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GENETICAL AND CHROMOSOMAL RELATIONSHIPS AMONG THE WHEATS AND THEIR RELATIVES

(aneuploid genetics, isozymes, gene and chromosome evolution, Triticum, wheat)

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SUMMARY

Results obtained from aneuploid genetic analyses of isozyme variation in hexaploid wheat have led to the construction of reasonable models for the genetic control and subunit structure of a large number of enzymes. Evidence has been obtained for the chromosomal locations of 57 isozyme structural genes in the cultivar Chinese Spring, including one duplicate and fifteen triplicate sets of homoeologous genes. Analyses of hexaploid wheat strains containing alien chromosomes have provided evidence for the chromosomal locations of approximately 20 isozyme structural genes in three other Triticeae species. Extensive inter-genomic variation between homoeologous structural genes has been detected in the tribe Triticeae. However, the chromosomal locations thus far determined for these genes in different genomes indicate that the ancestral Triticeae gene linkage relationships are largely conserved in the genomes that exist today.

INTRODUCTION

Aneuploid genetics is a method of analysis that utilizes the effects produced by dosage differences in chromosomes and chromosome segments to obtain genetic information. It was introduced by BRIDGES (1916) with his analysis of the phenotypes and chromosomal constitutions of *Drosophila melanogaster* sex chromosome aneuploids which conclusively demonstrated that the X chromosome of this species is the carrier of its sex-linked genes. Shortly thereafter BLAKESLEE et al. (1920) and BLAKESLEE and FARNHAM (1923) located a gene in a particular *Datura stramonium* chromosome by analysis of trisomics. This was the first use of the technique in the study of a plant species. Aneuploid

genetics has since played a major role in the study of these and many other organisms, including wheat, tomato, tobacco, cotton, maize, barley, and sorghum. It is probable that today its principles and attributes are less widely known than those of microbial, somatic cell, and Mendelian genetics. However, it deserves much wider recognition for its use greatly facilitates genetic investigations of many species.

Aneuploid genetic investigations of *Triticum aestivum* (hexaploid wheat) were begun in the late 1930's. They have disclosed the genetic basis of many character differences and the chromosomal locations of many genes (McINTOSH 1973; SEARS 1975). Classical Mendelian genetic analyses of *T. aestivum* are difficult to conduct because the species contains numerous triplicated gene loci. However, due to the diverse spectrum of aneuploid strains that are available in the species, developed in large part by Dr. E. R. SEARS of the University of Missouri (SEARS 1954, 1966a, b; SEARS and SEARS 1979), it is now a relatively simple matter to assign genes to chromosomes and to construct linkage maps which include the position of the centromere. As a consequence hexaploid wheat has become, as predicted (SEARS 1969), the most suitable material for certain types of investigations.

Tetraploid and hexaploid wheat are of great importance as world food crops. Although no other species in the genus *Triticum* ranks as a major crop plant, it has long been recognized that other species in the genus and as well species in other genera in the tribe Triticeae may be valuable sources of genetic material for the improvement of wheat varieties. There is thus much interest, both for the purpose of crop improvement and the acquiring of basic knowledge, in determining the genetic, evolutionary, and phylogenetic relationships that exist at the level of the genome, the chromosome, and the gene among the various *Triticum* species and among these species and their relatives in other Triticeae genera.

Analyses of the ability of chromosomes and chromosome arms to pair with each other and to compensate for one another have been effectively utilized for some time to study the relationships among the chromosomes of the three genomes of hexaploid wheat and among these chromosomes and those contained in other species (see SEARS 1975 for review). However, relationships among segments of chromatin smaller than chromosome arms have been difficult to analyze. The considerable number of genes concerned with morphological and physiological characters, chlorophyll abnormalities, disease resistance, color and other classical phenotypic characteristics that have been identified and located in chromosomes and in some cases mapped in hexaploid wheat have not by and large been useful for analyzing genetical and evolutionary relationships. The many interactions that take place because of the large quantity of triplicated genetic material that the species contains have made it very difficult to identify homologies between different loci for these types of genes. Evidence favoring duplicate and triplicate gene control has been obtained for a number of classical phenotypic differences but among the genes affecting such differences that are

listed in the wheat gene catalogue (McINTOSH 1973), only one group - a set of *chlorina* genes - are designated as homoeologous (related by descent).

Recent aneuploid genetic investigations of isozyme variation in hexaploid wheat and its relatives have identified and located in specific chromosomes and chromosome arms in hexaploid wheat and in other Triticeae species a large number of isozyme structural genes. Furthermore, homoeologies have been demonstrated for many of these genes between loci contained in the different genomes of hexaploid wheat and in different species in the genus *Triticum* and indeed in different genera in the tribe Triticeae. As a consequence, a considerable quantity of evidence regarding gene and chromosome evolution has been obtained. This paper will review this research, with particular emphasis on the findings that have been obtained concerning genetic and evolutionary relationships among the genomes and chromosomes of the wheats and their relatives.

SPECIES AND GENOMIC RELATIONSHIPS IN THE TRITICEAE

The wheat genus *Triticum* L. is a member of the subtribe Triticinae of the tribe Triticeae of the family Gramineae. Other genera in the Triticinae are *Agropyron* (the wheat grasses), *Secale* (rye), and *Haynaldia*. The classification used here, that of MORRIS and SEARS (1967) which follows with but minor changes that of BOWDEN (1959), relegates the species formerly included in the genus *Aegilops* to *Triticum*. The genus *Hordeum* (barley) is a member of the subtribe Hordeinae of the Triticeae.

The classification of MORRIS and SEARS (1967) recognizes 10 diploid *Triticum* species, three allopolyploid wheats, and some 10 other allopolyploid *Triticum* species (see also SEARS 1975). The relationships among the genomes contained in the *Triticum* species are sufficiently known so that a formula, in the form of a capital Roman letter, has been assigned to each genome in each species. *Triticum aestivum* (2n = 6x = 42) contains genomes A, B, and D. The D genome was contributed to *T. aestivum* by *T. tauschii* and the A and B genomes by *T. turgidum*. The A genome came from *T. monococcum*. The source of the B genome is as yet unknown. It appears probable that it came from a now extinct diploid or that it is a composite of chromosomes or parts of chromosomes from two or more species (SEARS 1975).

ANEUPLOID GENETICS OF HEXAPLOID WHEAT ISOZYMES

Material and Methods

The 21 chromosomes of hexaploid wheat belong to seven homoeologous groups of three chromosomes each, each group composed of one chromosome from each genome. The chromosomes are designated 1A-7A, 1B-7B, and 1D-7D. This classification is based on nullisomic-tetrasomic tests carried out by SEARS (1954, 1966a) in the cultivar Chinese Spring which showed that the deliterious effects of nullisomy for each chromosome of each ge-

nome are compensated for, at least in part, by tetrasomy for either of two related (homoeologous) chromosomes that belong one each to the other two genomes. No evidence for compensation was obtained when chromosomes from different homoeologous groups were combined in nullisomic-tetrasomic tests. These results established that the major genetic homoeologies between the genomes of hexaploid wheat lie within the homoeologous groups.

The chromosomal constitution of each of the six possible compensating nullisomic-tetrasomic combinations of one homoeologous group, namely, group 6, is shown in Table 1. Since there are seven homoeologous groups, there are 42 possible compensating nulli-tetra combinations. All of these types have been derived in Chinese Spring and all but four (nulli 2A-tetra 2B, 2A-2D, 4A-4B, and 4A-4D) are relatively easy to maintain as strains and are available for study.

Table 1. Chromosomal constitution of cv. Chinese Spring homoeologous group 6 compensating nullisomic-tetrasomic strains.

Strain	Chromosome Groups 1-5 and 7	Chromosomes 6A	6B	6D
Nulli-6A Tetra-6B	18"	Absent	1""	1"
" 6A " 6D	18"	Absent	1"	1""
" 6B " 6A	18"	1""	Absent	1"
" 6B " 6D	18"	1"	Absent	1""
" 6D " 6A	18"	1""	1"	Absent
" 6D " 6B	18"	1"	1""	Absent

The series of compensating nulli-tetra strains is a powerful tool for studying the genetic control of hexaploid wheat isozymes. By analyzing this series of strains and other aneuploids that substitute for the four unavailable nulli-tetra strains, the effects of zero, two, and four doses of each of the 21 chromosomes in the hexaploid wheat complement on the zymogram phenotype expressed by an enzyme may be determined. Powerful evidence for the chromosomal location of a structural gene comes from the finding that the level of expression of a specific gene product as observed with the zymogram technique varies concordantly with the dosage of a specific chromosome but is unaffected by variation in the dosage of each of the other chromosomes in the complement.

Strains possessing telocentric chromosomes (SEARS 1974; SEARS and SEARS 1979), especially ditelosomes, are also useful for studying the genetics of isozymes. Thirty-four of the 42 possible ditelosomic strains of Chinese Spring are available. After studies of nulli-tetra strains have provided evidence for the chromosomal locations in one or more homoeologous groups of the structural genes for an isozyme system, the appropriate ditelosomic strains may be efficiently used both to further test the results of the analyses of the nulli-tetra strains and to

obtain evidence for the chromosomal arm locations of the genes. Derivatives of dimonotelosomic strains may be used to obtain ditelosomic types that are not available since the added monotelosome in these strains will segregate each generation.

The manner in which aneuploids have been used to study the genetics of hexaploid wheat isozymes will be illustrated with two enzyme systems, namely, the aminopeptidases, which behave as monomeric enzymes, and the dimeric glutamic oxaloacetic transaminase-3 isozymes.

Diagrams of some of the zymogram phenotypes observed in an aneuploid analysis of the aminopeptidase (AMP) isozymes expressed in the shoots of 7-day-old etiolated seedlings are shown in Figure 1. The AMP phenotypes of five of the six possible homoeologous group 6 ditelosomic strains were also determined. It was found that the level of expression of each of the three AMP isozymes varies concordantly with the dosage of a specific group 6 chromosome and with the dosage of a specific arm of that chromosome but is unaffected by variation in the dosage of each of the other 20 chromosomes in the complement. The results obtained were entirely consistent with the proposal that each of the short (= p) arms of the homoeologous group 6 chromosomes possesses a gene which encodes a monomeric AMP isozyme. The genes located in 6Ap, 6Bp, and 6Dp were designated *Amp-A1*, *Amp-B1*, and *Amp-D1*, respectively, and the isozymes which they encode as AMP-1 (located at the site of band 1), AMP-3 (band 3), and AMP-2 (band 2), respectively (HART 1973; HART and LANGSTON 1977a).

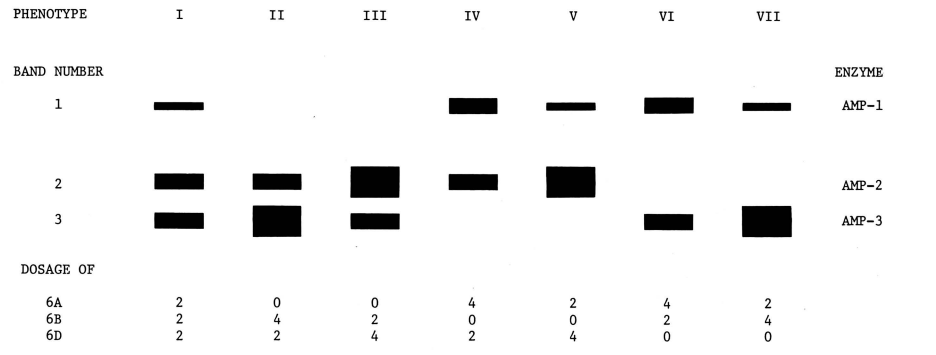

Figure 1. Diagram showing the relationships between dosages of the homoeologous group 6 chromosomes and the AMP zymogram phenotypes produced. (After HART and LANGSTON 1977a).

The relationships between zymogram phenotypes and dosages of chromosomes and chromosome arms that carry structural genes are considerably more complex for oligomeric than for monomeric enzymes. Diagrams of the zymogram phenotypes observed in an aneuploid analysis of the glutamic oxaloacetic transaminase-3 (GOT-3) isozymes that are expressed in the blade of the first foliage leaf of 7-day-old etiolated seedlings are shown in Figure 2. A similar pattern of variation has been observed for two

other GOT systems and for the alcohol dehydrogenase-1, glucose-phosphate isomerase-1, esterase-1, and phosphodiesterase systems (see Table 4 for references).

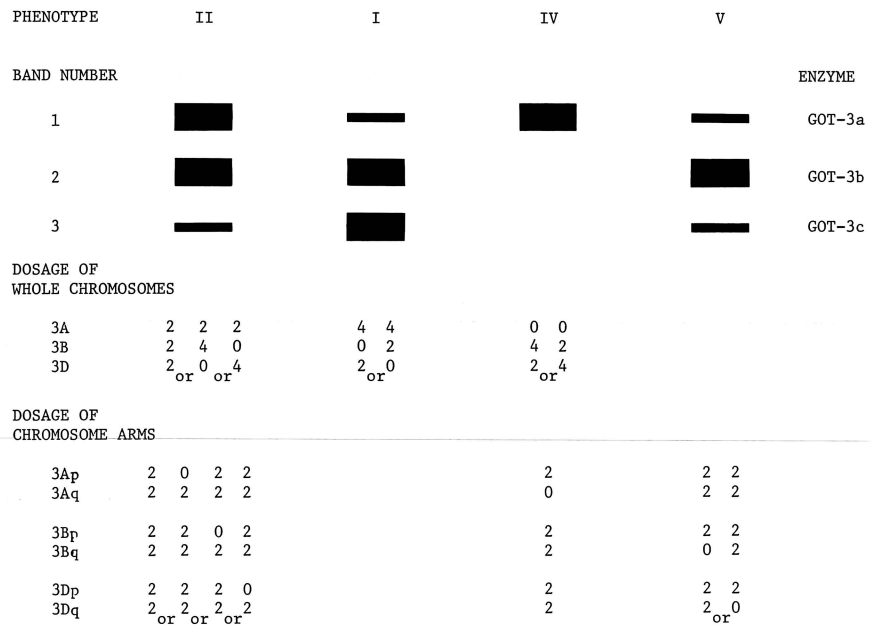

Figure 2. Diagram showing the relationships between dosages of the homoeologous group 3 chromosomes and chromosome arms and the GOT-3 zymogram phenotypes produced. (After HART 1975).

The variation observed in the GOT-3 zymogram phenotypes indicates that the isozymes are encoded by a minimum of three structural genes located one each in the q arms of the group 3 chromosomes and that the products of two of the three genes are electrophoretically identical and different from the product of the third gene (HART 1975). SHAW (1964), in his review of the use of Mendelian genetics in the analysis of the structure of isozymes of diploid organisms, presented the formula

$$i = \frac{(s+p-1)!}{p!(s-1)!}$$

in which i = number of isozymes produced by subunits which associate in all possible combinations, p = number of subunits per active enzyme molecule and s = number of different kinds of subunits. This formula may be used to estimate the number of subunits per active molecule for the enzymes of polyploid as well as diploid organisms. For the GOT-3 isozymes, s=2 and the value of 3 for i is obtained with p=2, indicating that the GOT-3 isozymes have a dimeric subunit structure.

The GOT-3 structural genes located in chromosome arms 3Aq, 3Bq, and 3Dq were designated *Got-A3*, *Got-B3*, and *Got-D3*, respec-

tively, and the subunits which they encode α^3, β^3, and δ^3, respectively. The expected distribution of the six possible dimeric molecules, assuming that each GOT gene present encodes the same quantity of subunit and that the subunits associate randomly into equally active molecules, is based on $(p + q + r)^2$, where p, q, and r represent the frequencies of α^3, β^3, and δ^3, respectively. Schematic models for the subunit composition and the quantitative distribution of the GOT-3 isozymes of Chinese Spring and of each of the group 3 aneuploid strains that were examined are shown in Tables 2 and 3. The excellent agreement

Table 2. Schematic model for the subunit composition of the GOT-3 isozymes produced by cv. Chinese Spring and by each of the homoeologous group 3 compensating nullisomic-tetrasomic types. (The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.) (From HART 1975).

ISOZYMES	CHINESE SPRING	NULLI-3B TETRA-3D	NULLI-3D TETRA-3B	NULLI-3B TETRA-3A	NULLI-3D TETRA-3A	NULLI-3A TETRA-3B or 3D
GOT-3a	4/9 $\beta^3\beta^3,\delta^3\delta^3,\beta^3\delta^3$	4/9 $\delta^3\delta^3$	4/9 $\beta^3\beta^3$	1/9 $\delta^3\delta^3$	1/9 $\beta^3\beta^3$	$\beta^3\beta^3,\delta^3\delta^3,\beta^3\delta^3$
GOT-3b	4/9 $\alpha^3\beta^3,\alpha^3\delta^3$	4/9 $\alpha^3\delta^3$	4/9 $\alpha^3\beta^3$	4/9 $\alpha^3\delta^3$	4/9 $\alpha^3\beta^3$	
GOT-3c	1/9 $\alpha^3\alpha^3$	1/9 $\alpha^3\alpha^3$	1/9 $\alpha^3\alpha^3$	4/9 $\alpha^3\alpha^3$	4/9 $\alpha^3\alpha^3$	
DOSAGE OF						
Got-A3	2	2	2	4	4	0 0
Got-B3	2	0	4	0	2	4 2
Got-D3	2	4	0	2	0	2 or 4

Table 3. Schematic model for the subunit composition of the GOT-3 isozymes produced by each of the cv. Chinese Spring homoeologous group 3 ditelosomic strains. (The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.) (From HART 1975).

ISOZYMES	Ditelo-3Aq or -3Bq or -3Dq	Ditelo-3Ap	Ditelo-3Bp	Ditelo-3Dp
GOT-3a	4/9 $\beta^3\beta^3,\delta^3\delta^3,\beta^3\delta^3$	$\beta^3\beta^3,\delta^3\delta^3,\beta^3\delta^3$	1/4 $\delta^3\delta^3$	1/4 $\beta^3\beta^3$
GOT-3b	4/9 $\alpha^3\beta^3,\alpha^3\delta^3$		2/4 $\alpha^3\delta^3$	2/4 $\alpha^3\beta^3$
GOT-3c	1/9 $\alpha^3\alpha^3$		1/4 $\alpha^3\alpha^3$	1/4 $\alpha^3\alpha^3$
DOSAGE OF				
Got-A3	2	0	2	2
Got-B3	2	2	0	2
Got-D3	2	2	2	0

between the models and the zymogram phenotypes observed (Figure 2) provides strong additional support for the hypothesis that the GOT-3 isozymes are dimeric molecules that are encoded by a set of three structural genes located one each in the q arms of the group 3 chromosomes.

Evidence similar to that just described for the GOT-3 isozymes has been obtained for the genetic control and subunit structure of each of the dimeric enzymes listed in Table 4. Further evidence that the active forms of the GOT-3 and ADH-1 isozymes are dimers was obtained in experiments in which the subunits of genetically controlled electrophoretic variants of the enzymes were dissociated and recombined in crude tissue extracts (HART 1971; HART and LANGSTON 1977b).

A series of zymogram phenotypes similar to those of the AMP's - phenotypes consistent with a monomeric subunit structure for the active isozymes - has been observed in aneuploid genetic studies of several wheat enzymes. The failure to detect one or more heterooligomeric forms of an enzyme on zymograms when two or more electrophoretically different gene products are present is evidence that the enzyme has a monomeric structure. Since wheat is self-fertilized, homozygosity is expected at the vast majority of isozyme structural gene loci. Consequently, heterooligomeric enzymes are expected to be the products of diverged duplicated genes rather than of alleles at a given locus. Homoeologous genes that encode oligomeric enzymes and are contained in different genomes could conceivably have diverged sufficiently from one another so that active enzymes are no longer formed by the association of their products. However, heterooligomers have been observed for all wheat enzymes tested that have been found in other higher organisms to have an oligomeric structure. The methods by which certain classes of enzymes are detected on gels (e.g., the esterases) are expected to detect a wide variety of enzymes with physiologically dissimilar functions and with different structures. Consequently both monomeric and oligomeric forms of these enzymes may exist.

Chromosomal Locations of Isozyme Structural Genes

A rigorous requirement for the assignment of an isozyme structural gene to a particular hexaploid wheat chromosome is that it be demonstrated in a test of *all possible nullisomic types* (using compensating nullisomic-tetrasomic strains and/or other appropriate aneuploids) that the product of the gene, as observed with the zymogram technique, is expressed by each strain in which the indicated chromosome is present (regardless of the absence or presence of each of the other chromosomes in the complement) and is not expressed by any strain which lacks the indicated chromosome (HART 1970; HART and LANGSTON 1977a). Table 4 lists 57 genes that have been identified in aneuploid genetic investigations of the cultivar Chinese Spring that have met this test or a close approximation to it. Each of the amylase gene locations are based on the finding in analyses of from 23 to 27 of the 42 possible ditelosomic strains that the product of the gene was expressed by all strains examined except those in which the indicated chromosome arm was absent. The compensating nulli-tetra series was the principal experimental material used to locate the other genes, along with selected ditelo strains. For some of the genes the nullisomic test was carried out only in part or not at all for chromosomes 2A and 4A due to the non-availability of the appropriate compensating nulli-tetra strains. Additional evidence for the chromosomal location of

Table 4. Chromosomal locations of isozyme structural genes of hexaploid wheat in cv. Chinese Spring

Chromosomal Location[a]	Gene or Gene Set	Enzyme[b]	References
Triplicate and duplicate sets			
1Ap, 1Bp, 1Dp	*Gpi-1*	*Glucosephosphate isomerase-1	HART 1979
3A, 3B, 3Dp	*Pde-1*	*Phosphodiesterase	WOLF et al. 1977
3Ap, 3Bp, 3Dp	*Est-1*[c]	*Esterase-1	BARBER et al. 1968, 1969; BERGMAN 1972
3Aq, 3Bq, 3Dq	*Got-3*	*Glutamic oxaloacetic transaminase-3	HART 1975
4Ap, 4Bp, 4Dp	*Adh-1*	*Alcohol dehydrogenase-1	HART 1970, 1973; HART and LANGSTON 1977a
" " "	*Lpx-1*	Lipoxygenase-1	HART and LANGSTON 1977a
4Aq, 4Dq	β-*Amy-1*[d]	β-Amylase	JOUDRIER and CAUDERON 1976
5Aq, 5Bq, 5Dq	*Lpx-2*	Lipoxygenase-2	HART and LANGSTON 1977a
" " "	*Adh-2*	Alcohol dehydrogenase-2	JAASKA 1978
6Ap, 6Bp, 6Dp	*Got-1*	*Glutamic oxaloacetic transaminase-1	HART 1975
" " "	*Amp-1*	Aminopeptidase	HART 1973; HART and LANGSTON 1977a
6Aq, 6Bq, 6Dq	*Got-2*	*Glutamic oxaloacetic transaminase-2	HART 1975
" " "	*Est-2*[d]	Esterase-2	MAY et al. 1973
" " "	α-*Amy-1*[c]	α-Amylase-1	NISHIKAWA and NOBUHARA 1971
7Aq, 7Bq, 7Dq	*Ep-1*	Endopeptidase-1	HART and LANGSTON 1977a
" " "	α-*Amy-2*[c]	α-Amylase-2	NISHIKAWA and NOBUHARA 1971
Other Genes			
4Aq, 4Bq, 4Dq	*Acph4&8, 2&3, 5&6*	Acid phosphatase	HART 1973; HART and LANGSTON 1977a
7Dp, 4Bp, 7Ap	*Per1, 2, 3*[d]	Peroxidase	KOBREHEL and FIELLET 1975; KOBREHEL 1978
7Bq	*Ep1*	Endopeptidase	HART and LANGSTON 1977a

[a]Chromosome arms are designated p and q consistent with the recommendations of SEARS and SEARS (1979).
[b]Either a monomeric or a dimeric subunit structure is indicated by the available evidence for each of the enzymes listed. Asterisks identify the known dimeric enzymes.
[c]Gene symbol differs from that given in the reference(s) cited.
[d]Symbols were not previously assigned to these genes.

most of the genes identified using the nulli-tetra series consisted of the finding that strains tetrasomic for the indicated chromosome express the gene product at an approximately two-fold higher level than do strains in which there are two doses of the chromosome present.

Discussion

Homologous genes can be identified by findings obtained from one or more of several possible types of investigations of isozymes. The strongest evidence comes from studies of (1) amino acid sequences, (2) immunological properties, (3) formation of heteropolymeric enzymes, and (4) chromosomal locations of structural genes. Studies of (5) tissue and developmental distribution, (6) subunit structure, (7) biochemical characteristics of enzyme activity, (8) subcellular location, and (9) peptide digests can also provide useful evidence. The relationships indicated for each of the genes listed in Table 4 are based on findings obtained from two or more of the types of studies numbered (3) through (7). The strongest evidence for homoeology is for the seven sets of genes that encode dimeric enzymes. Thus far the use of amino acid sequence, immunological property, subcellular location, and peptide digest comparisons for the identification and study of homoeologous wheat isozymes has not been reported.

Forty-five of the 57 isozyme structural genes listed in Table 4 are members of triplicate gene sets located in homoeologous chromosomes. A duplicate set of α-amylase genes located in homoeologous chromosomes is also identified. In addition, the six acid phosphatase genes that are located, in pairs, in the q arms of the group 4 chromosomes are probably homoeologous (HART 1973; TORRES and HART 1976; HART and LANGSTON 1977a). These findings indicate that each of the three members of most triplicate structural gene sets remain active in hexaploid wheat. Seven of the 15 triplicate gene sets encode dimeric enzymes and 8 encode monomeric enzymes. Among each of the former the products of two of the genes are electrophoretically indistinguishable while the product of the third gene differs from that of the other two. Among the latter, with one exception (LPX-1b and LPX-1c), the products of each of the three genes differ from one another in electrophoretic mobility. There is thus extensive homoeoallelic variation present in these gene sets and as a consequence a high level of biochemical diversity present in individual plants. The extent to which this variation is of functional significance has not been determined (see DISCUSSION in HART and LANGSTON 1977a).

Each of the products of most of the triplicate isozyme structural gene sets that have been identified appears to be expressed at a level that is approximately proportional to the number of copies present of the chromosome or chromosome arm that carries the gene encoding the product, regardless of the dosages of homoeologues or of other chromosomes (see e.g., HART and LANGSTON 1977a and MAY et al. 1973). This suggests that there has been no significant divergence among the genes which regulate most of these isozyme systems. WOLF et al. (1977) have reported

the only evidence for the regulation of an isozyme structural gene by an asyntenic gene or genes. They concluded that *Pde-D1*, a phosphodiesterase structural gene located in chromosome arm 3Dp, is regulated by genes located in chromosomes 5A, 5B, and 5D.

The results reported in Table 4 provide evidence at the genic level, in agreement with the findings of the earlier investigations of SEARS (1954, 1966a) of nullisomic-tetrasomic combinations, that the gene linkage relationships that existed in the ancestral wheat genome have in large part been conserved in each of the three genomes of the cultivar Chinese Spring. The gene locations provide no evidence for interchanges, either between the chromosomes of different genomes or between the chromosomes that compose any given genome. Furthermore, the gene locations provide evidence that, with the exception of the homoeologous group 4 chromosomes, the genetic content of the individual chromosome arms has been conserved. The location of one member of each of two triplicate sets of homoeologous genes (the *Adh-1* and *Lpx-1* sets) in the α arm of chromosome 4A (the arms of chromosome 4A are indistinguishable in length), the long arm of 4B and the short arm of 4D and of 2 *Acph* genes in each of the arms 4Aβ, 4BS and 4DL is evidence that these sets of arms are homoeologous. Tests of the ability of chromosome arms to pair conducted recently by Dr. L. M. S. SEARS (SEARS and SEARS 1979 and personal communication) have confirmed the homoeologies among 4Aα, 4BL, and 4DS (now designated 4Ap, 4Bp, and 4Dp, respectively) and among 4Aβ, 4BS, and 4DL (now designated 4Aq, 4Bq, and 4Dq, respectively). Dr. SEARS (personal communication) has also obtained evidence that 2BS is homoeologous with 2AL and 2DL and 2BL with 2AS and 2DS. The nature of the structural changes which have caused these anomolies between relatedness and arm length in groups 2 and 4 are unknown. However, with respect to the anomoly among the group 4 chromosomes, it is of interest to note that GERLACH et al. (1978) have concluded that the timopheevi and emmer tetraploid wheats had the same ancestral B genome but are distinguished by an apparent pericentric inversion involving chromosome 4B.

Linkage data for the isozyme structural genes that have been identified would be quite valuable. By use of telosomes, the genetic distance between gene and centromere can be readily determined (SEARS 1962, 1966b), provided allelic variation is present. However, to date linkage data have been reported for only one isozyme structural gene. Analysis of a series of *Triticum aestivum* - *Agropyron elongatum* translocation lines disclosed that *Got-D3* is located in the proximal region of the long (=q) arm of chromosome 3D, about 4 crossover units from the centromere (HART et al. 1976; see also SEARS 1977).

ANALYSIS OF ADDITION LINES

Materials and Methods

The range of aneuploid conditions which hexaploid wheat readily tolerates includes the addition and substitution of size-

able quantities of genetic material from other Triticeae species. This has allowed the construction of a large number of strains which contain alien chromatin in added or substituted chromosomes or telosomes or in segmental interchanges (see DRISCOLL 1975 for a compendium of the available wheat alien-chromosome lines). Investigations of these strains, in a manner analogous to that described above for intra-species hexaploid wheat aneuploids, has provided evidence for the chromosomal locations of isozyme structural genes in relatives of hexaploid wheat and information at the genic level regarding the genetic and evolutionary relationships among the chromosomes of hexaploid wheat and those contained in related species.

The most useful of the wheat alien-chromatin types for investigations of the genetics of isozymes are disomic chromosome addition lines. A complete disomic chromosome addition series for a 2n = 14 relative of hexaploid wheat consists of seven lines, each of which contains the full complement of 21 pairs of wheat chromosomes and an added pair of alien chromosomes. Complete disomic chromosome addition series have been produced for three different varieties of *Secale cereale* (rye), including the variety Imperial (DRISCOLL and SEARS 1971; see SEARS 1975 for references for the other two varieties), and one complete or nearly complete series has been produced for several other species, including *Agropyron elongatum* (DVORAK and KNOTT 1974) and *Hordeum vulgare* cv. Betzes (ISLAM, SHEPHERD, and SPARROW personal communication). The Imperial rye, *A. elongatum*, and Betzes barley chromosome addition series were each developed in the cultivar Chinese Spring of hexaploid wheat.

Determinations of the chromosomal locations of isozyme structural genes in a 2n = 14 relative of hexaploid wheat utilizing addition lines will preferably be based on comparisons of the recipient and donor varieties and their amphiploid hybrid and the seven possible disomic chromosome addition lines. Comparison of the zymogram phenotype of the amphiploid with those of the recipient and donor varieties identifies the genomic origin of each of the gene products expressed by the amphiploid and also reveals whether or not each of the products expressed by the individual parental varieties are also expressed when their genomes are combined in one organism. Comparison of the phenotypes of the addition lines reveals the chromosomal location in the donor genome of the isozyme gene products that the donor variety expresses.

The manner in which alien addition lines have been used to study isozyme genetics will be illustrated with two of the enzymes that have been studied in the wheat-barley addition series, namely, the monomeric AMP's and the dimeric alcohol dehydrogenase-1 (ADH) isozymes.

Diagrams of the aminopeptidase zymogram phenotypes produced by Chinese Spring wheat, Betzes barley, the Chinese Spring-Betzes heptaploid hybrid, and the six available disomic chromosome addition lines (disomic chromosome addition line G was not available) are shown in Figure 3 (extracts of shoots of 7-day-old etiolated seedlings were electrophoresed). Betzes produces

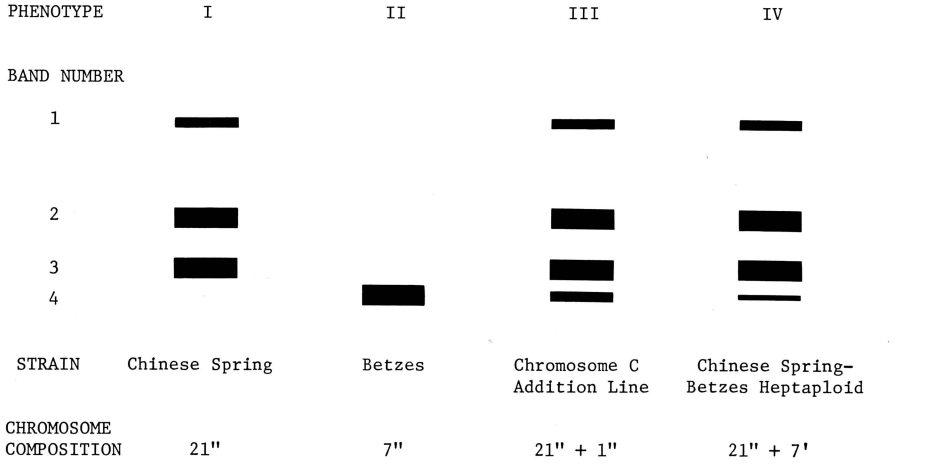

Figure 3. Diagram of the AMP zymogram phenotypes produced by wheat cv. Chinese Spring, barley cv. Betzes, the Chinese Spring-Betzes heptaploid, and disomic chromosome addition line C. (From HART, ISLAM, and SHEPHERD in preparation).

an aminopeptidase that migrates electrophoretically to a position cathodal to the three Chinese Spring isozymes. Among the chromosome addition lines, only the line carrying Betzes chromosome C expresses this isozyme. The other disomic addition lines express only the three Chinese Spring isozymes. No evidence for heterooligomer formation was obtained, consistent with the indicated monomeric structure of the hexaploid wheat aminopeptidases. It was concluded that Betzes chromosome C carries an aminopeptidase structural gene that is homoeologous to the AMP genes that are located in the three chromosomes of homoeologous group 6 of Chinese Spring (HART, ISLAM, and SHEPHERD unpublished).

Diagrams of the ADH phenotypes observed in the study of the Chinese Spring-Betzes addition series are shown in Figure 4. Two Betzes isozymes that are expressed at a low level in the tissue examined (scutella of grains germinated for 16 hours) are omitted from the diagram. The major Betzes form, ADH-3, migrates to a position cathodal to the Chinese Spring isozymes.

The three ADH isozymes of Chinese Spring are encoded by a triplicate set of genes (HART 1970; HART and LANGSTON 1977a). In addition to genetic evidence, several biochemical findings indicate that the isozymes have a dimeric subunit structure (HART 1971; LANGSTON et al. 1979). The phenotypes observed are fully consistent with the hypothesis that an ADH structural gene located in Betzes chromosome A encodes a product that associates in all possible combinations with the three wheat ADH gene products into active dimeric molecules. The expected distribution of the 10 possible dimeric molecules, assuming that each ADH gene present encodes the same quantity of subunit and that the subunits associate randomly into equally active molecules, is

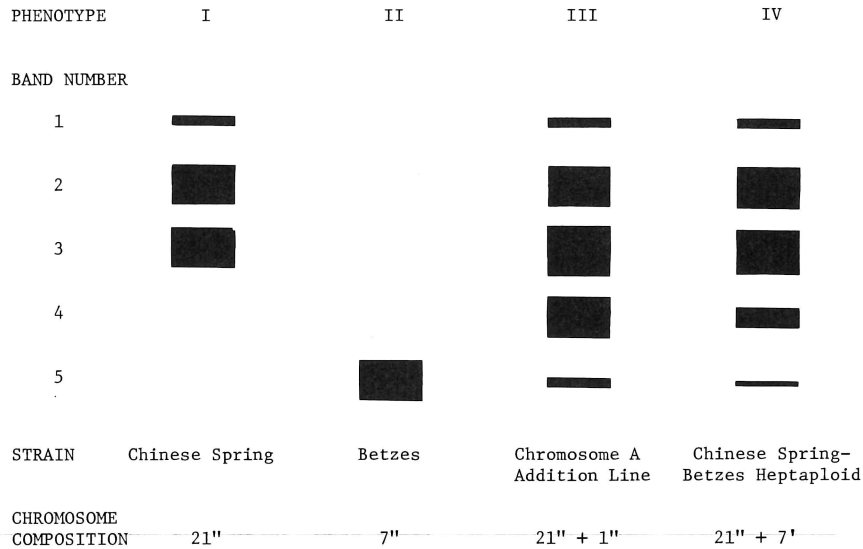

Figure 4. Diagram of the ADH zymogram phenotypes produced by wheat cv. Chinese Spring, barley cv. Betzes, the Chinese Spring-Betzes heptaploid, and disomic chromosome addition line A. (From HART, ISLAM, and SHEPHERD in preparation).

based on $(p + q + r + s)^2$, where p, q, r, and s represent the frequencies of the four types of subunits. Schematic models for the subunit composition and the quantitative distribution of the ADH isozymes of the recipient and donor varieties and their heptaploid hybrid and of chromosome addition line A are shown in Table 5. The excellent agreement between the models and the zymogram phenotypes observed (Figure 4) provides strong support for the stated hypothesis. Homoeology between the barley *Adh* gene and the three *Adh-1* genes of hexaploid wheat is strongly indicated by the evidence obtained for the association of the barley and wheat gene products into active heterodimers.

Table 5. Schematic model for the subunit composition of the ADH isozymes produced by cv. Chinese Spring, cv. Betzes, Chinese Spring-Betzes heptaploid, and disomic chromosome A addition line.
(ADH-1 and ADH-2 of cv. Betzes are not shown. The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.) (From HART, ISLAM, and SHEPHERD in preparation).

Chinese Spring			Betzes		Addition Line A			Heptaploid		
Isozymes		Subunit composition	Isozymes	Subunit composition	Isozymes		Subunit composition	Isozymes		Subunit composition
ADH-1	1/9	αα			ADH-1	1/16	αα	ADH-1	4/49	αα
ADH-2	4/9	αβ, αδ			ADH-2	4/16	αβ, αδ	ADH-2	16/49	αβ, αδ
ADH-3	4/9	ββ, δδ, βδ			ADH-3	6/16	ββ, δδ, βδ, αθ	ADH-3	20/49	ββ, δδ, βδ, αθ
					ADH-4	4/16	βθ, δθ	ADH-4	8/49	βθ, δθ
			ADH-3	θθ	ADH-5	1/16	θθ	ADH-5	1/49	θθ

Chromosomal Locations of Isozyme Structural Genes

The isozyme structural gene locations that have been determined in the chromosomes of *S. cereale* cv. Imperial, *H. vulgare* cv. Betzes, and *A. elongatum* and the locations of the homoeologous genes in cv. Chinese Spring, if known, are given in Table 6.

Table 6. Chromosomal locations of isozyme structural genes in four Triticeae species

Genes[a]	Chromosomal Location[b]			
	Triticum aestivum cv. Chinese Spring Genomes A, B, & D	*Hordeum vulgare* cv. Betzes Genome H	*Agropyron elongatum* Genome E	*Secale cereale* cv. Imperial Genome R
Homoeologous sets				
*Gpi-1**	1p		1S	1
*Est-1**	3p		VS + IVS	3
*Got-3**	3q			3
*Adh-1**	4p	A	4β	4/7S[c]
Amp-1	6p	C	6α	6/7
*Got-2**	6q	C	6β	6/7
Ep-1	7q	D	7β + IVL	6/7
Other genes				
Acph2-6 & 8	4q			
Acph1				7/4/6S
Est1 & 2		D		
Est3 & 4		F		
References	See Table 1.	HART, ISLAM, & SHEPHERD unpubl.	HART & TULEEN unpubl.	*Est-1:* BARBER et al. 1968, 1969; BERGMAN 1972. Other genes: TANG & HART 1975; HART 1978 & unpubl.

[a]Asterisks identify genes which encode known dimeric enzymes.
[b]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 (McINTOSH 1973). Chinese Spring is accepted as having the standard chromosome arrangement. Temporary designations are assigned to chromosomes (e.g., Roman numerals to the E genome chromosomes) until their relationships to those of Chinese Spring are established. The two arms of each chromosome are designated α and β or S and L or p and q. (see McINTOSH 1973 and SEARS and SEARS 1979).
[c]Three chromosomes of cv. Imperial appear to differ from those of their presumptive ancestor and from cv. Chinese Spring due to interchanges (see KOLLER & ZELLER 1976). The first Arabic numeral listed in the designation of these chromosomes identifies the segment containing the centromere.

Homoeology among the genes of hexaploid wheat, barley, *A. elongatum* and rye that encode the GPI-1, EST-1, GOT-3, ADH-1, and GOT-2 isozymes is strongly indicated since evidence for the formation of heterooligomers (by the association of the product of the alien gene with the products of the Chinese Spring triplicate gene set) has been obtained in an addition line and in the amphiploid hybrid between Chinese Spring and the related

species for each alien structural gene identified for these five enzymes. Homoeology among the AMP and EP-1 structural genes of the four species is indicated by the tissue and developmental distribution of their products. It appears probable that the ACPH gene located in the short arm of Imperial rye chromosome 7/4/6 is related to the ACPH genes located in the q arms of the Chinese Spring group 4 chromosomes but strong evidence for this relationship is not available. No evidence is available regarding the chromosomal locations of the Chinese Spring genes that are related to the EST genes located in Betzes chromosomes D and F.

Discussion

Evidence regarding the relationships between specific chromosomes of different genomes in the tribe Triticeae may be obtained from three types of investigations, namely, studies of the ability of chromosomes (or parts thereof) to compensate for each other and to pair with one another and studies of the chromosomal locations of specific genetic materials. The ability of specific chromosomes or chromosome segments of different genomes to pair with each other can be assessed by development of strains of hexaploid wheat which contain the appropriate alien chromatin and which lack or contain a suppressed chromosome 5B *Ph* locus (SEARS 1975). Substitution of an alien chromosome or chromosome arm for a chromosome or chromosome arm of hexaploid wheat allows the ability of the former to compensate for the latter to be assessed. Positive results in these tests provide strong evidence for homoeology between the segments of chromatin involved.

The compensation and pairing tests define both the contents and the dimensions of homoeologous regions of chromatin in a fairly gross sense, i.e., quantitatively rather than qualitatively. The greatest precision in defining relationships between different chromosomes lies in the location therein of specific genetic material.

Alien DNA contained in wheat may be detected by use of nucleic acid hybridization techniques. FLAVELL et al. (1978) have shown that rye-specific repeated sequence DNA in individual rye chromosomes and telosomes present in a complete complement of wheat chromosomal DNA can be readily detected by *in vitro* hybridization of radioactive DNA containing a large number of families of repeated sequences of the rye genome to unlabeled DNA of the wheat-rye addition lines. The findings reported indicate that repeated sequence DNA probes should be useful for detecting even small pieces of DNA incorporated into wheat from a related species. However, FLAVELL et al. (1978) note that radioactive DNA probes that contain a large number of repeated sequences of a genome are of little use in identifying the specific alien chromosome or chromosome segment that has been incorporated into a species.

Specific Triticeae chromosomes and chromosome segments may be identified by their metaphase C- and N-banding patterns (GILL and KIMBER 1974a; DARVEY and GUSTAFSON 1975; GERLACH 1977; LINDE-LAURSEN 1978). Also, GERLACH et al. (1978) have identified spe-

cific *Triticum* chromosomes and chromosome regions by *in situ* hybridization of radioactive RNA complementary to a conserved highly repeated DNA sequence isolated from *T. aestivum* cv. Chinese Spring. It is clear, however, that in general the banding patterns of homoeologous chromosomes in different Triticeae genomes are highly differentiated from each other (see GILL and KIMBER 1974b; DARVEY and GUSTAFSON 1975; GERLACH 1977; GERLACH et al. 1978) and thus that the patterns produced by the techniques thus far developed for banding Triticeae chromosomes have evolved at a sufficiently fast rate so that they are not useful for inferring relationships among the chromosomes of different genomes.

Definitive evidence regarding relationships between segments of chromatin contained in different chromosomes can be obtained by the location in each of homoeologous genes. Most suitable for this purpose are genes which encode isozymes. Their advantages in comparison with other genes are several. First, the techniques for genetic analysis of isozyme variation are relatively simple. Furthermore, strong evidence for homoeology among isozyme structural genes contained in different chromosomes may be obtained from one or more of several possible types of investigations of isozyme variation (see above). Finally, most of the chromosomes and about two-thirds of the chromosome arms of Chinese Spring are already marked by one or more isozyme structural genes (one or more sets of triplicate structural genes have been located in six of the seven homoeologous chromosome groups and in nine of the 14 sets of homoeologous chromosome arms) and it appears probable that soon each chromosome arm will be marked with at least one member of a set of homoeologous genes.

The region of homoeology identified by homoeologous genes is, in the strictest sense, only the segment occupied by the specific genes involved. Consequently, related segments of chromatin that are too small to be detected by a test of pairing or compensation may be detected when the chromosomal locations of homoeologous genes are determined. In most cases homoeologous genes will mark major regions of homoeology but, in the absence of other types of evidence, caution must be used in drawing conclusions as to the size of the related segments until several homoeologous genes have been located in them. The greatest progress in resolving the relationships between chromosomes of different genomes will come of course by the joint use of the methods of analysis available.

KOLLER and ZELLER (1976) have proposed that the chromosomes of *S. cereale* that were formerly designated CR, DR, and FR (herein designated 4/7, 7/4/6, and 6/7, respectively) are the products of two interchanges, between the ancestral chromosome arms 7RS and 4RL and between 7RL and 6RL. The isozyme gene locations that have been determined in these three chromosomes in Imperial rye (see Table 6) support this proposal (HART 1978).

The location of the *A. elongatum Est-1* gene in both IVS and VS and of the *Ep-1* gene in both IVL and 7β illustrates one of the desirable features of being able to readily detect the expression of individual alien genes in addition lines. These gene locations suggest that the chromosome designated IV was

formed by a centromeric fusion of the β arm of chromosome 7 with an arm of another chromosome, the arm that composes the short arm of the chromosome designated IV.

The gene locations reported in Table 6 suggest that the gene linkage relationships that existed in the ancestral Triticeae genome are largely conserved in the Triticeae genomes that exist today. With the exceptions that occur due to the two indicated interchanges in the R genome of Imperial rye, genes that are syntenic in the three genomes of Chinese Spring are syntenic in the E, H, and R genomes and genes that are asyntenic in Chinese Spring are asyntenic in the three related species. Of particular note are the *Amp-1* and *Got-2* genes; they are syntenic in each of the 6 genomes analyzed. Evidence for the conservation of gene synteny groups has also been reported for mouse and man (LALLEY et al. 1978).

No evidence has been obtained in these studies for the evolution of significant differences between hexaploid wheat and the E, H, and R genome species in the genes which regulate the isozyme structural genes under study. No evidence has been obtained for the failure of expression of any of the alien genes listed in Table 6 when present in a telosome or whole chromosome in hexaploid wheat. Also, the available evidence indicates that alien genes that encode subunits for oligomeric enzymes when present in addition lines encode about the same quantity of subunit as do the related wheat genes and that the wheat and alien subunits associate approximately randomly into approximately equally active molecules. It must be emphasized, however, with respect to the evolution of possible regulatory differences, that most of the studies conducted thus far have been of one or a few tissues at only one developmental stage. Thus significant regulatory differences may easily have escaped detection.

In closing, it is appropriate to note that, while the studies reviewed here have determined the chromosomal locations of more than 75 genes, the genes that have been located encode only about 10 classes of enzymes and the gene locations have been determined in only four species. Methodology is available for zymogram study of a considerable number of other classes of enzymes and several other complete or nearly complete wheat alien-species addition series are available. Also, gene location studies are now being conducted in other plant species, e.g., maize. It is thus highly probable that further studies of the type discussed here will enlarge considerably the current level of understanding of genetical and evolutionary relationships in the Triticeae and in higher plants in general.

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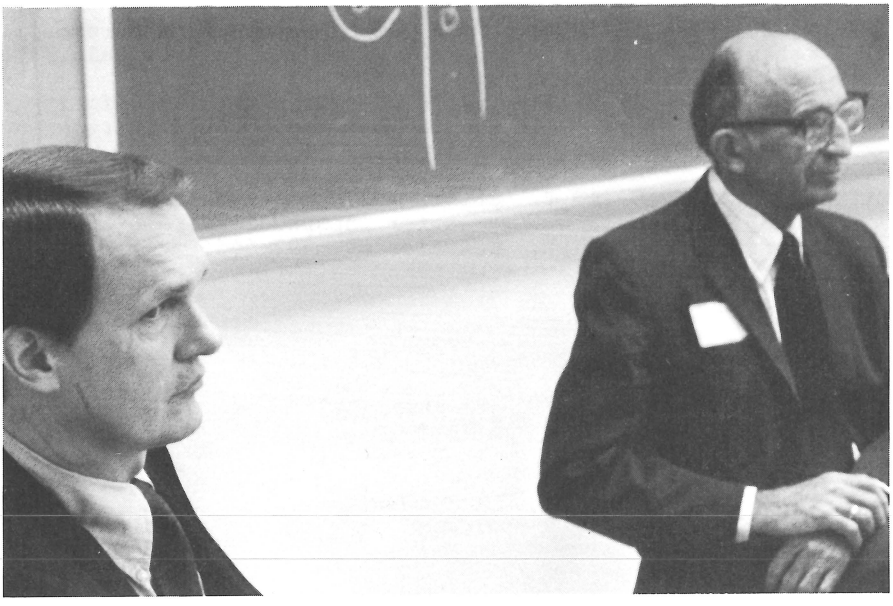

At the Symposium. (Dr. Gary E. Hart is at the lower right.)