Genet. Res., Camb. (1983), **41**, *pp.* 181–202
With 2 text-figures
Printed in Great Britain

Chromosomal locations of eleven *Elytrigia elongata* (= *Agropyron elongatum*) isozyme structural genes

By GARY E. HART* and NEAL A. TULEEN†

Department of Plant Sciences and Department of Soil and Crop Sciences,†, Texas A & M University, College Station, Texas, 77843*

(*Received 10 September 1982 and in revised form 23 November 1982*)

SUMMARY

The zymogram phenotypes of 11 enzymes were determined for 22 *Triticum aestivum* cv. Chinese Spring-*Elytrigia elongata* disomic and ditelosomic chromosome addition lines. Eleven isozyme structural genes were located in specific arms of six *E. elongata* chromosomes, as follows: *Gpi-E1* in 1ES, *Est-E1* in 3ES, *Got-E3* in 3EL, *Adh-E1* and *Lpx-E1* in 4ES, *Adh-E2* and *Lpx-E2* in 5EL, *Amp-E1* in 6Eα, *Adh-E3* and *Got-E2* in 6Eβ, and *Ep-E1* in 7EL. The *E. elongata* chromosomes present in five disomic addition lines have previously been designated 1E, 2E, 4E, 6E, and 7E to indicate their homoeology with Chinese Spring chromosomes. The results of this study support these designations. The development of disomic putative 3E and 5E addition lines is reported. The added chromosomes designated IV, V, and VI that are present in three of the seven original disomic *T. aestivum-E. elongata* addition lines are translocated. Evidence that VL and VIL are opposite arms of 2E and that IV is partially homoeologous to 3E has been published. The results reported in this paper indicate that IVS = 3ES, IVL = 7EL, VS = 3ES, and VIS = 5ES and are consistent with VL and VIL being opposite arms of 2E. The synteny relationships of the 11 *E. elongata* isozyme genes identified in this study are fully consistent with those of homoeologous *T. aestivum* cv. Chinese Spring genes and thus provide evidence that the gene synteny groups which these two species inherited from their common ancestor are conserved. This study further documents the valuable role that studies of isozyme genes can play in the isolation, characterization, and maintenance of alien chromosomes, telosomes, and chromosomal segments in wheat strains.

1. INTRODUCTION

The relatives of the cultivated wheats in the tribe Triticeae are a rich potential source of genetic variation for many agriculturally significant characteristics. Successful wide crosses among different Triticeae genera are relatively easily accomplished (Sakamoto, 1973) and cytogenetic techniques have been developed for transferring intact alien chromosomes and parts of alien chromosomes into common bread wheat (*Triticum aestivum*, $2n = 6x = 42$, genomes A, B, and D)

(O'Mara, 1940; Sears, 1956; Riley, Chapman & Johnson, 1968; see also Riley & Kimber, 1966; Sears, 1975). Consequently, the genetic variation of the whole of the tribe, comprising approximately 15 genera (Sakamoto, 1973), is potentially available for wheat improvement.

A prerequisite for the efficient exploitation of alien genetic material in wheat improvement is a high level of understanding of the genetic constitution and evolutionary relationships of Triticeae species, genomes, and chromosomes. Unfortunately, most Triticeae relatives of the cultivated wheats are as yet largely unexplored genetically. Only in cultivated barley (*Hordeum vulgare*, $2n = 14$, genome H) have more than a few genes been identified and located in chromosomes.

The available knowledge of the genetic content of individual chromosomes of Triticeae relatives of the cultivated wheats, other than *H. vulgare*, has been derived largely from studies of the ability of alien chromosomes to substitute for and to pair with specific wheat chromosomes. The degree of similarity in fertility, morphology, vigor, and other characteristics between euploid wheat plants and plants which contain a particular alien chromosome substituted for a wheat chromosome is an indication of the degree of genetic relationship between the substituted alien chromosome and the missing wheat chromosome. Chromosomes from several relatives, including cultivated rye (*Secale cereale*, $2n = 14$, genome R) (see, for example, Riley, 1965; Sears, 1968), cultivated barley (Islam, 1980; Shepherd & Islam, 1981), and *Elytrigia elongata* (= *Agropyron elongatum*, $2n = 14$, genome E) (Dvořák, 1980) have been successfully substituted for wheat chromosomes. Tests of the ability of alien and wheat chromosomes to pair are usually carried out by constructing plants which contain one copy of both the alien chromosome of interest and a known wheat telocentric chromosome and which also either lack or contain a suppressed chromosome 5B *Ph* locus (see Kimber & Sears, 1980). Pairing of the wheat telosome in these circumstances indicates that it and the alien chromosome are in some part homoeologous. Some alien chromosomes are capable of a substantial amount of homoeologous pairing with wheat chromosomes, e.g. several *E. elongata* chromosomes (Dvořák, 1979). However, the chromosomes of rye will pair little if at all with their wheat homoeologues (Riley & Kimber, 1966; Beilig & Driscoll, 1970). When the results of either of the two aforementioned types of tests provide evidence that an alien and wheat chromosome are to some extent homoeologous, a portion of the genetic content of the alien chromosome is tentatively identified, based on the known genetic content of the wheat chromosome. However, only a few individual alien genes have been directly identified in these types of studies.

The most effective means currently available for identifying alien genes and determining their chromosomal locations is the study of isozyme expression in wheat lines that contain alien genetic material (Hart, 1979*a*). This method has been used to identify and determine the chromosomal locations of several isozyme structural genes of rye (Barber *et al.* 1968, 1969; Driscoll & Sears, 1971; Irani & Bhatia, 1972; Tang & Hart, 1975; Hart, 1978; Rao & Rao, 1980) and barley (Hart, Islam & Shepherd, 1980; Powling, Islam & Shepherd, 1981) and of one wild relative

of the wheats, *E. pontica* ($2n = 10x = 70$) (Hart, McMillin & Sears, 1976; Kobrehel, 1978). (For a discussion of the taxonomic status of *E. pontica*, also named *Ag. elongatum*, see Dvořák, 1981*a*, *b*). Most of the alien isozyme structural genes thus far identified have been shown to be orthologous with the members of a triplicate set of paralogous hexaploid wheat genes whose chromosomal locations are known. (Orthologous genes are homologous genes present in different species while paralogous genes are homologous genes present in the same species (Fitch & Margoliash, 1970; Fitch, 1973).) This ability to define chromosomal relationships in terms of specific genetic material is among the advantages the study of isozyme expression has over studies of chromosomal pairing and genetic compensation. Also an advantage is that evidence for wheat-alien chromosome homoeology can be obtained by analyses of disomic chromosome addition lines; these are produced and maintained with comparative ease relative to the efforts required to construct the plants needed for studies of chromosome pairing and genetic compensation.

This paper reports the results of a zymogram study of several enzymes that was carried out on twenty-two strains of *T. aestivum* which contain different added pairs of *E. elongata* telosomes or whole or translocated chromosomes. Included among the strains were the five whole chromosome addition lines developed by Dvořák & Knott (1974) and Dvořák (1980) and two putative whole chromosome additional lines developed by us (Tuleen & Hart, in preparation). The principal findings and analyses reported are as follows: (1) Evidence for the structural gene basis of 11 *E. elongata* enzymes; (2) models for the subunit structure of five dimeric *E. elongata* enzymes and of heterodimers formed between wheat and *E. elongata* protomers; (3) the chromosomal arm locations of 11 isozyme structural genes in six *E. elongata* chromosome; (4) evidence regarding the composition of the translocated *E. elongata* chromosomes that are present in three of the seven disomic addition lines developed by Dvořák & Knott (1974); and (5) deductions regarding the evolutionary relationships among *E. elongata* and *T. aestivum* chromosomes that stem from the gene locations reported. A brief account of some of this research was published in a review (Hart, 1979*a*).

2. MATERIALS AND METHODS

The manner in which wheat lines containing alien genetic material may be used to determine the chromosomal locations of isozyme genes in wheat relatives and to establish homoeologies between wheat and alien genes and chromosomes was recently reviewed (Hart, 1979*a*). Only a brief summary is given here.

For the study of a given relative of the wheats the most useful of the various possible wheat-alien chromatin types is a complete disomic chromosome addition series. For a $2n = 14$ relative, this consists of seven lines, each of which contains the full complement of 21 pairs of hexaploid wheat chromosomes and an added pair of alien chromosomes. The two parental strains and the wheat-alien species amphiploid hybrid, to the extent that they are available, should also be analysed. Studies are preferably conducted on enzymes whose subunit composition and

structural gene basis in hexaploid wheat are known. With this information available, comparison of the zymogram phenotype of the amphiploid with that of the parental wheat strain will usually disclose the specific origin of each of the gene products expressed in the amphiploid and the addition lines, including protomers contained in hetero-oligomers. Confirmation of these findings and disclosure of the chromosomal locations of the genes that encode the alien isozymes and protomers can usually be accomplished by comparing the phenotypes of the chromosome addition lines. Appropriate ditelosomic addition lines, if available, may be used to further locate the alien genes in chromosome arms. Study of the alien parental strain, if available (or of other derivatives of the related species), may provide additional evidence for the specific origin of the gene products

Table 1. *Enzymes studied*

Name	Abbreviated name	E.C. number
Alcohol dehydrogenase-1	ADH-1	1.1.1.1
Alcohol dehydrogenase-2	ADH-2	1.1.1.91
Alcohol dehydrogenase-3	ADH-3	1.1.1.90
Aminopeptidase-1	AMP-1	3.4.11.–
Endopeptidase-1	EP-1	3.4.21-24.–
Esterase-1	EST-1	3.1.–.–
Glutamic-oxaloacetic transaminase-2	GOT-2	2.6.1.1
Glutamic-oxaloacetic transaminase-3	GOT-3	2.6.1.1
Glucosephosphate isomerase-1	GPI-1	5.3.1.9
Lipoxygenase-1	LPX-1	1.13.11.12
Lipoxygenase-2	LPX-2	1.13.11.12

expressed in the amphiploid and the addition lines. However, except when the related species is naturally self-fertilizing and the alien parental strain is available for study, the addition series may well carry at any gene locus an allele that is not present in any of the plants of the related species that are examined.

Dvořák & Knott (1974) used the procedure of O'Mara (1940) to produce from an amphiploid hybrid of *T. aestivum* cv. Chinese Spring *x E. elongata* (= *Agropyron elongatum*; see Dvořak, 1980) four disomic addition lines containing chromosomes 1E* (formerly designated I), 4E (III), 6E (VII), and 7E (II) and three other disomic addition lines containing chromosomes designated IV, V, and VI. Dvořák later (1980) developed a disomic 2E addition line and we (Tuleen & Hart, in preparation) have developed disomic putative chromosome 3E and 5E addition lines (designated

* Genomes in the Triticeae are designated by capital Roman letters and chromosomes by Arabic numerals (1–7), with related chromosomes in different genomes assigned the same Arabic numeral. Chinese Spring is accepted as having the standard chromosome arrangement (McIntosh, 1973). In this paper, the arms of *T. aestivum* chromosomes are designated p and q to indicate homoeologies, consistent with the recommendations of Sears & Sears (1979), and also S and L or α and β, consistent with the recommendations contained in McIntosh (1973). S and L designate short and long arms, respectively, while α and β designate arms whose length relationships are not known, α designating the arm first isolated as a telosome. *E. elongata* chromosome arms are designated only with the symbols S and L or α and β.

herein as VIII and IX, respectively) from the same amphiploid. The zymogram phenotypes of 11 enzymes, listed in Table 1, were determined for each of the aforementioned ten lines, for the *T. aestivum* cv. Chinese Spring–*E. elongata* amphiploid, and for Chinese Spring. The *E. elongata* strain used to develop the wheat–*E. elongata* amphiploid is not available. Accession 524 of Chinese Spring (obtained from Dr E. R. Sears, University of Missouri, Columbia) was used in zymogram studies of each of the 11 enzymes. Accession 818 of Chinese Spring (obtained from Dr K. W. Shepherd, University of Adelaide, Australia) was also studied in the analyses of GPI-1. This latter accessions was used as the wheat parent in the initial crosses with the *T. aestivum–E. elongata* amphiploid and in ensuing backcrosses during the development of the putative 3E and 5E addition lines. Subsequently we determined that A818 expresses a different GPI-1 zymogram phenotype than A524. Recently, Dr M. Gale and colleagues (personal communication) have determined that the genetic basis for this difference is the presence of a null allele at the *Gpi-D1* locus in A818.

The zymogram phenotypes of one or more ditelosomic addition lines were also determined for each enzyme, as follows: 1ES (GPI-1), 4EL (ADH-1 and LPX-1), IXL (= putative 5EL) (ADH-2 and LPX-2), 6Eα and 6Eβ (ADH-3, AMP-1, and GOT-2), 7ES and 7EL (EP-1), IVS and IVL (EP-1 and EST-1), VS and VL (EST-1), and VIS (ADH-2 and LPX-2). (Telosome 4EL was initially designated as the α arm of 4E and 7ES as the α arm of 7E.) These ditelosomic addition lines were developed by Dvořák & Knott (1974), with the exception of 7EL, which was produced by Dvořák (1979), and IXL and 6Eβ, which were produced by Tuleen & Hart (in preparation). All of the lines, with the exception of 7EL, were developed with hexaploid wheat cv. Chinese Spring.

The ADH-1 and GPI-1 analyses were conducted with extracts of scutella obtained from grains germinated for 16 h and 48 h, respectively. The extracts used for the study of ADH-3 were obtained from the first foliage leaf of 5-day-old seedlings and for the study of AMP-1, EP-1, EST-1, GOT-2, and GOT-3 from the first foliage leaf of 7-day-old seedlings. A combined shoot-root extract obtained from 7-day-old seedlings was used for the study of ADH-2. Coleoptiles of 4-day-old seedlings were the source of LPX-1 and LPX-2. All grains and seedlings were grown on moist filter paper in Petri dishes in an incubator at 23 °C in the dark, except the seedlings used for the study of EST-1 which were grown under continuous light after they reached 2 days of age. Extracts were obtained by maceration of tissue with sand in a mortar with pestle in a pH 7·5 buffer containing 0·1 M-tris-(hydroxymethyl)aminomethane-HCl, 0·1 M-KCl, 0·005 M-EDTA, 0·04 M-2-mercaptoethanol, and 0·1 M-sucrose. Each scutellum used for ADH-1 analysis was macerated in 125 μl and each used for GPI-1 analysis in 300 μl of the extraction buffer. A weight : volume ratio of tissue : buffer of 1 : 2 was used for all other enzymes except the LPXs and GOTs for which ratios of 1 : 5 and 1 : 6 were used, respectively. The slurry obtained by maceration was centrifuged at 12000 × g for 20 min. The supernatant obtained was used directly for electrophoresis. Extraction, centrifugation, and electrophoresis were carried out at 2–5 °C. The zymogram

phenotype of each enzyme was determined for a minimum of three grains (ADH-1 and GPI-1) or plants of each chromosomal type.

For the determination of the ADH-1, ADH-2, ADH-3, EST-1, GOT-2, GOT-3, LPX-1, and LPX-2 zymogram phenotypes, extracts were electrophoresed in disk acrylamide gels (100 μl of supernatant/gel for ADH-1, ADH-3, and EST-1, 200 μl/gel for ADH-2, LPX-1, and LPX-2, and 50 μl/gel for GOT-2 and GOT-3) using procedures described by Hart (1975). The separation gel was made to 0·5 % Electrostarch for study of LPX-1 and LPX-2. Gels were stained for ADH-1 as described by Hart (1970), except for the omission of NaCN and $MgCl_2$ from the staining solution. The procedure of Jaaska (1978) was used to stain ADH-2 and also, using an equal molar amount of NAD in place of NADP, for ADH-3. ADH-1 isozymes stain faintly on ADH-3 zymograms and also partially overlap the isozymes of ADH-3 in electrophoretic mobility. Consequently, in studying the latter enzyme, it is necessary to carefully distinguish between the bands produced by the two enzymes. EST-1 was stained as described by Kahler & Allard (1970), GOT-2 and GOT-3 as described by Yang (1971), and LPX-1 and LPX-2 using the procedure of Guss *et al.* (1968) as modified by Hart & Langston (1977).

The AMP-1, EP-1, and GPI-1 zymogram phenotypes were determined in starch gels (Electrostarch, 12 %, w/v) using methods described by Hart (1973) for AMP-1 and EP-1 and by Gottlieb (1973) as modified by Hart (1979*b*) for GPI-1. (A 0·05 M-tris-0·007 M-citric acid buffer and a 0·02 M-lithium hydroxide-0·19 M-boric acid buffer, prepared using the quantities of reagents specified by Scandalios (1969), from whom these buffers are taken (Gottlieb, 1973), were used for electrophoresis of GPI-1. Both of these buffers are erroneously described as being '0·2 M' in Hart (1979*b*), Gottlieb (1973), and in Scandalios (1969).) The differences in electrophoretic mobility among certain AMP-1 and certain EP-1 isozymes are quite small. Consequently, electrophoresis was continued for these enzymes until the most anodal AMP-1 isozyme had migrated 18–20 cm and the most anodal EP-1 isozyme 20–22 cm from the sample slots. AMP-1 and EP-1 were stained as described by Hart (1973) and by Tang & Hart (1975) and GPI-1 as described by Hart (1979*b*).

All zymograms were routinely observed and their phenotypes recorded early in the staining process when the level of their staining was still low since it is during this period of time that the relative levels of staining activity of different bands most accurately reflect the relative levels of activity of the isozymes which produce the bands.

3. RESULTS

The available evidence (see below) indicates that five of the enzymes studied are active as monomers and six as dimers. Each of the monomeric enzyme zymogram phenotypes observed in this study is either diagrammed in the body of Fig. 1 or described, with the aid of the diagrams, in the legend to the same figure. Fig. 2 contains diagrams of the observed dimeric enzyme zymogram phenotypes. Table 2 reports the phenotype expressed for each monomeric enzyme and Table 3 the phenotype expressed for each dimeric enzyme by each of the strains examined.

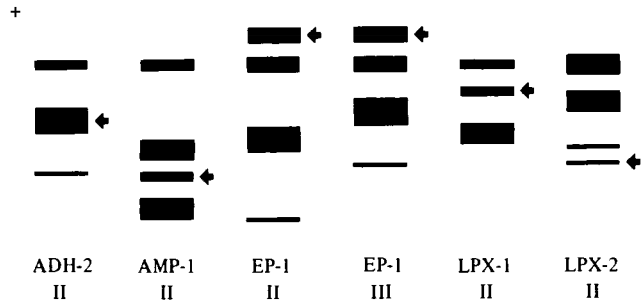

Fig. 1. Diagrams of some of the zymogram phenotypes observed for the five monomeric enzymes studied. Phenotype I of ADH-2 (not shown) differs from phenotype II by a major reduction in intensity of the band indicated by the arrow relative to the other two bands. For each of the other enzymes, phenotype I (not shown) differs from phenotype II by the absence of the band indicated by the arrow. The vertical positioning of the ADH-2, AMP-1, EP-1, LPX-1, and LPX-2 diagrams relative to one another does not indicate the true relative electrophoretic mobilities among the multiple forms of these enzymes. However, the relative mobilities among the two groups of EP-1 isozymes are as indicated by the vertical positioning of the two diagrams.

Table 2. *Zymogram phenotypes expressed by the strains examined for the five monomeric enzymes studied**

Strain	Enzyme				
	ADH-2	AMP-1	EP-1	LPX-1	LPX-2
Chinese Spring	I	I	I	I	I
Chinese Spring–*E. elongata* amphiploid	II	II	II	II	II
Disomic addition lines					
1E	I	I	I	I	I
2E	I	I	I	I	I
VIII (= putative 3E)	I	I	I	I	I
4E	I	I	I	II	I
IX (= putative 5E)	II	I	I	I	II
6E	I	II	I	I	I
7E	I	I	II†	I	I
IV	I	I	II	I	I
V	I	I	I	I	I
VI	I	I	I	I	I
Ditelosomic addition lines					
4EL	—	—	—	I	—
IXL (= putative 5EL)	II	—	—	—	II
6Eα	—	II	—	—	—
6Eβ	—	I	—	—	—
7ES	—	—	I	—	—
7EL	—	—	III	—	—
IVS	—	—	I	—	—
IVL	—	—	II	—	—

* See the body and legend of Fig. 1 for either a diagram or a description of each phenotype.

† Line 7E when originally received was segrating for three EP-1 phenotypes, namely I, II, and a third phenotype in which the anodal band has a relative staining intensity intermediate to that present in phenotypes I and II. See Results.

https://doi.org/10.1017/S0016672300021212 Published online by Cambridge University Press

(i) *Monomeric enzymes*

The enzymes ADH-2, AMP-1, EP-1, and LPX-2 are expressed in three molecular forms and the enzyme LPX-1 in two molecular forms in the tissues of *T. aestivum* cv. Chinese Spring from which these enzymes were obtained for this study. Aneuploid genetic studies of Chinese Spring have provided evidence that the active forms of each of these enzymes are monomers that are encoded by the members of triplicate sets of paralogous genes (for references, see Table 5).

The AMP-1, EP-1, LPX-1, and LPX-2 zymogram phenotypes of the amphiploid hybrid between Chinese Spring and *E. elongata* differ from those of Chinese Spring by the presence of an additional band. The ADH-2 phenotypes of both Chinese Spring and the amphiploid consist of three bands; however, the intermediate band has greater staining intensity relative to the other two bands in the amphiploid phenotype than it does in the phenotype of Chinese Spring (see Fig. 1 and Table 2). For each enzyme one of the disomic chromosome addition lines among the group of seven that includes 1E, 2E, VIII (= putative 3E), 4E, IX (= putative 5E), 6E, and 7E expresses the same phenotype as the amphiploid (phenotype II) while six of the lines express a phenotype indistinguishable from that of Chinese Spring (phenotype I; see Fig. 1 and Table 2).

We interpret these findings as indicating that for each of these enzymes the difference between phenotypes I and II is the expression in the latter of an *E. elongata* isozyme that is encoded by a gene located in the *E. elongata* chromosome that is present in the disomic addition line that expresses phenotype II.

(*a*) *Alcohol dehydrogenase-2*

Disomic addition line IX, the putative 5E addition line, was derived from the amphiploid using the *E. elongata* form of LPX-2 as a chromosome marker (see *Lipoxygenase-2* below). The expression of ADH-2 phenotype II by the amphiploid and by disomic addition line IX and ditelosomic line IXL indicates that the *E. elongata* structural gene for this enzyme is located in the long arm of IX. Consistent with our conclusion that this *E. elongata* gene is orthologous to the members of the *T. aestivum Adh-2* set, i.e. to *Adh-A2*, *Adh-B2*, and *Adh-D2*, we designate it as *Adh-E2*, in accordance with the recommended rules for gene symbolization in wheat (McIntosh, 1973).

(*b*) *Aminopeptidase-1*

We designate the *E. elongata* AMP-1 structural gene as *Amp-E1*. The expression of phenotype II by lines 6E and 6Eα and of I by 6Eβ indicates that *Amp-E1* is located in 6Eα.

(*c*) *Endopeptidase-1*

We designate the *E. elongata* EP-1 structural gene as *Ep-E1*. The expression of EP-1 phenotype III by ditelosomic addition line 7EL and of phenotype I by line 7ES indicates *Ep-E1* is located in 7EL. (The differences in electrophoretic

mobility between the two cathodal EP isozymes of line 7EL and of Chinese Spring presumably are due to a genetic difference(s) between Chinese Spring and the wheat strain that was used in the development of line 7EL.)

In contrast with the ditelo results, anomolous results were obtained in studies of certain presumed disomic 7E addition lines. The EP-1 zymogram phenotypes were determined for several seedlings in each of seven F_1 populations produced from seven of the ten seeds of addition line 7E received from Dr J. Dvořák. In three populations (numbers 1, 2 and 3) each seedling examined expressed phenotype II, indicative of homozygosity for *Ep-E1*, while each of the seedlings examined in one population (number 4) expressed phenotype I, indicative of either the lack of expression or the absence of *Ep-E1*. In each of three other populations (numbers 5, 6, and 7) three phenotypes were observed, namely I and II and a third phenotype in which the *E. elongata* band was present but of reduced relative intensity, a phenotype indicative of the expression of one rather than two *Ep-E1* genes.

We have determined that the alien chromosome present in population number 4 will pair with chromosome 7E, with telosome 7ES, and with chromosome VI and will not pair with telosome 7EL and also that chromosomes 7E and VI will not pair. In addition, we have evidence that VIS = 5ES (unpublished results of Tuleen & Hart; see also Discussion). Thus, since plants of population number 4 do not have waxless foliage or tenacious glumes, characters which are determined by genes located in VIL (Dvořák & Knott, 1974; Dvořák, 1980), it is likely that population number 4 is homozygous for a translocated chromosome consisting of 7ES and 5ES and that both this chromosome and an intact chromosome 7E are present in populations 5, 6, and 7, homozygosity for 7E producing phenotype II, homozygosity for the translocated chromosome producing phenotype I, and heterozygosity for these two chromosomes producing the third phenotype.

Lines IV and IVL express *Ep-E1* (phenotype II) while line IVS does not (phenotype I), suggesting that IVL is 7EL. The results of chromosome pairing tests support this proposal (see Discussion).

(*d*) *Lipoxygenase-1*

Expression of LPX-1 phenotype II by line 4E and of phenotype I by 4EL indicates that *Lpx-E1*, the *E. elongata* LPX-1 structural gene, is located in 4ES.

(*e*) *Lipoxygenase-2*

An *E. elongata* form of LPX-2 is expressed by the amphiploid hybrid between Chinese Spring and *E. elongata* but not by disomic addition lines 1E, 2E, 4E, 6E or 7E or by lines IV, V, or VI. This suggested to us that this isozyme, the Chinese Spring molecular forms of which are encoded by a triplicate set of homoeologous chromosome group 5 genes, could be used as a marker to isolate chromosome 5E from the amphiploid. Following a cross of the amphiploid with Chinese Spring and appropriate backcrosses, a disomic addition line which expresses LPX-2 phenotype II was isolated (Tuleen & Hart, in preparation). The added chromosome present in this line is assigned IX as a temporary designation. The available evidence (see

Discussion) indicates that this chromosome is an intact chromosome 5E. Pairing tests with the chromosome are now underway. A long arm ditelosomic derivative of chromosome IX expresses phenotype II, thus locating the LPX-2 structural gene, which we designate as *Lpx-E2*, in this arm.

(ii) *Dimeric enzymes*

The enzymes ADH-1, ADH-3, EST-1, GPI-1, GOT-2, and GOT-3 are each expressed in three molecular forms in the tissues of *T. aestivum* cv. Chinese Spring from which these enzymes were obtained for this study. Aneuploid genetic studies of Chinese Spring (for references, see Table 5) and other anlyses have provided evidence that the active forms of each of these enzymes are dimers produced by the association in all possible combinations of promoters encoded by a minimum of three paralogous genes. Three isozymes, rather than a larger number, are produced due to the coincident electrophoretic mobilities of the products of two of the three genes. For the enzymes ADH-1, ADH-3, GOT-2, and GOT-3, the relative staining intensities of the zymogram bands of Chinese Spring and of various aneuploid derivatives of Chinese Spring are in good agreement with the distribution of isozymes expected to result from the random association in all possible combinations of three protomers produced in equal quantities per gene copy present by three genes located one each in homoeologous chromosome arms. However, the relative staining intensities of the bands that compose the GPI-1 and the EST-1 phenotypes are consistent with the production of a twofold greater quantity per relevant chromosome arm present of one protomer than of the other two, indicating that one member of each of the two triplicate sets of genes that encode these enzymes may be duplicated (see below).

For each dimeric enzyme, the amphiploid hybrid between Chinese Spring and *E. elongata* expresses a zymogram phenotype which differs from that of Chinese Spring. The difference for four enzymes (ADH-3, GPI-1, GOT-2, and GOT-3) is only in the relative staining intensities of the zymogram bands while for two enzymes (ADH-1 and EST-1) both the number of bands and their relative staining intensities differ (see Fig. 2 and Table 3). For each of these six enzymes one of the disomic addition lines among the group of seven that includes 1E, 2E, VIII (= putative 3E), 4E, IX (= putative 5E), 6E, and 7E expresses the same phenotype as the amphiploid while six of the lines express a phenotype indistinguishable from that of Chinese Spring (see Fig. 2 and Table 3).

The simplest genetic hypothesis consistent with the aforementioned findings is that for each dimeric enzyme the isozymes expressed by the amphiploid and by the phenotypically identical addition line are composed of dimeric combinations of four protomers, three of which are encoded by wheat genes and one of which is encoded by an orthologous gene located in the *E. elongata* chromosome present in the addition line.

Schematic models for each of these enzymes, except EST-1 (see below), for the subunit composition of the isozymes expressed by Chinese Spring and by the

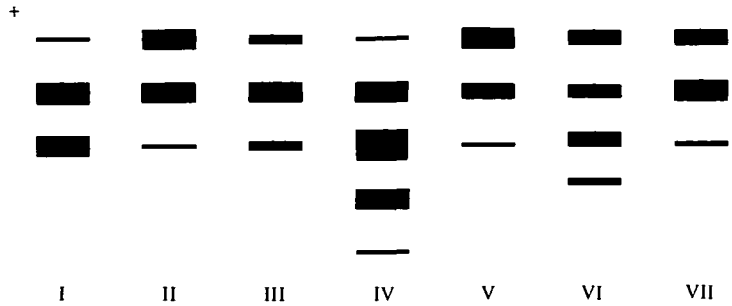

Fig. 2. Diagrams of the zymogram phenotypes observed for the six dimeric enzymes studied. Two or three of these phenotypes were observed for each enzyme (see Table 3). For each *individual* enzyme the vertical positioning of the diagrams relative to one another indicates the approximate relative electrophoretic mobilities among the isozymes expressed in different phenotypes. However, the vertical positioning of the diagrams does not indicate the relative electrophoretic mobilities among the multiple forms of *different* enzymes.

Table 3. *Zymogram phenotypes expressed by the strains examined for the six dimeric enzymes studied**

Strain	Enzyme					
	ADH-1	ADH-3	EST-1	GPI-1	GOT-2	GOT-3
Chinese Spring						
Accession 524	I	II	V	III	I	II
Accession 818	—	—	—	I	—	—
Chinese Spring–*E. elongata* amphiploid	IV	III	VI	VII	III	III
Disomic addition lines						
1E	I	II	V	VII	I	II
2E	I	II	V	III	I	II
VIII (= putative 3E)	I	II	VI	I	I	III
4E	IV	II	V	III	I	II
IX (= putative 5E)	I	II	V	I	I	II
6E	I	III	V	III	III	II
7E	I	II	V	III	I	II
IV	I	II	VI	III	I	II
V	I	II	VI	III	I	II
VI	I	II	V	III	I	II
Ditelosomic addition lines						
1ES	—	—	—	VII	—	—
4EL	I	—	—	—	—	—
6Eα	—	II	—	—	I	—
6Eβ	—	III	—	—	III	—
IVS	—	—	VI	—	—	—
IVL	—	—	V	—	—	—
VS	—	—	VI	—	—	—
VL	—	—	V	—	—	—

* See Fig. 2 for a diagram of each phenotype.

https://doi.org/10.1017/S0016672300021212 Published online by Cambridge University Press

Table 4. *Schematic models for the subunit composition of the ADH-1, ADH-3, GPI-1, GOT-2, and GOT-3 isozymes produced by Chinese Spring and by the Chinese Spring–*E. elongata *amphiploid*

(The multiple forms of each enzyme are identified with lower case letters assigned consecutively starting with the most anodal isozyme. The expected quantitative distribution of each group of isozymes is indicated by the ratios preceding the dimers).

Isozymes	Chinese Spring accession 524	Amphiploid	Chinese Spring accession 818
ADH-1a	1/9 $\alpha\alpha$	1/16 $\alpha\alpha$	—
ADH-1b	4/9 $\alpha\beta,\alpha\delta$	4/16 $\alpha\beta,\alpha\delta$	—
ADH-1c	4/9 $\beta\beta,\delta\delta,\beta\delta$	6/16 $\beta\beta,\delta\delta,\beta\delta,\alpha\epsilon$	—
ADH-1d	—	4/16 $\beta\epsilon,\delta\epsilon$	—
ADH-1e	—	1/16 $\epsilon\epsilon$	—
ADH-3a	4/9 $\beta\beta,\delta\delta,\beta\delta$	1/4 $\beta\beta,\delta\delta,\beta\delta$	—
ADH-3b	4/9 $\alpha\beta,\alpha\delta$	2/4 $\alpha\beta,\alpha\delta,\epsilon\beta,\epsilon\delta$	—
ADH-3c	1/9 $\alpha\alpha$	1/4 $\alpha\alpha,\epsilon\epsilon,\alpha\epsilon$	—
GPI-1a	1/4 $\alpha\alpha,\delta\delta,\alpha\delta$	9/25 $\alpha\alpha,\delta\delta,\epsilon\epsilon,\alpha\delta,\alpha\epsilon,\delta\epsilon$	1/9 $\alpha\alpha$
GPI-1b	2/4 $\alpha\beta,\delta\beta$	12/25 $\alpha\beta,\delta\beta,\epsilon\beta$	4/9 $\alpha\beta$
GPI-1c	1/4 $\beta\beta$	4/25 $\beta\beta$	4/9 $\beta\beta$
GOT-2a	1/9 $\delta\delta$	1/4 $\delta\delta,\epsilon\epsilon,\delta\epsilon$	—
GOT-2b	4/9 $\alpha\delta,\beta\delta$	2/4 $\alpha\delta,\beta\delta,\alpha\epsilon,\beta\epsilon$	—
GOT-2c	4/9 $\alpha\alpha,\beta\beta,\alpha\beta$	1/4 $\alpha\alpha,\beta\beta,\alpha\beta$	—
GOT-3a	4/9 $\beta\beta,\delta\delta,\beta\delta$	1/4 $\beta\beta,\delta\delta,\beta\delta$	—
GOT-3b	4/9 $\alpha\beta,\alpha\delta$	2/4 $\alpha\beta,\alpha\delta,\beta\epsilon,\delta\epsilon$	—
GOT-3c	1/9 $\alpha\alpha$	1/4 $\alpha\alpha,\epsilon\epsilon,\alpha\epsilon$	—

amphiploid (and by other lines which carry two doses of the *E. elongata* gene) are presented in Table 4. In each of the models, α, β, and δ designate the protomers encoded by paralogous wheat genes located in genomes A, B, and D, respectively, while ϵ designates the protomer encoded by the *E. elongata* gene.

The expected quantitative distributions of the isozymes shown in Table 4 are based on $(p+q+r+s)^2$, where p, q, r, and s represent the frequencies of α, β, δ, and ϵ, respectively. Table 5 cites references in which the schematic models for the Chinese Spring isozymes were derived. For the amphiploid for ADH-1, ADH-3, GOT-2, and GOT-3, $p = q = r = s = 1/4$. This assumes that the four protomers are produced in equal quantities and associate randomly in all possible combinations to produce active dimeric molecules. The expected quantitative distribution of the GPI-1 isozymes of the amphiploid is derived with $p = r = s = 1/5$ and $q = 2/5$ since the available evidence (Hart, 1979*b*; see also above) is consistent with the production in Chinese Spring of a twofold greater quantity of the β protomer than of the α and δ protomers.

The amphiploid and one or more addition lines express dimeric enzyme phenotype IV for ADH-1, phenotype III for ADH-3, GOT-2, and GOT-3, and phenotype VII for GPI-1. The relative staining intensities of the bands which compose these phenotypes are in good accord with the expected quantitative

distribution shown in Table 4 for each of these enzymes. Consequently, we accept for these enzymes the genetic hypothesis stated above.

(*a*) *Alcohol dehydrogenase-1*

We designate the *E. elongata* ADH-1 structural gene as *Adh-E1*. The expression of phenotype IV by disomic addition line 4E and of phenotype I by line 4EL indicates that *Adh-E1* is located in 4ES.

(*b*) *Alcohol dehydrogenase-3*

Expression of phenotype III by addition lines 6E and 6Eβ and of phenotype II by 6Eα indicates that *Adh-E3*, the structural gene for ADH-3, is located in 6Eβ.

(*c*) *Esterase-1*

T. aestivum contains numerous esterases. EST-1 of Chinese Spring consists of three isozymes that form a discrete group at the anodal end of zymograms prepared as described in this paper. A model for their subunit composition has been proposed (Barber *et al.* 1968) and strong evidence has been obtained for the formation in chromosome addition lines of active EST-1 molecules by the association of Chinese Spring protomers with protomers encoded by Imperial rye (Barber *et al.* 1968, 1969) and by other rye strains and *T. longissimum* (Hart & Tuleen, unpublished).

The zymogram phenotype expressed in the EST-1 zone by the Chinese Spring–*E. elongata* amphiploid and by disomic addition lines IV and V, designated as phenotype VI, is diagrammed in Fig. 2. The genetic basis of this phenotype is still being investigated. We do not have definitive evidence that the variation between this phenotype and that of Chinese Spring is due to an *E. elongata* gene that is orthologous to the Chinese Spring *Est-1* set of genes. However, based on the fact that the phenotypes differ not only in the number of bands but as well in the relative staining intensities of the three anodal bands and on the existence of strong evidence (cited above) for the location of an orthologous *Est-1* gene in various other relatives of wheat, we tentatively adopt the conclusion that the strains which express phenotype VI carry two doses of an *E. elongata Est-1* gene, the products of which associate with *T. aestivum Est-1* protomers to produce active molecules. We designate this gene as *Est-E1*.

Disomic addition lines IV and V and ditelosomic addition lines IVS and VS both express phenotype VI while ditelosomic addition lines IVL and VL both express phenotype V, indicating that an *Est-E1* locus is present in both IVS and VS. The presence of a copy of *Ep-E1*, a 7EL gene, in IVL (see *Endopeptidase-1* above) and the lack of expression of an *E. elongata Got-3* gene (the *Got-3* genes of Chinese Spring are located in the 3q (= 3L) arms and the *Est-1* genes in the 3p (= 3S) arms) in any of the seven original disomic addition lines or in line 2E (see *Glutamic-oxaloacetic transaminase-3* below) suggested to us that an intact chromosome 3E

is not present in any of these lines. We thus initiated a project to isolate such a chromosome from the amphiploid, using first *Est-E1* alone as a chromosome marker and then later the *E. elongata Got-3* gene also. This endeavour resulted in the isolation of a disomic addition line which expresses both a *Got-3* gene (see below) and *Est-E1* (Tuleen & Hart, in preparation). The added chromosome in this line has been assigned VIII as a temporary designation. The available evidence (see Discussion) indicates that this chromosome is an intact chromosome 3E. Pairing tests with the chromosome are now underway. A ditelosomic derivative of line VIII has not yet been produced. However, we have obtained good evidence that *Est-E1* and the *E. elongata Got-3* gene are located in the short and long arms, respectively, of chromosome VIII (Tuleen & Hart, in preparation).

Table 5. *Chromosomal locations of* Elytrigia elongata *isozyme structural genes and of orthologous* Triticum aestivum *genes in cv. Chinese Spring*

E. elongata gene	Chromosomal location	Chromosomal locations* of orthologous *T. aestivum* genes in cv. Chinese Spring	Reference(s) for *T. aestivum* gene locations†
Gpi-E1	1S	1p	(7)
Est-E1	3S	3p	(1,2,3)
Got-E3	3L	3q	(6)
Adh-E1	4S	4p	(4,5,9)
Lpx-E1	4S	4p	(9)
Adh-E2	5L	5q	(10)
Lpx-E2	5L	5q	(9)
Amp-E1	6α	6p	(5,9)
Adh-E3	6β	6q	(8)
Got-E2	6β	6q	(6)
Ep-E1	7L	7q	(9)

* p = S and q = L except for homoeologous chromosome group 4 for which p designates the α arm of 4A, the L arm of 4B, and the S arm of 4D.

† (1) Barber *et al.* (1968); (2) Barber *et al.* (1969); (3) Bergman (1972); (4) Hart (1970); (5) Hart (1973); (6) Hart (1975); (7) Hart (1979*b*); (8) Hart (in preparation); (9) Hart & Langston (1977); (10) Jaaska (1978).

(*d*) *Glucosephosphate isomerase-1*

Disomic addition line 1E and ditelosomic addition line 1ES both express phenotype VII as does the Chinese Spring–*E. elongata* amphiploid. The other disomic addition lines, with the exceptions of VIII and IX, express the same phenotype as accession 524 of Chinese Spring. Disomic addition lines VIII and IX express phenotype I, the same phenotype as the accession of Chinese Spring, namely, 818, that was used in the development of these lines from the amphiploid. These results indicate that *Gpi-E1*, the *E. elongata* GPI-1 structural gene, is located in 1ES.

(*e*) *Glutamic-oxaloacetic transaminase-2*

We designate the *E. elongata* GOT-2 structural gene as *Got-E2*. The expression of phenotype III by lines 6E and 6Eβ and of phenotype I by 6Eα indicates that *Got-E2* is located in 6Eβ.

(*f*) *Glutamic-oxaloacetic transaminase-3*

Expression of phenotype III by line VIII indicates that *Got-E3*, the structural gene for GOT-3, is located in the *E. elongata* chromosome present in this line. As noted above (see *Esterase-1*), the available evidence indicates that this chromosome is an intact 3E and that *Got-E3* is located in 3EL.

(iii) *Chromosomal locations of* E. elongata *genes*

Table 5 lists each gene location reported for genome E in this study and also the locations of orthologous *T. aestivum* genes in genomes A, B, and D of Chinese Spring.

4. DISCUSSION

(i) *Homoeologous genes*

Homoeologous genes can be identified by one or more of several types of investigations of the products which they encode (MacIntyre, 1976; Hart, 1979*a*). Similarity in developmental and tissue specificity of expression, the absence of expression of other similar gene products in the tissue examined, and similarity in electrophoretic behaviour were used as criteria for each of the genes identified in this study. In addition, good evidence in support of a model for the formation of active enzymes by the association of *E. elongata* and *T. aestivum* protomers was obtained for five of the six dimeric enzymes that were studied.

Our findings indicate that each of the 11 *E. elongata* genes identified in this study is orthologous to the members of a known set of paralogous *T. aestivum* genes (and thus orthologous also to other Triticeae genes previously shown to be orthologous to the same set of *T. aestivum* genes). Consequently, the symbol we have assigned to each *E. elongata* gene designates it to be a member of a specific homoeologous set of Triticeae genes (see McIntosh, 1973).

(ii) *Homoeology between* E. elongata *and* T. aestivum *chromosomes*

In the Triticeae, related chromosomes in different genomes are assigned the same Arabic numeral with Chinese Spring accepted as having the standard chromosome arrangement (McIntosh, 1973). The gene locations we report here are in full agreement with the designations previously assigned to chromosomes 1E, 2E, 4E, 6E, and 7E on the basis of other investigations, principally tests of genetic compensation and pairing (Dvořák, 1979, 1980). The gene synteny and asynteny

relationships determined for the seven genes located in chromosomes 1E (one gene), 4E (two genes in one arm), 6E (three genes, two in one arm and one in the other), and 7E (one gene) are entirely consistent with the locations of orthologous genes in Chinese Spring (see Table 5). None of the 11 genes studied is located in chromosome 2E. This is in itself significant since genes orthologous to these 11 genes mark eight of the 12 arms in Chinese Spring homoeologous chromosome groups 1, 3, 4, 5, 6, and 7. The location of any of the 11 genes in 2E would be evidence for a translocation difference(s) between *E. elongata* and Chinese Spring.

Our results and those of Dvořák (1980) indicate that the added chromosome present in three of the seven original disomic *E. elongata* addition lines developed by Dvořák & Knott (1974) is translocated, consisting in each case of parts of two *E. elongata* chromosomes. It is probable that in each case the translocation resulted from the fusion of telosomes that were produced by misdivisions of centromeres of univalents during meiosis (Sears, 1973).

Chromosome IV has *Est-E1*, a homoeologous group 3 gene, in the short arm (see Results) and will compensate in the gametophyte for 3A and 3D (Dvořák, 1980). However, it has *Ep-E1*, a group 7 gene, in the long arm (see Results) and also compensates in the gametophyte for 7A (Dvořák, 1980). We have evidence that both chromosome IV and telosome IVL will pair with 7E and that IVS will not pair with 7E (our results of these and various other pairing tests conducted with *E. elongata* chromosomes and telosomes will be published *in extenso* elsewhere). We also have evidence that IV will not pair with 7ES. In tetraploid hybrids between ditelosomic Chinese Spring–*E. elongata* addition lines and *T. speltoides* (= *Aegilops speltoides*, $2n = 14$, genome S), telosomes 7EL and IVL were determined to be of the same length (79·4 % and 79·5 %, respectively, of the length of 5Bq (= 5BL)) and to pair at the same frequency in pollen mother cells (see table 1 in Dvořák, 1979). It thus appears highly probable that IVS is an intact 3ES arm and that IVL is an intact 7EL.

Dvořák (1980) has concluded that VL is an arm of 2E. Our findings are in agreement with this conclusion. Only one of the 11 genes that were studied was located in V and this gene, *Est-E1*, is located in VS. We have evidence that IVS and VS will pair and that IVS will not pair with VL and that IVL will not pair with VS. In tetraploid hybrids between ditelosomic Chinese Spring–*E. elongata* addition lines and *T. speltoides* it was determined that telosomes IVS and VS have approximately the same length (50·3 % and 54·4 %, respectively, of the length of 5Bq (= 5BL)) and pair at similar, although significantly different, frequencies (23·6 % versus 27·6 %, respectively, which differs at the 5 % probability level) (see table 1 in Dvořák, 1979). It appears likely that the short arms of both chromosomes IV and V are the same intact 3E arm and thus that V is composed of an arm of 2E and the short arm of 3E.

Dvořák (1980) has concluded that VIL is the second arm of 2E. None of the 11 genes identified in this study were located in VI. Consequently, VIS is likely to be one of the four E genome chromosome arms that are, in addition to the arms of 2E, unmarked by one or more isozyme structural genes; that is, VIS is likely

to be either 1EL, 4EL, 5ES, or 7ES. We have evidence that a short arm telosome derived from chromosome IX, the putative 5E chromosome, will pair with VIS. Also, previously reported root tip telosome and chromosome length measurements are inconsistent with VIS being 1EL, 4EL, or 7ES. Both of the latter two telosomes are approximately 75% longer than VIS (table 1 in Dvořák, 1979). The length of 1EL has not been determined directly but the chromosome length and arm ratio measurements reported for chromosomes I (= 1E) and VI by Dvořák & Knott (1974, table 1) indicate that 1EL is significantly longer than VIS. It is thus quite probable that VIS is 5ES.

The location of *Got-E3* and *Est-E1* in different arms of chromosome VIII provides direct evidence that VIII is 3E since these two genes are orthologous to two triplicate sets of genes located in opposite arms of the Chinese Spring homoeologous group 3 chromosomes. Furthermore, if chromosomes 1E, 2E, 4E, 6E, and 7E have been correctly identified and each of the arms of VIII is intact, then VIII must be composed entirely of genetic material from one or both of the remaining two unidentified E genome chromosomes since VIII contains genes not found in any of the identified chromosomes and does not contain any genes known to be located in the five identified chromosomes. Our data are consistent with VIII being an intact 3E. However, it is also possible, although quite unlikely, that reciprocally translocated 3E and 5E chromosomes naturally exist in *E. elongata*, or are at least present in the Chinese Spring–*E. elongata* amphiploid, and that a translocation between two such chromosomes following centromere misdivisions during the development of addition line VIII produced a chromosome homoeologous to the group 3 chromosomes of Chinese Spring.

Adh-E2 and *Lpx-E2* are located in the long arm of chromosome IX, suggesting that IXL is 5EL and also that IX is 5E. However, given the observed high frequency of production of translocated chromosomes by centric fusions following centromere misdivisions during the production of addition lines, caution must be exercised in identifying the components of IX. If IXS is an intact translocated chromosome arm, then it must be one of the arms in which an isozyme structural gene has not been located, namely, 1EL, 2ES, 2EL, 4EL, or 7ES (see Table 5). But we have noted above that IXS and VIS pair with each other and appear to be the same chromosome arm and also that 1EL, 4EL, and 7ES are all significantly longer than VIS. Both IVL and VL, the two arms of 2E, are also approximately twice the length of VIS (Table 1 in Dvořák, 1979). It is thus probable that the added chromosome present in addition line IX is 5E. Definitive identification of the components of chromosomes VIII and IX should be obtained in pairing and compensation tests now underway.

(iii) *Conservation of gene synteny groups*

Sears (1966) concluded from his studies of numerous nullisomic-tetrasomic combinations that genetic homology in Chinese Spring is largely confined to homoeologous groups and thus that differentiation of the chromosomes of the three

genomes has involved few if any translocations. Anomolous behavior was observed for chromosomes 2A, 4A, and 6B that may be due to translocation but, in the case of 4A and 6B, deficiency in their homoeologues could produce the same effect. Riley & Chapman (1960) have shown that there are no interchanges in Chinese Spring that involve D genome chromosomes. They observed regular formation of seven bivalents and the complete absence of multivalents in a hybrid between Chinese Spring and *T. tauschii* ($2n = 14$, genome D). Chapman & Riley (1966) have concluded that some interchanges between A and B genome chromosomes exist in Chinese Spring, based on the observation of trivalents in AABD hybrids between Chinese Spring and *T. thaoudar* ($2n = 14$, genome A). The frequent participation of chromosomes 2A and 3A in trivalents indicates they are two of the chromosomes involved.

The chromosomal locations of 18 triplicate sets and one duplicate set of paralogous isozyme structural genes have been determined in Chinese Spring (Hart, 1982). One or more sets of genes are located in six of the seven homoeologous chromosome groups and in 10 of the 14 homoeologous chromosome arm groups. No isozyme gene that is a member of a paralogous set has thus far been located in chromosome 2A (or in 2B or 2D or in the 1q (= 1L) or 7p (= 7S) arms). However, four genes that are members of paralogous sets have been located in 3A (three in the p (= S) arm and one in the q (= L) arm), three genes in 4A (two in the p (= α) arm and one in the q (= β) arm) and six genes in 6B (two in the p (= S) arm and four in the q (= L) arm).

The locations of the 19 paralogous sets of isozyme genes thus far identified provide no evidence for the occurrence of translocations between the chromosomes of either the same or different genomes subsequent to the divergence of genomes A, B, and D from a common ancestral genome. The members of each set of paralogous genes are located one each in homoeologous chromosome arms.

Dvořák (1980) has presented a speculative account of the possible role of translocations in the differentiation of the three genomes of hexaploid wheat from genome E and from each other (the chromosome referred to as 2B by Sears (1966), now known to be 2A (Chapman & Riley, 1966), is erroneously referred to as 2B in this account). Although Dvořák discusses a considerable number of possible and probable translocations, he concludes by suggesting that translocations have played a relatively minor role in the differentiation of these genomes.

The 11 isozyme structural gene locations reported in this paper (Table 5) provide no evidence for the involvement of translocations in the differentiation of genome E from the genomes of Chinese Spring. Complete conservation of gene synteny relationships is indicated. There is also no evidence for the involvement of pericentric inversions in the differentiation of the E genome chromosomes from those of Chinese Spring. There is conservation of gene locations in chromosome arms, including short versus long arms (4B and also 2B are anomolous in this respect relative to their Chinese Spring homoeologues (Hart, 1979*a*; Sears & Sears, 1979; Dr L. M. S. Sears, personal communication)).

The chromosomal locations of a number of other genes orthologous to *T.*

aestivum genes have been reported for cv. Imperial of *S. cereale* (Barker *et al.* 1968, 1969; Tang & Hart, 1975; Hart, 1978, 1979*a*) and cv. Betzes of *H. vulgare* (Hart *et al.* 1980; Powling *et al.* 1981). Although there is cytological and genetic evidence (Riley, 1955; Tang & Hart, 1975; Koller & Zeller, 1976; Hart, 1978) for two translocation differences between the R genome of *S. cereale* and the genomes of *T. aestivum*, the gene locations reported to date for *S. cereale*, *H. vulgare*, and *E. elongata* support the proposal (Hart, 1979*a*) that the gene synteny relationships that existed in the ancestral Triticeae genome are largely conserved in the Triticeae genomes that exist today.

(iv) *Isozyme genes as chromosomal markers*

The valuable role that studies of isozyme structural genes can play in the isolation, characterization, and maintenance of alien chromosomes, telosomes, and chromosomal segments in wheat strains has been documented in several earlier investigations. Barber *et al.* (1968) showed, by study of an esterase, that evidence for wheat–alien chromosome homoeology can be obtained by determining the chromosomal locations of the related alien and wheat genes which encode a given enzyme (see also Irani & Bhatia, 1972). Driscoll & Sears (1971) demonstrated that an isozyme can be used as a chromosomal marker during the development of an alien chromosome addition line. The utility of using several isozyme structural genes to determine the presence or absence of alien chromosomes in existing addition lines and to obtain evidence for homoeology between alien and wheat chromosomes was shown by Tang & Hart (1975). Information concerning the physical nature of several translocated wheat–alien chromosomes was obtained by study of an isozyme structural gene locus contained in the translocated chromosomes (Hart *et al.* 1976). The value of using isozyme structural genes during the development of an alien chromosome addition series to distinguish among presumptive addition lines and to identify and characterize isolated chromosomes was demonstrated by Hart *et al.* (1980).

Isozyme structural genes would be valuable as markers in alien chromosomes even if translocation differences between different Triticeae genomes were frequent. However, given that the gene synteny relationships and the chromosomal arm locations of isozyme structural genes appear to be largely conserved in different Triticeae genomes, they are especially valuable since, during the development of addition lines, selection for specific isozyme genes may be used to attempt to isolate specific alien chromosomes. Furthermore, the gene synteny relationships in and among isolated chromosomes provide evidence regarding possible translocations. Three of the original seven *E. elongata* addition lines contain translocated chromosomes. The isozyme gene locations detected in two of these three chromosomes, namely, IV and V, were alone sufficient to suggest translocations. Also, the gene locations detected in these and the other four original lines and in chromosome 2E suggested as well the absence of intact 3E and 5E chromosomes. We report here the use of isozymes as markers to isolate putative 3E and 5E addition lines.

Ideally, in the development of addition lines selection would be practiced for at least two genes in each chromosome that are orthologous to genes located in opposite chromosome arms in the genomes of Chinese Spring. Unfortunately, paralogous gene sets have not yet been reported in four homoeologous arm groups (1q (= 1L), 2p (= 2AS, 2BL, and 2DS), 2q (= 2AL, 2BS, and 2DL), and 7p (= 7S)). The identification of genes in these arms must be a high priority for future research.

We are grateful to Dr J. Dvořák for generously supplying us with seed stocks of most of the strains used in this study and to Ms. Wendy Kring for excellent technical assistance. Appreciation is also expressed to the personnel of the Waite Agricultural Research Institute of the University of Adelaide, Australia, for the facilities and assistance provided to G. E. H. while he was in residence as a Hannaford Research Fellow at the Institute, where part of this research was conducted.

This paper is based upon work supported in part by the U.S. Department of Agriculture under Agreement No. 59-2485-1-1-642-0 and is Technical article no. 17990 of the Texas Agricultural Experiment Station.

REFERENCES

BARBER, H. N., DRISCOLL, C. J., LONG, P. M. & VICKERY, R. S. (1968). Protein genetics of wheat and homoeologous relationships of chromosomes. *Nature* **218**, 450–452.

BARBER, H. N., DRISCOLL, C. J., LONG, P. M. & VICKERY, R. S. (1969). Gene similarity of the *Triticinae* and the study of segmental interchanges. *Nature* **222**, 897–898.

BEILIG, L. M. & DRISCOLL. C. J. (1970). Substitution of rye chromosome $5R^L$ for chromosome 5B and its effect on chromosome pairing. *Genetics* **65**, 241–247.

BERGMAN, J. W. (1972). Chromosome locations of genes controlling esterase and malate dehydrogenase isozymes in *Triticum*. Ph.D. dissertation, North Dakota State University, U.S.A.

CHAPMAN, V. & RILEY, R. (1966). The allocation of the chromosomes of *Triticum aestivum* to the A and B genomes and evidence of genome structure. *Canadian Journal of Genetics and Cytology* **8**, 57–63.

DRISCOLL, C. J. & SEARS, E. R. (1971). Individual addition of the chromosomes of 'Imperial' rye to wheat. *Agronomy Abstracts*, p. 6.

DVOŘÁK, J. (1979). Metaphase I pairing frequencies of individual *Agropyron elongatum* chromosome arms with *Triticum* chromosomes. *Canadian Journal of Genetics and Cytology* **21**, 243–254.

DVOŘÁK, J. (1980). Homoeology between *Agropyron elongatum* chromosomes and *Triticum aestivum* chromosomes. *Canadian Journal of Genetics and Cytology* **22**, 237–259.

DVOŘÁK, J. (1981*a*). Chromosome differentiation in polyploid species of *Elytrigia*, with special reference to the evolution of diploid-like chromosome pairing in polyploid species. *Canadian Journal of Genetics and Cytology* **23**, 287–303.

DVOŘÁK, J. (1981*b*). Genome relationships among *Elytrigia* (= *Agropyron*) *elongata*, *E. stipifolia*, '*E. elongata* 4*x*', *E. caespitosa*, *E. intermedia*, and '*E. elongata* 10*x*'. *Canadian Journal of Genetics and Cytology* **23**, 481–492.

DVOŘÁK, J. & KNOTT, D. R. (1974). Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. *Canadian Journal of Genetics and Cytology* **16**, 399–417.

FITCH, W. M. (1973). Aspects of molecular evolution. *Annual Review of Genetics* **7**, 343–380.

FITCH, W. M. & MARGOLIASH, E. (1970). The usefulness of amino acid and nucleotide sequences in evolutionary studies. *Evolutionary Biology* **4**, 67–109.

GOTTLIEB, L. D. (1973). Enzyme differentiation and phylogeny in *Clarkia franciscana*, *C. rubicunda*, and *C. amoena*. *Evolution* **27**, 205–214.

GUSS, P. L., MACKO, V., RICHARDSON, T. & STAHMANN, M. A. (1968). Lipoxidase in early growth of wheat. *Plant and Cell Physiology* **9**, 415–422.

HART, G. E. (1970). Evidence for triplicate genes for alcohol dehydrogenase in hexaploid wheat. *Proceedings of the National Academy of Sciences, U.S.A.* **66**, 1136–1141.

HART, G. E. (1973). Homoeologous gene evolution in hexaploid wheat. *Proceedings of the Fourth International Wheat Genetics Symposium*, pp. 805–810.

HART, G. E. (1975). Glutamate oxaloacetate transaminase isozymes of *Triticum*: Evidence for multiple systems of triplicate structural genes. In *Isozymes*, vol. III (ed. C. L. Markert), pp. 637–657. Academic Press.

HART, G. E. (1978). Chromosomal arm locations of *Adh-R1* and an acid phosphatase structural gene in Imperial rye. *Cereal Research Communications* **6**, 123–133.

HART, G. E. (1979*a*). Genetical and chromosomal relationships among the wheats and their relatives. *Stadler Genetics Symposium* **11**, 9–29.

HART, G. E. (1979*b*). Evidence for a triplicate set of glucosephosphate isomerase structural genes in hexaploid wheat. *Biochemical Genetics* **17**, 585–598.

HART, G. E. (1982). Genetics and evolution of multilocus isozymes in hexaploid wheat. *Proceedings of the 4th International Congress on Isozymes.* (In the Press.)

HART, G. E., ISLAM, A. K. M. R. & SHEPHERD, K. W. (1980). Use of isozymes as chromosome markers in the isolation and characterization of wheat–barley chromosome addition lines. *Genetical Research* **36**, 311–325.

HART, G. E. & LANGSTON, P. J. (1977). Chromosomal location and evolution of isozyme structural genes in hexaploid wheat. *Heredity* **39**, 263–277.

HART, G. E., MCMILLIN, D. E. & SEARS, E. R. (1976). Determination of the chromosomal location of a glutamate oxaloacetate transaminase structural gene using *Triticum–Agropyron* translocations. *Genetics* **83**, 49–61.

IRANI, B. N. & BHATIA, C. R. (1972). Chromosomal location of alcohol dehydrogenase gene(s) in rye, using wheat–rye addition lines. *Genetica* **43**, 195–200.

ISLAM, A. K. M. R. (1980). Identification of wheat–barley addition lines with *N*-banding of chromosomes. *Chromosoma* **76**, 365–373.

JAASKA, V. (1978). NADP-dependent aromatic alcohol dehydrogenase in polyploid wheats and their diploid relatives. On the origin and phylogeny of polyploid wheat. *Theoretical and Applied Genetics* **53**, 209–217.

KAHLER, A. L. & ALLARD, R. W. (1970). Genetics of isozyme variants in barley. I. Esterases. *Crop Science* **10**, 444–448.

KIMBER, G. & SEARS, E. R. (1980). Uses of wheat aneuploids. In *Polyploidy: Biological Relevance* (ed. W. K. Lewis), pp. 427–443. Academic Press.

KOBREHEL, K. (1978). Identification of chromosome segments controlling the synthesis of peroxidases in wheat seeds and in transfer lines with *Agropyron elongatum. Canadian Journal of Botany* **56**, 1091–1094.

KOLLER, O. L. & ZELLER, F. J. (1976). The homeologoous relationships of rye chromosomes 4R and 7R with wheat chromosomes. *Genetical Research* **28**, 177–188.

MACINTYRE, R. J. (1976). Evolution and ecological value of duplicate genes. *Annual Review of Ecology and Systematics* **7**, 421–468.

MCINTOSH, R. A. (1973). A catalogue of gene symbols for wheat. *Proceedings of the 4th International Wheat Genetics Symposium*, pp. 893–937.

O'MARA, J. G. (1940). Cytogenetic studies on Triticale. I. A method for determining the effects of individual Secale chromosomes on Triticum. *Genetics* **25**, 401–408.

POWLING, A., ISLAM, A. K. M. R. & SHEPHERD, K. W. (1981). Isozymes in wheat–barley hybrid derivative lines. *Biochemical Genetics* **19**, 237–254.

RAO, I. N. & RAO, M. V. P. (1980). Evidence for duplicate genes coding for 6-phosphogluconate dehydrogenase in rye. *Genetical Research* **35**, 309–312.

RILEY, R. (1955). The cytogenetics of the differences between some *Secale cereale* species. *Journal of the Science of Food and Agriculture* **46**, 377–383.

RILEY, R. (1965). Cytogenetics and plant breeding. *Genetics Today, Proceedings of the 11th International Congress on Genetics*, vol. 3, pp. 681–688.

RILEY, R. & CHAPMAN, V. (1960). The D genome of hexaploid wheat. *Wheat Information Service* **11**, 18–19.

RILEY, R., CHAPMAN, V. & JOHNSON, R. (1968). The incorporation of alien disease resistance in wheat by genetic interference with the regulation of meiotic chromosome synapsis. *Genetical Research* **12**, 199–219.

RILEY, R. & KIMBER, G. (1966). The transfer of alien genetic variation to wheat. *Annual Report of Plant Breeding Institute, Cambridge*, 1964–1965, pp. 6–36.

SAKAMOTO, S. (1973). Patterns of phylogenetic differentiation in the tribe Triticeae. *Seiken Ziho* **24**, 11–31.

SCANDALIOS, J. G. (1969). Genetic control of multiple molecular forms of enzymes in plants: a review. *Biochemical Genetics* **3**, 37–79.

SEARS, E. R. (1956). The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. *Brookhaven Symposia in Biology* **9**, 1–22.

SEARS, E. R. (1966). Nullisomic–tetrasomic combinations in hexaploid wheat. In *Chromosome Manipulations and Plant Genetics* (ed. R. Riley and K. R. Lewis), pp. 29–45. Edinburgh: Oliver and Boyd.

SEARS, E. R. (1968). Relationships of chromosomes 2A, 2B, and 2D with their rye homoeologue. *Proceedings of the 3rd International Wheat Genetics Symposium*, pp. 53–61.

SEARS, E. R. (1973). Translocations through union of newly formed telocentric chromosomes. *Genetics* **74**, s247.

SEARS, E. R. (1975). The wheats and their relatives. In *Handbook of Genetics*, vol. II (ed. R. C. King), pp. 59–91. Plenum Press.

SEARS, E. R. & SEARS, L. M. S. (1979). The telocentric chromosomes of common wheat. *Proceedings of the 5th International Wheat Genetics Symposium*, vol. 2, pp. 389–407.

SHEPHERD, K. W. & ISLAM, A. K. M. R. (1981). Wheat:barley hybrids – the first eighty years. In *Wheat Science – Today and Tomorrow* (ed. L. T. Evans and W. J. Peacock), pp. 107–128. Cambridge University Press.

TANG, K. W. & HART, G. E. (1975). Use of isozymes as chromosome markers in wheat–rye addition lines and in triticale. *Genetical Research* **26**, 187–201.

YANG, S. Y. (1971). *Appendix: Studies in Genetics. University of Texas Publications* **6** (7103), 85–90.