosome 3D (3DL, formerly 3Da).

Five additional lines containing other translocation chromosomes (described in Sears (1973) and in the Results section of this paper) derived from this same sequence of crosses were also analyzed with the zymogram technique. Four of the five lines—those carrying chromosomes 10, 12, 13, and 20—were homozygous for the translocation chromosome while the fifth carried chromosome 9 in the heterozygous condition. Translocation chromosome 12, which is composed of segments of both 3B and 3D of wheat as well as 3Ag, was substituted for chromosome 3D in the
line examined. In each of the other four lines, the translocation chromosome was substituted for the wheat chromosome involved in the translocation.

Also determined were the GOT-3 zymogram phenotypes of a chromosome substitution line (carrying 20’”+1”3Ag(3D)) derived from TAP 67 by a cross and three backcrosses to “Chinese Spring” and of a chromosome addition line (carrying 21’”+1”3Ag) derived from TAP 67 by a cross and five backcrosses to “Chinese Spring”. The Agropyron accession whose chromosome is contained in these lines is no longer available.

Extracts were obtained for electrophoresis from shoots of nine-day-old etiolated seedlings grown in moist paper toweling at 23°. Preparation of the tissue extracts, disc acrylamide gel electrophoresis, and staining for GOT activity were performed as described by Hart (1975). A minimum of three plants of each line was analyzed.

RESULTS

Zymogram analyses

Among the strains examined, a total of three GOT-3 zymogram phenotypes was observed (Figure 1). The phenotypes are distinguished solely by major differences in the relative staining intensities of the bands which compose them. Differences in number or in electrophoretic mobilities of bands was not observed. I designates the phenotype in which the two more cathodal bands (#’s 2 and 3) stain much more intensely than the anodal band (#1), II the phenotype in which the two more anodal bands stain much more intensely than the cathodal band, and III the phenotype in which the band of intermediate mobility stains with greater intensity than the approximately equally intense anodal and cathodal bands.

Table 1 reports the GOT-3 phenotype of each strain.

Cytological analyses

Cytological data obtained since 1973 on the 15 homozygous 3D/3Ag translocations (combined with the previous findings into Table 2) are chiefly of note for the failure of translocation chromosomes 6 and 16 to pair with “Chinese Spring” telocentric-3DL, and for the excellent pairing with “Chinese Spring”

**Figure 1.** Photograph of the three GOT-3 zymogram phenotypes observed. Roman numerals identify the phenotypes while the bands which compose the phenotypes are identified by numbers. Migration was toward the anode from the origin, as indicated by the arrow.
## TABLE 1

GOT-3 zymogram phenotypes of Triticum-Agropyron lines examined

<table>
<thead>
<tr>
<th>Substitution and addition Phenotype</th>
<th>3D/3Ag translocations Line Phenotype</th>
<th>Other translocations Line Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20(^{+}1^{3}3Ag(3D))</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>21(^{+}1^{3}3Ag)</td>
<td>III</td>
<td>II</td>
</tr>
</tbody>
</table>

The latter result supports the idea that the short arm of each of the 3D/3Ag translocation chromosomes is either an intact 3DS or includes a rather long terminal segment of 3DS. The seven chromosomes checked showed very good pairing with the telocentric for the Agropyron arm (3AgL) that carries the gene \(Lr\) for leaf-rust resistance. In fact, each of the seven showed better pairing with the telocentric than with the

## TABLE 2

Meiotic metaphase I pairing of Triticum-Agropyron 3D/3Ag translocation chromosomes

<table>
<thead>
<tr>
<th>Translocation chromosome number</th>
<th>Telo-3DL No. %</th>
<th>Telo-3DS No. %</th>
<th>3Ag No. %</th>
<th>Telo-3AgL No. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 11</td>
<td></td>
<td>100 91</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>200 28</td>
<td>74 89</td>
<td>64 84</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>138 64</td>
<td>100 93</td>
<td>43 84</td>
<td>100 95</td>
</tr>
<tr>
<td>4</td>
<td>89 0</td>
<td></td>
<td>125 83</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>41 0</td>
<td>61 95</td>
<td>43 84</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100 0</td>
<td>100 97</td>
<td>100 77</td>
<td>50 100</td>
</tr>
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</tr>
<tr>
<td>8</td>
<td>100 0</td>
<td>62 97</td>
<td>200 77</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>100 0</td>
<td>100 93</td>
<td>100 80</td>
<td>50 100</td>
</tr>
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<td>14</td>
<td>200 70</td>
<td>100 95</td>
<td>76 72</td>
<td>100 94</td>
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<td>15</td>
<td>100 1</td>
<td></td>
<td>100 75</td>
<td>90 98</td>
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<tr>
<td>16</td>
<td>50 0</td>
<td>82 94</td>
<td>43 70</td>
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</tr>
<tr>
<td>18</td>
<td>100 0</td>
<td>100 99</td>
<td>200 76</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>100 28</td>
<td>100 93</td>
<td>100 86</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>29 0</td>
<td>100 95</td>
<td>98 47</td>
<td>50 100</td>
</tr>
</tbody>
</table>
complete 3Ag chromosome. This was probably an artifact resulting from the impossibility of distinguishing between an unpaired 3Ag chromosome and various wheat chromosomes unpaired by chance, which were therefore scored as instances of 3Ag asynapsis. Telo-3AgL could always be distinguished from chance univalents, because of its shortness and its terminal centromere.

The Triticum chromosome(s) involved in three of the five other translocations have now been determined. Translocation 12, on the basis of its ability to pair with not only 3D but also some other chromosome, was believed to be the result of exchange between 3D and not only 3Ag but also 3B or 3A (SEARS 1973). Subsequent studies have established that 3B is the third chromosome involved in the translocation. A plant which showed 19 bivalents and 1 trivalent (19"+1") at meiosis and which was known to be nullisomic for 3D and to have one dose of the translocation chromosome and two doses of each of the other chromosomes was crossed to "Chinese Spring" mono3B. The resistant 41-chromosome offspring had predominantly 20"+1" while 19"+1"+1" was found in the resistant 42 chromosome sibs. This identified the trivalent, already known to include the translocation chromosome, as also involving two 3B chromosomes. The univalent had to be 3D, which is known to pair only rarely with the translocation chromosome.

When combined with chromosome 3B (or with telocentric-3BS), translocation chromosome 12 paired in about 90% of the microsporocytes. With telo-3BL and telo-3DS, it paired not at all, and with telo-3DL about 3%. In tests with chromosomes 3 and 14, chromosome 12 showed about 95% pairing.

Translocation chromosomes 10 and 13 have now been found to involve chromosome 3B. From a cross of a translocation 10 heterozygote onto "Chinese Spring" monosomic-3B, a rust-resistant, monosomic F1 plant was selected and carried into F2, where, in a family of 29, only the eight apparent nullisomics (two confirmed cytologically) were susceptible. This was as expected if the F1 monosome was the translocation chromosome, but was beyond expectation (only one-fourth of the nullisomics susceptible) if chromosome 3B was not involved in the translocation. From an F2 monosomic, one F3 seedling with 42 chromosomes was tested and found resistant to rust, while a sister seedling with 40 chromosomes (presumably nullisomic) was susceptible, again as expected.

Results with translocation 13 were similar. In the F2 from "Chinese Spring" mono-3B × translocation line, 26 plants were resistant and one, the only nullisomic, susceptible. A disomic proved homozygous as expected, when tested in F3.

The translocation 13 F2 monosomics and the disomic, with one or two doses, respectively, of the translocation chromosome and none of the intact 3B, were synaptic. "Chinese Spring" chromosome arm 3BL carries a gene, symbolized Syn, required for normal synapsis. Presumably translocation chromosome 13 carries this 3BL gene, for indications are that 3AgL has a weaker allele. Translocation 12, which is believed to include nearly the entire long arm of 3Ag (see discussion below), does not suppress asynapsis when substituted monosomic for disome 3B. Disomics and monosomics for translocation 10 are synaptic, thus this chromosome, like translocation 13, apparently carries the 3BL Syn allele.
The wheat chromosomes involved in translocations 9 and 20 have not been identified. Either 3A or 3B is probably involved in translocation 20, but translocation 9 now seems much more likely to involve 3D than either 3A or 3B (Sears unpublished data).

**DISCUSSION**

The triplicate set of structural genes which encode the isozymes of the GOT-3 system of hexaploid wheat was localized to specific arms of the homoeologous group 3 chromosomes by zymogram analysis of appropriate aneuploid derivatives of the cultivar "Chinese Spring" (Hart 1975). The results of the study were consistent with the proposal that the three genes produce approximately equal quantities of subunits, that the subunits randomly dimerize to produce the active GOT-3 isozymes, and that each of the six dimeric forms produced are approximately equally active. The structural genes located in chromosome arms 3Aα, 3BL, and 3DL have been designated Got-A3, Got-B3, and Got-D3, respectively, and the subunits which they encode as α3, β3, and δ3, respectively. The three GOT-3 isozymes of hexaploid wheat are designated GOT-3a (β3β3, δ3δ3, and β3δ3 dimers), GOT-3b (α3β3 and α3δ3) and GOT-3c (α3α3). “Chinese Spring” produces the GOT-3 zymogram phenotype II, with GOT-3a, -3b, and -3c occurring at the sites of bands 1, 2, and 3, respectively (Figure 2).

The 20'+1'3Ag(3D) chromosome substitution line expresses phenotype I (Table 1). Aneuploid derivatives of “Chinese Spring” which are tetrasomic for chromosome 3A and nullisomic for either 3D or 3B, and which consequently possess four doses of Got-A3 and but two doses of either Got-D3 or Got-B3, also express phenotype I (Hart 1975). Expectations which are entirely consistent with the phenotypes produced by each of the strains in this study are generated by the hypothesis that chromosome 3Ag carries a GOT-3 structural gene whose phenotypic expression as determined by the zymogram technique is indistinguishable from that of Got-A3. We designate the gene as Got-Ag3 and the subunit which it encodes as ε3.

With respect to the chromosome substitution line, the hypothesis predicts that the random association of α3, β3, and ε3 subunits produces the three isozymes GOT-3a (β3β3 dimers), GOT-3b (α3β3 and ε3β3), and GOT-3c (α3α3, ε3ε3, and α3ε3) (Figure 2). The expected quantitative distribution of the isozymes will be based on \((p + q)^2\), where \(p = 1/3 = \) the frequency of the β3 subunit and \(q = 2/3 = \) the frequency of the α3 and ε3 subunits combined. The observed relative staining intensities of the three bands which compose the GOT-3 zymogram phenotype of the substitution line are in good agreement with the expected 1:4:4 distribution of the three GOT-3 substitution line isozymes.

The chromosome addition line expresses phenotype III (Table 1). According to the hypothesis proposed, it should possess four GOT-3 structural genes and active dimers composed of all possible combinations of the subunits β3, δ3, α3, and ε3 (Figure 2). The expected proportions of the three GOT-3 isozymes will be based on \((p + q)^2\), where \(p = 1/2 = \) the frequency of the β3 and δ3 sub-
**CHROMOSOMAL LOCALIZATION OF Got-D3**

**Table 1**

<table>
<thead>
<tr>
<th>Isozymes</th>
<th>Triticum aestivum cv 'Chinese Spring'</th>
<th>Chromosome Substitution Line</th>
<th>Chromosome Addition Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOT-3a</td>
<td>4/9 ( \beta^3, 3^3 ), ( \varepsilon^3 )</td>
<td>1/9 ( \beta^3, 3^3 )</td>
<td>1/4 ( \beta^3, 3^3, 3^3 )</td>
</tr>
<tr>
<td>GOT-3b</td>
<td>4/9 ( \alpha^3, 3^3 )</td>
<td>4/9 ( \alpha, 3^3 )</td>
<td>2/4 ( \alpha^3, 3^3, 3^3 )</td>
</tr>
<tr>
<td>GOT-3c</td>
<td>1/9 ( \alpha^3 )</td>
<td>4/9 ( \alpha, 3^3, 3^3 )</td>
<td>1/4 ( \alpha, 3^3, 3^3, 3^3 )</td>
</tr>
</tbody>
</table>

**Figure 2**—Schematic model for the subunit composition of the GOT-3 isozymes produced by *T. aestivum* cv. "Chinese Spring", the chromosome substitution line and the chromosome addition line. Dimers on the same line in the figure have coincident electrophoretic mobility. The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.

The degree of similarity between Got-Ag3 and the genes of the Got-3 set of hexaploid wheat is, however, striking. Indeed, there has been no greater divergence in those properties observable with the zymogram technique between the product of Got-Ag3 and the product of the hexaploid wheat homoeoalleles than there has been between the products of the three members of the hexaploid wheat Got-3 set. And the latter have been observed to have diverged only in that the Got-A3 subunit has a different electrophoretic mobility from that of the other two subunits. The evidence available to date indicates that Got-Ag3 and the three *T. aestivum* genes each produce approximately the same quantity of subunit, that the four types of subunits randomly associate to produce the active dimeric molecules, and that each of the dimeric types produced is equally active. This
suggests that there has been no significant divergence between *A. elongatum* and *T. aestivum* in the genes which regulate GOT-3 and but little divergence in the gene which specifies the GOT subunit. Essentially identical findings have been reported for four homoeologous *Secale cereale-T. aestivum* structural gene sets. These include the sets which encode the GOT-2 and GOT-3 isozymes (Tang and Hart 1975), alcohol dehydrogenase (Iranì and Bhatia 1972; Tang and Hart 1975), and an esterase system (Barber et al. 1968, 1969). Secale, like Agropyron, is a member of the subtribe Triticinae, tribe Triticeae, which includes Triticum.

Each 3D/3Ag translocation line possesses two doses of both *Got-A3* and *Got-B3*. The composition of each line with respect to *Got-D3* and *Got-Ag3* was determined at the time of the origin of the translocated chromosome, and is a function both of the location of the crossover between 3D and 3Ag and of the location of the two genes in their respective chromosomes. Since differences in gene order may exist between 3D and 3Ag, a given crossover between the two chromosomes could conceivably produce a translocated chromosome possessing both or neither of the two genes, or a chromosome possessing *Got-D3* or *Got-Ag3* alone.

A translocation line carrying two doses of both *Got-D3* and *Got-Ag3* would possess a GOT-3 genotype identical to that of the chromosome addition line and thus, like it, would be expected to express phenotype III. A relative staining intensity of 1:2:1 for bands 1, 2, and 3, respectively, is also expected for a line carrying neither *Got-D3* nor *Got-Ag3*, since the dimers produced by the random association of $a^3$ and $b^3$ would be expected to be distributed among the three GOT-3 isozymes based on $(p + q)^2$, where $p = 1/2 = \text{frequency of the } a^3\text{ subunit and } q = 1/2 = \text{frequency of the } b^3\text{ subunit}$. However, two such lines should be distinguishable, since the intensity of staining of the GOT-3 isozymes relative to other GOT isozymes present in the gels will be twice as great for lines possessing both genes as for lines possessing neither gene. We have observed this difference between the chromosome addition line and the “Chinese Spring” derivative designated ditelosomic-3DS, a strain known to lack *Got-D3*.

Each of the 15 3D/3Ag translocation lines expresses either phenotype I or II (Table I), a finding which allows the conclusion that each line carries two doses of either *Got-D3* or *Got-Ag3*. With respect to the Got-3 genes, lines which express II are identical to “Chinese Spring”—they carry *Got-A3*, -B3, and -D3—while lines which express I are identical to the chromosome substitution line—they carry *Got-A3*, -B3, and -Ag3.

Since it is highly probable that all of the 3D/3Ag chromosomes possess a substantial terminal segment of 3AgL (Sears 1973), the finding that several of the lines express *Got-D3* (Table I) suggests that *Got-D3* is situated in the proximal portion of 3DL. This would place the GOT locus proximal to the 3Ag segment in 3D/3Ag lines possessing *Got-D3* and distal to the 3D segment in lines possessing *Got-Ag3*. The finding that lines expressing *Got-D3* retain larger segments of 3DL than do lines expressing *Got-Ag3* would constitute evidence in favor of this suggestion.

The ability of each of the 3D/3Ag chromosomes to synapse with telocentric-
3DL has been assessed by observations made at meiotic metaphase I (Table 2). Of the seven 3D/3Ag chromosomes that have been observed to pair with 3DL, five (chromosomes 1, 2, 3, 14, and 19) carry Got-D3 (See Table 1). The percentage of pairings observed among these five chromosomes varied in the range from 11 to 70. Chromosomes 7 and 15, observed to pair with 3DL in but 2% and 1%, respectively, of the cells examined (based on 100 observations for each chromosome), carry Got-Ag3. These findings are quite significant in that they allow no reasonable conclusion but that Got-D3 is located in the proximal portion of 3DL, distal to the points of exchange in 3D/3Ag-7 and 3D/3Ag-15 and proximal to the point of exchange in 3D/3Ag-1, a chromosome which shows 11% pairing with 3DL and carries Got-D3.

Fortunately, the amount of crossing over between the centromere and the exchange point has been computed for translocation chromosome 7 (Table 2, Sears 1973). The value determined, 4.3%, is based on a small sample. However, it is reasonably consistent with the amount of crossing over expected on the basis of the frequency of metaphase I pairing observed, given that metaphase determinations of pairing frequencies are known to substantially underestimate actual chiasma frequencies in chromosomes of this type (Fu and Sears 1973). Thus a location for Got-D3 somewhat greater than 4.3 crossover units from the centromere on 3DL is indicated.

The position of Got-D3 is further localized by the fact that Got-D3 is carried by two of the eight translocation chromosomes which have not been observed to pair with 3DL. The possession of Got-D3 by translocation chromosomes 4 and 6 indicates that each retains a larger proximal segment of 3DL than does either chromosome 7 or 15. However, the failure to observe pairing of chromosomes 4 and 6 with telocentric-3DL in 89 and 100 cells, respectively (Table 2), indicates that they must retain but slightly larger segments of 3DL than chromosomes 7 and 15, since the latter two chromosomes were observed to pair with telocentric-3DL in 2% and 1%, respectively, of the cells examined. In view of the low frequency of pairing of chromosomes 7 and 15 with telocentric-3DL observed at metaphase I, small differences between chromosomes 4, 6, 7, and 15 is the true rate at which they form chiasmata with 3DL could readily be obscured by environmental effects or by observation of the chromosomes at different substages of metaphase I. Consequently, a location for Got-D3 only slightly greater than 4.3 crossover units from the centromere in chromosome arm 3DL is indicated.

The location proposed for Got-D3 may be yet more accurately defined by determination of the amount of crossing over between the centromere and the exchange point in translocation chromosomes 4, 6, and 15. A maximum centromere—Got-D3 genetic distance will be set by the lower of the two crossover values determined for chromosomes 4 and 6 and a minimum of the higher of the two values determined for chromosomes 7 and 15.

The approximate constitution of the 15 3D/3Ag chromosomes suggested by the GOT zymogram and meiotic pairing results reported herein and by meiotic
pairing, male transmission, and exchange point-centromere genetic distance determinations reported earlier (Sears 1973) is shown in Figure 3.

It was pointed out above that differences in gene order may exist between chromosomes 3D and 3Ag. However, the results of this study provide no evidence for structural divergence between the two chromosomes. Four genetic exchanges have occurred closely adjacent to Got-D3, two on the distal and two on the proximal side, but the translocation chromosome recovered in each case possesses but one Got-3 structural gene. This would seem to indicate that Got-D3 and Got-Ag3 are situated in chromosomal regions which retain the ancestral gene order.

The gene-centromere genetic distance determined for Got-D3 in this study may be a considerable underestimate of the amount of crossing over that occurs in this interval in plants which possess two complete 3D chromosomes. The value of 4.3 crossover units was determined in plants heterozygous for telocentric-3DL and 3D/3Ag-7. There is evidence that the amount of crossing over near the centromere is considerably reduced in bivalents which include a telocentric chromosome (Sears 1972b; Driscoll and Sears 1965; Endrizzi and Kohel 1966).

Translocation lines 10 and 13, each homozygous for a 3B/3Ag chromosome,
each express GOT-3 phenotype II (Table 1). Got-B3 and Got-D3 have equal and interchangeable effects on the zymogram phenotype, as do Got-A3 and Got-Ag3. Consequently, expression of phenotype II by lines 10 and 13 indicates that both 3B/3Ag-10 and 3B/3Ag-13 carry Got-B3 rather than Got-Ag3. The lines would express phenotype I if the translocation chromosomes possessed the Agropyron homoeoallele in place of Got-B3. It may therefore be concluded that the exchange point in both 3B/3Ag-10 and 3B/3Ag-13 is distal to the Got-B3 locus (Figure 3). Also, a location for Syn, the gene required for normal synapsis, in the proximal portion of chromosome arm 3BL is indicated, since it appears that both of these translocation chromosomes carry this 3BL gene.

Translocation line 12, homozygous for a 3B/3Ag/3D double translocation chromosome substituted for 3D, expresses GOT-3 phenotype I (Table 1). This indicates that this translocation chromosome carries Got-Ag3 rather than Got-B3 or Got-D3. Two doses each of Got-A3 (carried by 3A), Got-B3 (carried by 3B), and Got-Ag3 (carried by 3B/3Ag/3D-12) constitutes a Got-3 genotype identical to that of the chromosome substitution line, known to produce phenotype I (Figures 1 and 2), while two doses of Got-A3 plus either two doses each of Got-B3 and Got-D3 or four doses of Got-B3 would produce phenotype II.

It was originally assumed that chromosome 3D contributed the middle portion, including the centromere, to translocation chromosome 12, and the chromosome arms 3AgL and 3BS (or 3AS) the end portion (Sears 1973). Since all of the other translocations were alike in having the distal portion of 3AgL, it seemed reasonable to assume that translocation 12 had involved a 3D-3Ag crossover in the same region as the other chromosomes, but that in addition there had been a crossover between the short arm of 3D and that of 3B (or 3A). However, recent findings indicate that the order suggested for the 3B, 3D, and 3Ag chromosome segments is incorrect; the middle segment is almost surely 3Ag chromosomal material. This is established by the fact that Rodríguez-Loperena, A et al. (1976) found that a line with a pair of translocation 12 chromosomes substituted for the 3D pair lacks a 3DS protein gene and has the corresponding 3AgS gene or genes. Thus translocation 12 must have part of both of the chromosome arms 3AgS and 3AgL. Presumably it has the 3Ag centromere and the proximal portion of both 3Ag arms, with the 3B and 3D segments both being terminal; otherwise, an improbable double crossover in one arm would have been necessary. The possession of Got-Ag3 by chromosome 12 is consistent with this alignment of the segments and makes a double crossover in the long arm particularly improbable.

That the 3D and 3B segments of translocation chromosome 12 are from the long and short arms, respectively, as shown in Figure 3, is demonstrated by the ability of this chromosome to pair with telocentric-3BS and telo-3DL but not with telo-3BL or telo-3DS. If any other 3D/3Ag translocations occurred with the breakpoint distal to Lr (that is, resulting in largely 3Ag chromosomes, with only the end of their long arm from 3D) they must have escaped detection because of too little pairing with 3D. Indeed, translocation 12 was only recovered because of the substantial segment of 3B comprising the distal portion of its other arm.
In all of the 3D/3Ag translocations, the 3Ag segment is evidently long enough to support regular pairing with chromosome 12, for the two with the shortest segments, chromosomes 3 and 14, pair regularly with chromosome 12. Crossing over between chromosome 12 and any of the others should give rise to a recombined chromosome with the 3DL end of 12 replacing the 3AgL end of the other.

The wheat chromosomes involved in translocations 9 and 20 have not yet been identified. The line carrying translocation 9 in the heterozygous condition and the line homozygous for translocation 20 both express GOT-3 zymogram phenotype II (Table 1). This suggests only that each line carries two doses of either Got-A3 or Got-Ag3 and four doses of the other two Got-3 genes combined (i.e., either two doses of each or four doses of one). Consequently, the GOT-3 zymogram phenotype of the lines suggests neither the wheat chromosomes involved nor the location of the exchange point in translocation chromosomes 9 and 20.

The available evidence concerning the relative degree of genetic relationship of chromosomes 3A, 3B, and 3D to 3Ag is as yet insufficient to allow a firm conclusion. At this time the evidence suggests that 3B is more closely related to 3Ag than is 3A, but there is little basis for assigning 3D a position in this grouping.

Chromosome 3Ag substituted spontaneously for 3D in TAP 67 (Bakshi and Schlehuber 1959), in “Chinese Spring” it perhaps pairs best with 3B (Sears 1973), and it has the same GOT homoeoallele as 3A. The substitution for 3D occurred only once, insofar as is known, and perhaps it could just as well have been for 3A as 3D. Presumably a substitution for 3B would result in asynapsis and sterility and would therefore be eliminated. However, this is not necessarily true for, while “Chinese Spring” carries a gene essential for normal synapsis on 3B, in another variety this gene might be masked.

If translocations 9 and 20 prove to involve chromosome 3B rather than 3A (giving a total of five translocations with 3B involvement), then the conclusion that 3Ag pairs preferentially with 3B rather than 3A seems justified. However, even if five of the 20 translocations involve 3B, this may not mean a higher level of homoeology between 3Ag and 3B than between 3Ag and 3D. It is not known whether, when 5B and the attendant Ph locus are absent, the presence of a homologue of a particular chromosome interferes with the pairing of that chromosome with a homologue any more than a third homologue interferes with pairing of the other two.

The possession by chromosome 3Ag of the same homoeoallele as 3A, an allele which differs from that carried by 3B and 3D, may be of physiological significance. However, knowledge of several systems is required before the allelic constitution of sets of homoeologous genes may be utilized to assess the relative degree of genetic relationship of homoeologous chromosomes.

LITERATURE CITED


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