INTROGRESSION IN WHEAT VIA TRIPLOID HYBRIDS*

ALIZA VARDI and DANIEL ZOHARY

Department of Botany, the Hebrew University, Jerusalem, Israel

Received 24.xii.66

1. INTRODUCTION

In several recent papers (for review see Zohary, 1965), the hypothesis was advanced that the apparent evolutionary success of polyploid entities in the wheat group (*Triticum* and *Aegilops*) was largely dependent on introgression. It was assumed that these polyploids were able to evolve rapidly and successfully colonise new habitats because their chromosome system had the advantage of being well buffered to absorb alien genetic material. It was demonstrated (Zohary and Feldman, 1962; Feldman, 1965; Pazy and Zohary, 1965) that *Aegilops* polyploids are not simple amphidiploids. Hybridisation between amphidiploids sharing a common genome was found to be a regular device for generating variation and ensuring genetic flexibility.

The present paper aims to demonstrate that additional sources of variation are present in the wheat group—which supplement the polyploid-to-polyploid gene transfer previously described. This work deals with direct introgression from diploids to tetraploids via triploid bridges. The process has been studied in two representative species combinations: by examination of the interspecific triploid hybrids and the types of F_2 and F_3 progenies they produced.

2. METHODS

Meiosis in p.m.c.s served both for determination of chromosome numbers and for the study of chromosome pairing. Anthers were fixed in 3:1 alcohol-acetic acid for 24 hours, stored in 70 per cent. alcohol and stained in acetocarmine.

Since chromosome numbers varied from plant to plant, pairing is expressed as "associations per chromosome" and not "chiasmata per cell". In each plant this value is based on only four randomly picked metaphases. Only in the triploids themselves and in the F_2 parents of the F_3 families samples of 30 cells were employed. We found that with the large number of chromosomes present (27-35), variation in chiasmata numbers between cells within plants was relatively small; and the sampling error due to samples of only four cells negligible for the purpose of the present work.

Pollen fertility was determined by dissecting mature anthers soaked in 4 per cent. acetocarmine and scoring c. 500 pollen grains per plant. Grains were considered normal when they were rounded and well stained.

Seed fertility was determined by examination of the two lower florets in the spikelet. A floret was considered fertile if a well-developed kernel was found in it. In the case of more fertile plants a sample of 100 florets (*i.e.* 50 spikelets) was employed. In the semi-sterile plants, and particularly in the triploids themselves, seed set was determined by examination of all available spikes.

* The material presented in this paper comprises a part of the Ph.D. thesis work of A. Vardi, to be presented to the Hebrew University, Jerusalem.

These two combinations present the two main classes of interspecific triploid hybrids encountered in the wheat group. They differ markedly from one another in the degree of chromosome pairing:

Triploid combination (i) is genomically AAB and contains two sets of more or less homologous chromosomes. This is a case of closely related diploid and tetraploid species, where one of the *durum* genomes (genome A) is similar to the chromosome set of diploid wild einkorn.

Triploid combination (ii) is ABS¹. It thus contains three different sets of chromosomes and shows very little chromosome pairing in meiosis. This combination is representative of cases where the diploid species is not directly related to the tetraploid one and apparently did not participate in its amphidiploid formation.

These artificial F₁ triploid hybrids were grown in Jerusalem in 1963. They were planted intermixed with the tetraploid wheat and were isolated from any other *Triticum* or *Ægilops* species. Thus the male sterile triploids were massively exposed to (back-cross) pollination by their tetraploid wheat parent. The occasional, rare back-cross seeds produced by the triploids were planted in 1964 and the second generation of hybrid derivatives was grown and analysed. These F₂ plants were also interplanted with tetraploid wheats. But since some of them were already semi-fertile, the seed they produced were a mixture of selfed and second back-cross products. Selected families of third hybrid generation derivatives were grown in 1965. The following diagram sketches the experimental design employed.

The results obtained are described in the following two sections.

(i) AAB triploid combination *T. durum* × *T. baeticum*

(a) F₁ hybrids

The four *T. durum* × *T. baeticum* triploids (genomic constitution AAB) showed marked vegetative vigour under Jerusalem nursery conditions and exceeded both parental lines in height and in number of tillers produced. Each produce 25-40 well-developed flowering spikes. Meiosis in the four plants was uniform and showed the characteristic features of wheat triploids where one genome is found in double dose: from four to seven bivalents (see plate III, fig. 1) and occasionally also a single trivalent. Pollen abortion was 98-99 per cent. and subsequently anthers did not dehisce. Thus the triploids were

TABLE 1
Cytology and fertility of parental species and their triploid F₁ hybrids

	No. of cells examined	Univalents	Bivalents	Trivalents	No. of chiasmata per cell	Assoc. per chromosome	Per cent. of normal pollen	Per cent. of seed-set
PARENTAL LINES								
<i>T. durum</i> cultivar <i>Nursit 163</i> (genomes AABB)	30	0.06 (0.2)	13.97 (13.14)	...	27.43 ± 1.55	1.95	95	98
<i>T. boeoticum</i> ssp. <i>thaoudar</i> (genome AA)	30	...	7	...	14.5 ± 1.06	2.07	97	92
<i>Æ. longissima</i> (genome S'S ¹)	30	...	7	...	13.5 ± 0.42	1.93	98	94
F ₁ TRIPLOID HYBRIDS								
<i>T. durum</i> × <i>T. boeoticum</i> (genomes AAB)	50	9.88 (3.15)	5.22 (2.6)	0.16 (0.1)	8.70 ± 1.66	0.83	2.0	1.6
<i>T. durum</i> × <i>Æ. longissima</i> (genomes ABS ¹)	46	18.63 (11.21)	1.15 (0.5)	0.02 (0.1)	1.21 ± 1.17	0.11	1.4	4.1

Note.—Values given for chromosome associations indicate means per microsporocyte; those in parentheses represent ranges observed.

completely male sterile. Pollination was assured by surrounding tetraploid wheat plants. The four vigorous triploids with their dozens of ears produced 2700 well-developed spikelets and 99 back-cross seed (table 1).

(b) *Second-generation hybrid derivatives*

The F_2 generation was started by planting the (back-cross) seed secured from the *durum* $\times$ *bæoticum* triploids.

Growth and development. A wide range of variation in germination, development and vigour was noted. Several kernels failed to germinate or develop, but the majority of individuals (75 altogether) reached maturity. Many showed pronounced vigour and even exceeded the original parental lines in size and development. Morphologically the larger proportion of the plants resembled the tetraploid wheat parent both in their erect habit and the shape of their ears (plate I).

Cytology. Chromosome numbers in the progeny of the *durum* $\times$ *bæoticum* triploids ranged from 27 to 30 with the mode of 28 (table 2). All these numbers are higher than the mode of 24-25 chromosomes expected from a random segregation in a triploid ($2n = 21$) $\times$ tetraploid ($2n = 28$) cross.

A striking improvement in chromosome pairing was apparent in the progeny when compared with their triploid parents. This is reflected in the frequency distribution of associations per chromosome (Plate III). Significantly, 45 of the 51 F_2 plants were characterised by a predominance of cells with 12-13 and sometimes even 14 bivalents (including occasional trivalents) plus 2-6 univalents. Moreover, most of the bivalents observed were of the ring type. Thus in general terms, the F_2 plants demonstrated pairing in 24-28 of their 27-30 chromosomes (table 3, figs. 2 and 3).

The cytological information on the F_2 generation also indicates what types of viable products the AAB F_1 triploids are able to produce. Pollen for the formation of all F_2 plants was standard, namely $n = 14$ AB *durum* pollen. Since the majority of the F_2 plants (with their 27-30 chromosomes) showed prevalence of 12 to 14 bivalents, they should have inherited, from their maternal AAB triploid parent, two more or less full chromosome sets (13-16 chromosomes), one of genome A and one of B. Thus the predominance in the F_2 generation of plants with 28-29 chromosomes apparently reflects a mechanism of genomic buffering (see Discussion).

Fertility. Parallel to the improvement in chromosome pairing remarkable restoration of fertility is apparent in the *durum* $\times$ *bæoticum* second-generation plants. This is true both for pollen scores (table 4) and for seed fertility (table 5). A wide variation in pollen and seed set was observed between plants, and individuals ranged from almost sterile to fully fertile. But significantly 69 out of 72 F_2 plants showed more than 15 per cent. of normally stained pollen—and their anthers at least partly dehisced. Moreover, several plants showed normal

TABLE 2
Chromosome numbers in second and in third hybrid generations: frequency distribution of chromosome types

Triploid combination and accession numbers	Chromosome No. in parent	Total plants examined	Chromosomal types															
			27	28	29	30	31	32	33	34	35	36	37	38	39	40		
A. SECOND HYBRID GENERATION (i) <i>T. durum</i> × <i>T. boeoticum</i> 455A, 455B, 455C, 455D (ii) <i>T. durum</i> × <i>Æ. longissima</i> 463A, 463C	21	56	7	31	14	4	...	...	...	...	...	...	...	...	...	...		
	21	59	...	...	5	5	8	6	18	7	10	...	...	...	...	...		
B. THIRD HYBRID GENERATION (i) <i>T. durum</i> × <i>T. boeoticum</i> 455B2, 455B8 455B1, 455B5, 455B14, 455B21 455B3, 455B10, 455B11 455B9 (ii) <i>T. durum</i> × <i>Æ. longissima</i> 463A22, 463A23, 463C25, 463C27 463C15, 463C16 463A1, 463A8, 463A17, 463A29	27	23	3	18	2	...	...	...	...	...	...	...	...	...	...	...		
	28	29	2	21	6	...	...	...	...	...	...	...	...	...	...	...		
	29	28	...	26	2	...	...	...	...	...	...	...	...	...	...	...		
	30	9	...	9	...	...	...	...	...	...	...	...	...	...	...	...		
	33	27	...	...	...	8	12	5	2	...	...	...	...	...	...	...		
	34	16	...	...	1	2	4	6	...	...	1	...	1	...	...	...		
	35	22	...	...	1	3	3	5	2	3	1	...	...	1	1	2		

TABLE 3
Chromosome pairing in second and in third hybrid generations: frequency distribution of "associations per chromosome" values

Triplod combination and accession numbers	Chromosome No. in parent	No. of plants examined	Associations per chromosome												
			0.9-1.0	1.0-1.1	1.1-1.2	1.2-1.3	1.3-1.4	1.4-1.5	1.5-1.6	1.6-1.7	1.7-1.8	1.8-1.9	1.9-2.0	2.0-2.1	
A. SECOND HYBRID GENERATION (i) <i>T. durum</i> × <i>T. boeoticum</i> 455A, 455B, 455C, 455D (ii) <i>T. durum</i> × <i>Ae. longissima</i> 463A, 463C	21	51	...	...	...	1	...	7	6	14	14	7	2	...	
	21	53*	2	1	6	2	11	24	5	1	...	...	...	...	
B. THIRD HYBRID GENERATION (i) <i>T. durum</i> × <i>T. boeoticum</i> 455B2, 455B8 455B1, 455B5, 455B14, 455B21 455B3, 455B10, 455B11 455B9	27	21	...	...	...	...	...	...	...	2	7	5	7	...	
	28	26	...	...	...	...	...	2	...	1	7	3	12	1	
(ii) <i>T. durum</i> × <i>Ae. longissima</i> 463A22, 463A23, 463C25, 463C27 463C15, 463C16 463A1, 463A8, 463A17, 463A29	29	27	...	...	...	...	...	...	...	...	1	9	13	4	
	30	7	...	...	...	...	...	...	...	...	1	3	3	...	
	33	24	...	1	1	...	...	1	6	6	7	2	...	...	
	34	14	...	...	...	1	...	2	1	6	3	1	...	...	
	35	21	...	...	1	1	...	1	6	3	7	2	...	...	

* Includes one plant with 0.5-0.6 associations per chromosome.

or almost normal pollen fertility. The restoration is obvious also from the seed set data (table 5): the majority of the individuals were semi-fertile and some showed 75-100 per cent. seed set. A rough correlation between bivalent formation and fertility was noted. Plants with prevalence of 13-14 bivalents tended to be more fertile. This was particularly true in $2n = 28$ plants.

(c) *Third-generation hybrid derivatives*

Ten representative families of third-hybrid generation plants of the *durum* $\times$ *baeoticum* combination were raised. Each F_3 family consisted of a sample of 13-15 progenies grown from seed produced by an individual F_2 plant. Two such F_3 families were derived from $2n = 27$ parents, four from F_2 parents with 28 chromosomes, three from parents with 29 chromosomes and the tenth had a $2n = 30$ seed parent (table 6). These parents represent the various chromosome types and fertility classes of the second hybrid generation of the *durum* $\times$ *baeoticum* combination. They all showed some degree of pollen fertility with anthers at least partly dehiscent. Since the ten parents were also exposed to pollen of tetraploid wheats, the F_3 generation should be considered as a mixture of selfed products and second back-cross derivatives.

Growth and development. All F_3 families were strikingly similar to the tetraploid wheat parent. With the exception of 3-4 freaks, plants showed normal development. Segregation occurred both between and within families, in characters such as vegetative vigour, shape of spikes, brittleness and hairiness. The majority of the individuals in all ten families were vegetatively vigorous, many even taller than their tetraploid *durum* parent, and produced large, long ears.

Cytology. Chromosome numbers in the F_3 representatives varied from 27 to 29 with a conspicuous trend to stabilise the chromosome number at $2n = 28$ (table 2). Progenies derived from $2n = 27$ parents increased their chromosomes: 18 out of the 23 progenies examined had $2n = 28$. Progenies from $2n = 28$ parents remained centred around their parents' number, while of those from $2n = 29$ and $2n = 30$ parents all but two had 28 chromosomes.

A general trend for further improvement in chromosome pairing characterises all the ten F_3 *durum* $\times$ *baeoticum* families. Chromosome association in the majority of plants was almost normal, particularly in the $2n = 28$ derivatives. More than half of the $2n = 28$ plants commonly had 13-14 bivalents, and only occasional univalents. Some showed regular formation of 14 bivalents (see plate III, fig. 4) and were similar in chromosome behaviour to the original tetraploid *durum* stock. This improvement in chromosome pairing is reflected in the associations per chromosome (table 3). Most F_3 individuals scored values higher than their F_2 parents and very few scored less. Forty out of the 81 plants had values higher than 1.9 (means of 26-29 chiasmata per p.m.c.), and thus entered the normal range of chromosome pairing found in the tetraploid *durum* wheat.

TABLE 4
Frequency distribution of pollen fertility values scored by second and third hybrid generations

Triploid combination and accession numbers	Chromosome No. in parent	No. of plants examined	Pollen fertility (in per cent.) classes							
			0-5	6-15	16-30	31-45	46-60	61-75	76-90	91-100
A. SECOND HYBRID GENERATION (i) <i>T. durum</i> × <i>T. boeoticum</i> 455A, 455B, 455C, 455D (ii) <i>T. durum</i> × <i>Æ. longissima</i> 463A, 463C	21	72	2*	1	12	11	10	10	18	8
	21	58	11*	16	15	6	2	7	1	...
B. THIRD HYBRID GENERATION (FAMILIES) (i) <i>T. durum</i> × <i>T. boeoticum</i> 455B2 455B8 455B1 455B5 455B14 455B21 455B3 455B10 455B11 455B9	27	14	...	...	1*	2	2	2	5	2
	27	12	...	...	...	*	...	2	2	7
	28	15	...	...	...	*	2	1	3	6
	28	15	...	*	...	...	...	2	5	8
	28	14	...	1	...	1	2	2*	7	1
	28	13	...	...	...	...	...	...	4	9*
	29	15	...	...	...	*	1	5	9	...
	29	14	...	...	...	...	...	...	5*	9
	29	15	...	...	...	...	...	...	5	10*
	29	15	...	...	...	...	1	...	9	4*
	30	15	...	...	...	...	...	1	...	...
	33	8	2	4*	2	...	...	...	...	...
	33	9	2	3*	4	...	...	...	...	...
(ii) <i>T. durum</i> × <i>Æ. longissima</i> 463A22 463A23 463C25 463C27 463C15 463C16 463A1 463A8 463A17 463A29	33	12	7	2	3	*	...	...	...	...
	33	10	...	4*	5	...	1	...	...	...
	34	9	...	...	4	...	...	2*	3	...
	34	9	2	3	2	...	...	1*	...	...
	35	2	...	...	...	*	1	1	...	...
	35	15	1	...	...	...	...	4*	5	5
	35	4	1	...	3	...	*	...	...	...
	35	6	...	...	1	1	1*	1	2	...
	35	6	...	...	...	...	...	...	...	...
	35	6	...	...	...	...	...	...	...	...

* The asterisks mark the pollen fertility of the parents.

TABLE 5
Frequency distribution of seed set values scored by second and third hybrid generations

Triplet combination and accession numbers	Chromosome No. in parent	Total plants examined	Seed-set (in per cent.) classes								
			0-5	6-15	16-30	31-45	46-60	61-75	76-90	91-100	
A. SECOND HYBRID GENERATION											
(i) <i>T. durum</i> × <i>T. boeoticum</i> 455A, 455B, 455C, 455D	21	72	1*	4	12	12	9	8	15	11	
(ii) <i>T. durum</i> × <i>Æ. longissima</i> 463A, 463C	21	58	56*	2	...	...	...	...	...	...	
B. THIRD HYBRID GENERATION (FAMILIES)											
(i) <i>T. durum</i> × <i>T. boeoticum</i>											
455B2	27	14	...	...	1	1*	3	...	2	7	
455B8	27	12	1	...	...	*	4*	...	3	4	
455B1	28	15	...	...	...	...	2	1	2	12	
455B5	28	15	...	...	*	...	...	1	6	6	
455B14	28	14	...	...	*	1	...	1*	3	9	
455B21	28	13	...	...	...	...	...	1*	4	9	
455B3	29	15	...	...	...	...	...	1*	2	12	
455B10	29	14	...	...	...	...	...	1	1*	12	
455B11	29	15	...	...	...	...	...	...	3	12*	
455B9	30	15	...	...	...	...	...	1	5	9*	
(ii) <i>T. durum</i> × <i>Æ. longissima</i>											
463A22	33	8	8*	...	...	...	...	...	...	...	
463A23	33	9	3*	6	...	...	...	...	...	...	
463C25	33	12	9*	3	...	...	...	...	...	...	
463C27	33	10	7*	3	...	...	...	...	...	...	
463C15	34	9	5*	2	1	...	1	...	...	...	
463C16	34	9	7*	2	...	...	...	...	...	...	
463A1	35	9	2*	...	...	...	...	...	...	...	
463A8	35	15	8	5*	2	...	...	...	...	...	
463A17	35	4	3*	1	...	...	...	...	...	...	
463A29	35	6	3*	...	2	1	...	...	...	...	

* The asterisks mark the seed set of the parents.

TABLE 6
Cytology and fertility of the *ten durum* × *boeoticum* F₂ plants which served as parents for the F₃ families

F ₂ parent (accession number)	Chromosome No. (2n)	Chromosome association in metaphase I							Fertility	
		No. of cells examined	Univalents	Bivalents	Trivalents	Quadri- valents	No. of chiasmata per cell	Associations per chromosome	Per cent. of normal pollen	Per cent. of seed set
455B-2	27	30	3·3 (3·5) 1·16	11·73 (10·12) 12·86	0·06 (0·1) ...	...	21·8 ± 2·24	1·60	19·2	32·8
455B-8	27	30	(1·3) 4·75 (2·9)	(11·13) 11·25 (8·13)	...	...	26·26 ± 2·05	1·94	40·1	33·3
455B-1	28	4	5 (4·6)	11·5 (11·12)	0·02 (0·1) ...	...	21·75	1·55	44·4	51·7
455B-5	28	4	2·1 (1·4)	12·9 (12·13)	0·03 (0·1) ...	...	20·5	1·46	15·8	18·9
455B-14	28	30	0·06 (0·2)	13·96 (13·14)	...	...	25·13 ± 1·12	1·79	62·9	19·5
455B-21	28	30	1·46 (0·4)	13·26 (10·14)	0·33 (0·1) ...	...	26·86 ± 1·68	1·92	94·8	98·5
455B-3	29	30	1·66 (1·3)	13·66 (13·14)	...	...	25·43 ± 2·53	1·75	31·2	63·6
455B-10	29	30	3·12 (1·5)	12·37 (11·14)	0·6 (0·1) 0·5	0·12 (0·1) ...	27·26 ± 1·86	1·88	84·1	87·2
455B-11	29	8	4 (1·3)	13 (12·14)	0·5 (0·1)	...	22·12	1·52	92·6	93·0
455B-9	30	4					25·0	1·66	94·4	97·1

Note.—Values given for chromosome associations indicate *means* per microspore; those in parentheses represent *ranges* observed.

Thus, in all the ten representative F_3 families of the *durum* $\times$ *baeoticum* combination there is a marked trend for stabilisation of chromosome number at the euploid tetraploid level. Moreover, many individuals already show a pattern of chromosome pairing characteristic of the tetraploid *durum* parental stock.

Fertility. Parallel to the trend for chromosome stabilisation just described, further improvement of fertility occurs in all the ten F_3 families (tables 4 and 5). In fact, the larger proportion of the third-generation plants were found to be fully fertile or almost fully fertile.

(ii) ABS^1 triploid combination (*T. durum* $\times$ *Æ. longissima*)

(a) F_1 hybrids

The two *T. durum* $\times$ *Æ. longissima* triploids (genomic constitution ABS^1) showed conspicuous vegetative vigour under Jerusalem nursery conditions; each produced numerous tillers and dozens of long, well-developed spikes. Meiosis in both plants was characteristic of wheat triploids containing three different genomes. There was very little chromosome pairing (table 1). Most common were p.m.c.s with a single-rod bivalent plus 19 univalents (plate III, fig. 5); cells with 21 I or 2 II + 17 I were also rather frequent. Chiasmata per cell values were thus very low—around 1.2. At the end of first meiotic division 2-4 per cent. of the cells contained restitution nuclei. Such nuclei, with the full set of 21 elements, were apparently formed in p.m.c.s which previously contained univalents only (plate III, fig. 6). In addition, cells with almost complete restitution nuclei were also frequent. The latter apparently have been produced by cells with a single bivalent which many times showed at the end of the first division a restitution aggregate of univalents at their centre, while the bivalents' separating products were paradoxically excluded as micronuclei at the poles (plate III, fig. 6). Similar to the AAB combination, the ABS^1 triploids were fully male sterile. Anthers did not dehisce and contained only 1.0-1.5 per cent. of stainable pollen. But plants were massively exposed to pollen of tetraploid wheat and produced 2-4 per cent. of back-cross seed.

(b) Second-generation hybrid derivatives

The F_2 generation was started by planting the (back-cross) seed secured from the *durum* $\times$ *longissima* triploids.

Growth and development. A very wide range of variation in germination, development and vegetative vigour was noted. Sixty seeds were planted and all but one reached maturity. Wide morphological segregation was apparent, and in contrast to the *durum* $\times$ *baeoticum* case many F_2 plants had a "wild type" appearance, *i.e.* spreading or semi-spreading habit and very elongate, cylindrical or loose spikes. Some even showed the strong terminal awns characteristic of the *longissima* spike (plate II).

Cytology. The F_2 generation from *durum* $\times$ *longissima* had chromosome numbers ranging from 29 to 35, with a majority (35 out of 53) containing 33-35 chromosomes. These values are higher by 5-7 chromosomes than those found in the progeny of the previous *durum* $\times$ *baeoticum* combination.

F_2 plants showed remarkable improvement in chromosome pairing compared to their F_1 parents, but also varied widely in this character. Most plants, however, showed a high frequency of univalents (7-12) and many (40 out of 53) had 9-14 bivalents (including occasional trivalents). Moreover, bivalents were mainly of the ring type (plate III, figs. 7 and 8). Thus the major difference between the F_2 from the AAB combination and that from the ABS¹ combination was not in the number of paired chromosomes present, but in the additional univalents present in the ABS¹ derivatives. This was particularly conspicuous in pentaploid or almost pentaploid F_2 individuals. It is also reflected in the "associations per chromosome" scored by the *durum* $\times$ *longissima* F_2 plants (table 3). The lower values of the ABS¹ derivatives are mainly due to the presence of additional univalents, not the lack of bivalents. In mean chiasmata per cell, F_2 progenies from *durum* $\times$ *longissima* scored values only slightly lower than those of their *durum* $\times$ *baeoticum* counterparts.

The cytological data assembled enabled conclusions to be drawn on the types of viable gametes produced by the ABS¹ triploids. The pollen source was standard, and all F_2 plants resulted from back pollination by $n = 14$ AB *durum* pollen. Since the larger part of viable F_2 plants were pentaploid or almost pentaploid (33-35 chromosomes), the most frequent class of viable female gametophytes (or gametophytes that gave rise to viable zygotes) produced by the ABS¹ triploid were those with unreduced or almost unreduced nuclei. That pentaploid F_2 plants had actually a rough AABBS¹ constitution is shown also by the frequent occurrence of 14 bivalents + 7 univalents (plate III, fig. 8). Thus, in this combination also the operation of genomic buffering is apparent; but in this case largely gametophytes with approximately three full sets of chromosomes have survived.

Fertility. In contrast to the semi-fertility of the *durum* $\times$ *baeoticum* derivatives most of the F_2 plants from the *durum* $\times$ *longissima* were almost sterile (tables 4 and 5).

(c) Third-generation hybrid derivatives

Ten F_3 families from the *durum* $\times$ *longissima* combination were raised and each family was derived from a single, representative F_2 plant. Some of these families are smaller than those in the previous (*durum* $\times$ *baeoticum*) combination. This was determined by the lower fertility of the F_2 generation mentioned above. The parents of the F_3 families were selected to represent the common chromosome types and fertility classes encountered in the F_2 generation. Four families are derived from parents with $2n = 33$ chromosomes, two from parents

TABLE 7
Cytology and fertility of the ten durum $\times$ longissima F_2 plants which served as parents for the F_3 families

F ₂ parent (accession number)	Chromosome No. (2n)	Chromosome association in metaphase I						Fertility		
		No. of cells examined	Univalents	Bivalents	Trivalents	Quadri- valents	No. of chiasmata per cell	Associations per chromosome	Per cent. of normal pollen	Per cent. of seed set
463A-22	30	33	9.2 (7-11)	11.6 (10-13)	0.06 (0-1)	0.1 (0-1)	22.63 ± 1.40	1.37	15.2	2.3
463A-23	30	33	6.67 (3-11)	10.8 (9-13)	1.2 (0-3)	0.26 (0-2)	23.3 ± 1.59	1.43	15.4	1.7
463C-25	30	33	7.03 (6-9)	12.9 (12-13)	...	0.03 (0-1)	24.56 ± 1.46	1.43	41.7	4.33
463C-27	30	33	7 (6-8)	11.33 (10-13)	1.03 (0-2)	0.03 (0-1)	23.33 ± 1.96	1.42	10.2	4.9
463C-15	30	34	5.26 (3-8)	13.7 (12-15)	0.36 (0-2)	0.06 (0-1)	26.3 ± 1.60	1.70	66.3	5.2
463C-16	30	34	8.0 (6-12)	12.8 (11-14)	0.06 (0-1)	0.1 (0-1)	24.9 ± 1.60	1.46	68.0	5.0
463A-1	30	35	7.33 (5-9)	13.7 (12-15)	0.1 (0-1)	...	25.33 ± 1.54	1.45	39.7	3.3
463A-8	30	35	6.7 (5-9)	13.7 (12-15)	0.2 (0-2)	0.06 (0-1)	26.06 ± 1.46	1.48	73.6	7.3
463A-17	30	35	6.66 (4-9)	13.36 (11-15)	0.53 (0-2)	...	26.93 ± 1.46	1.54	51.5	0.7
463A-29	30	35	6.9 (5-9)	14.0 (13-15)	0.03 (0-1)	...	26.7 ± 1.16	1.52	47.7	2.2

Note.—Values given for chromosome associations indicate means per microsporocyte; those in parentheses represent ranges observed.

with $2n = 34$ and four from parents with the pentaploid $2n = 35$ (table 7).

Growth and development. F_3 plants were much closer in their general morphology to the tetraploid wheat parent than the F_2 generation. But less so than the F_3 generation of the *durum* $\times$ *bæoticum* combination. Variation was wider here, both within and between families in morphology and in vegetative vigour. Several (7-8) individuals (in several families) were retarded in their growth, while many were quite vigorous, some even exceeded their *durum* parent in size. Segregation was also noted in habit. About one-third of the plants were erect, the others were semi-spreading or spreading. The genetic contribution of the diploid *Æ. longissima* was still obvious, particularly in the shape of the ear. More than half of the F_3 plants had loose or semi-cylindrical spikes, some were brittle, some were tough.

Cytology. Chromosome numbers in the F_3 families varied widely, and individuals from $2n = 29$ to $2n = 40$ were encountered (table 2). In the four F_3 families derived from parents with $2n = 33$ there is a trend to decrease in chromosome numbers and the families show mean values some 2-3 chromosomes fewer than their respective F_2 parents. Thus these four families seem to follow the same trend towards stabilisation at the 28 chromosome level characteristic of the *durum* $\times$ *bæoticum* combination, although in this case stabilisation has not yet been achieved.

The F_3 families raised from the pentaploid ($2n = 35$) or almost pentaploid ($2n = 34$) parents show a somewhat different picture. Here two trends are apparent. There is an obvious major trend to decrease chromosome numbers parallel to that in the previous four families. But there is also a trend to increase chromosomes since five individuals had chromosome numbers ranging from 38 to 40. Moreover, the distribution of chromosome types among F_3 individuals seems bimodal (table 2).

Compared to their F_2 parents, plants of the F_3 families showed some improvement in chromosome pairing. This is apparent from the frequency distribution of "associations per chromosome" scored (table 3). However, parallel to the situation in F_2 the F_3 plants lag in their scores behind those of the *durum* $\times$ *bæoticum* F_3 sample. The large majority of plants in all 10 families were slightly more regular in the formation of ring bivalents than their F_2 parents. The majority of F_3 plants with 29-32 chromosomes showed prevalence of 12-14 bivalents, while 15-18 bivalents were common in F_3 plants with 39-40 chromosomes. One plant with $2n = 40$ even showed p.m.c.s with 19 II + 2 I (plate III, fig. 9).

Fertility. Most F_3 plants were very poor in seed set (table 5), and about two-thirds of them scored seed fertility values of 5 per cent. or less. This is in sharp contrast to the predominance of semi-fertile or almost fertile plants in the F_3 representatives of the AAB combination. But even the low values obtained, represent some degree of restoration

compared to the values obtained in the F_2 generation. Significantly a few F_3 individuals achieved seed set values in the range of 15-50 per cent. Thus there is a general agreement in the F_3 generation of the ABS¹ combination between the cytological data and the fertility scores. F_3 plants have not yet reached either full chromosomal stabilisation or full pollen or seed fertility.

5. DISCUSSION

The results indicate that in the wheat (*Triticum-Aegilops*) group, interspecific triploid hybrids can serve as efficient bridges for gene transfer from diploid to tetraploid species. Further, field observations indicate that such introgression actually operates in nature. The data on various triploid hybrids, and particularly on numerous F_2 and F_3 progenies from two combinations tested also indicate what happens in the critical stages of introgression: how sterility barriers are crossed and how the drastic segregational unbalance is overcome.

Interspecific triploid wheat hybrids are not completely sterile. When massively exposed to parental pollen (as they are in nature) such hybrids set occasional back-cross seed. This was the case when diploid and tetraploid species shared a common genome (the AAB combination of *T. durum* $\times$ *T. baoticum*). It was also true when three different genomes constituted the triploid (the ABS¹ combination of *T. durum* $\times$ *Ae. longissima*). Thus by virtue of back pollination (by polyploid parents) an intrigression process is effectively initiated.

The choice of two different triploid combinations clarified what actually happens in the critical stage of the introgression process—when the F_2 generation is formed. The large samples of F_2 plants examined in each combination show what products escape the segregational “shock”, when meiosis in the F_1 triploids drastically disrupts the original chromosome complements. Similar to the process of tetraploid-to-tetraploid introgression in *Aegilops* (Pazy and Zohary, 1965) the operation of genomic buffering is apparent here. This is reflected by the high chromosome numbers encountered in F_2 plants. It is mainly gametophytes possessing an approximately full chromosome complement which are able to survive or form viable F_2 zygotes. From the data gathered the following picture emerges:

(i) In the AAB combination introgression is apparently effectively initiated by the survival of 13-16 chromosome, roughly AB female gametophytes. That such gametophytes, possessing more or less the full content of A and B genomes, are the main viable products of the F_1 triploids is clearly indicated by the chromosome numbers of the F_2 plants and the pairing they show (see p. 545). Moreover, the recovery of such F_2 segregants is in full accord with the meiotic behaviour of the triploid parents themselves. The two sets of genome A chromosomes form mainly bivalents; this leads to more or less regular A:A separation in anaphase I. Irregular separation is the lot of the single

B set chromosomes which occur mainly as univalents. From the mode of anaphase distribution of the univalents one expects a small fraction of meiotic segregants incorporating 6-7 B genome chromosomes. This fits well with the seed fertility values found in the AAB triploids. When such essentially AB female gametophytes are fertilised by standard $n = 14$ AB *durum* pollen, stabilisation at the tetraploid level is achieved—and in a single step! This is reflected in the F_2 generation by the concentration around 28 chromosomes, the conspicuous improvement in chromosome pairing and the spectacular restoration of fertility. What still remains unbalanced is further corrected in the subsequent F_3 and F_4 generations. But these F_3 and F_4 corrections (*i.e.* elimination of 1-2 surplus chromosomes, increase from 27 to 28 chromosomes, improvement in pairing, further recovery of fertility) are indeed relatively minor adjustments. All in all gene transfer from diploid *T. baeticum* to tetraploid *T. durum* via a triploid hybrid bridge is a neat, basically one-step process. Paradoxically it is well canalised by the high sterility of the triploid, *i.e.* by the drastic selection which weeds out meiotic products other than the rare, chromosomally balanced AB-type female gametophytes. When stabilisation is attained and fertility restored, selection coupled with self-pollination would rapidly fix favourable recombinations and complete the introgression cycle.

(ii) In the ABS^1 combination diploid to tetraploid introgression is obviously a longer process. But here too a mechanism of genomic balancing buffers the critical stage. Examination of F_2 plants clearly shows that the main class of viable products produced by the ABS^1 triploids is that of unreduced or almost unreduced gametophytes. The main difference between the AAB and the ABS^1 combinations is thus in the number of genomes that go together to form viable gametophytes. When one genome is shared, the main likelihood is for two-set AB products. But when three different genomes combine in the F_1 hybrid the most common viable products are three-set or almost three-set ABS^1 gametophytes. Why triple dosage prevails (rather than balanced products with only two genomes) is indicated also by the meiotic behaviour of the ABS^1 F_1 triploids. In contrast with AAB F_1 triploids one finds here almost no chromosome pairing (see p. 552). Subsequently restitution nuclei with 33-35 chromosomes are regularly produced—in the order of few per cent. On the other hand, when 21 univalents are present, the chance for segregation of more or less two full sets is very small indeed.

When such roughly ABS^1 constituted female gametophytes are fertilised by $n = 14$ *durum* AB pollen the resulting F_2 generation is mainly pentaploid or near pentaploid, and stabilisation is a much longer process. In contrast with the F_2 generation of the previous combination, F_2 plants here are yet largely sterile. Even the F_3 families contain mostly unbalanced products, but improvement is apparent. There is a main trend among F_3 individuals to decrease chromosome numbers and restore fertility. Significantly F_3 individuals

with 29-33 chromosomes include semi-fertile plants, many of which are characterised already by the prevalence of 13-14 bivalents. Future development seems to be determined. Such semi-fertile plants can be expected to further decrease their chromosome numbers in one or two generations and to become stabilised at the tetraploid $2n = 28$ *durum* level. That such products could easily incorporate alien substitution segments donated by the S^1 genome is indicated by the occurrence of trivalents both in the F_1 and F_2 generations. Moreover, the stabilisation process might be further hastened by additional back-cross pollination of either the F_2 or the F_3 plants. All in all, one is led to the conclusion that gene transfer from diploid *Æ. longissima* to tetraploid *T. durum* via a triploid bridge can be achieved in a matter of 3-4 generations. It is not a quick one-step process, as the previous bridge was. Yet it is relatively efficiently achieved and well buffered, in its critical stage, by F_2 pentaploidy.

Apart from the F_2 trend to decrease chromosome numbers toward stabilisation at the $2n = 28$ *durum* level, the ABS^1 combination F_3 plants indicate an alternative development of chromosome increase. This trend is clear in the families obtained from pentaploid, $2n = 35$ $AABBS^1 F_2$ individuals. F_3 families of such $2n = 35$ parents show bimodal distribution of chromosome numbers (table 2) and chromosome numbers as high as 40. One F_3 $2n = 40$ plant showed 19 bivalents + 2 univalents. Such F_3 individuals might lead to fixation at the hexaploid level and give rise to a new, $2n = 42$, $AABBS^1 S^1$ amphidiploid. More data are needed before this trend of chromosome increase could be critically evaluated. But even at this stage one is tempted to suggest that hexaploid entities in *Triticum* and *Aegilops* could have originated in such a fashion, and are not necessarily exclusively products of somatic chromosome doubling in triploid F_1 hybrids. If so, hexaploid cultivated wheat *T. aestivum* could have evolved in a similar way—upon back-pollination of a tetraploid wheat $\times$ *Æ. squarrosa* spontaneous hybrid. Such ABD triploid could have given rise to a $AABBD F_2$ pentaploid. This in turn could have produced a stabilised $AABBDD$ amphidiploid.

As to the actual evolutionary role of diploid to tetraploid introgression in variation build-up in polyploid *Triticum* and *Aegilops* species—it is yet difficult to arrive at any definite evaluation. Our main aim in this paper is to point out the fact that such gene-flow is possible and could have added significantly to the build up of variation and to the evolutionary success of polyploid entities here. Situations like *Æ. variabilis*-*Æ. longissima* clearly indicate its occurrence in nature. But from our experience with *Aegilops* and *Triticum* species in the Middle Eastern countries it seems that diploid to tetraploid connections are far less frequent than connections between the tetraploids themselves. Only the later interconnections (see Zohary, 1965) are very frequent and conspicuous in nature. Yet one has also to bear in mind that when a common genome is shared by the diploid donor and the tetraploid

Representative spikes from the parental species, F_1 triploid hybrid and F_2 derivatives in *T. durum* $\times$ *T. boeoticum* AAB combination. Note the close morphological resemblance of the F_2 plants to their *durum* parent.

Representative spikes from the parental species F_1 , triploid hybrid and F_2 derivatives in *T. durum* $\times$ *Ae. longissima* ABS¹ combination. Note the wide segregation in the F_2 generation.

Plate III

FIGS. 1-4. Meiosis in *durum* $\times$ *bæoticum* AAB combination.

FIG. 1.—6 II+9 I in F_1 triploid.

FIG. 2.—13 II+2 I in $2n = 28$ F_2 plant.

FIG. 3.—14 II+1 I in $2n = 29$ F_2 plant.

FIG. 4.—14 II in stabilised $2n = 28$ F_3 plant.

FIGS. 5-9. Meiosis *durum* $\times$ *longissima* ABS¹ combination.

FIG. 5.—1 II+19 I in F_1 triploid.

FIG. 6.—Microsporocyte with a full restitution nucleus and microsporocyte with almost full restitution nucleus at the end of first division in the F_1 triploid.

FIG. 7.—1 IV+8 II+11 I in $2n = 31$ F_2 plant.

FIG. 8.—14 II+7 I in pentaploid F_2 plant.

FIG. 9.—19 II+2 I in $2n = 40$ F_3 plant.

Magnification: fig. 6 *c.* $\times 300$; all other figs. $\times 750$.

receptor introgression via triploids is not only a quick but, as already noted by Harlan (1964), also a subtle process. While F_1 triploids can be detected in natural mixed stands it is very hard to recognise morphologically the F_2 derivatives. Thus one can easily overlook such introgression. In addition direct diploid to tetraploid introgression could have played a decisive role in the past, when polyploids were just formed and when it could have greatly assisted their initial spread into areas yet occupied by diploids alone—in a similar way that it did apparently help tetraploids in the *Dactylis glomerata* complex (Zohary and Nur, 1959) to achieve their distribution.

Finally it seems worth while to suggest some practical applications for the two triploid bridges described. Such experimental design can serve in wheat breeding work. The AAB case can serve as a model for an easy transfer of favourable genes from diploid einkorn or diploid *Æ. speltoides* to tetraploid *T. durum*. The results obtained with ABS^1 combination indicate also what could be done with genes on the D genome of diploid *Æ. squarrosa*. Instead of attempting to produce colchicine doubled amphidiploids, *squarrosa* genes could be transferred to hexaploid *T. aestivum* by securing AABBD pentaploid F_2 plants from a *T. durum* $\times$ *Æ. squarrosa* triploid and crossing them to hexaploid AABDD *T. aestivum*.

6. SUMMARY

1. Diploid to tetraploid introgression by means of interspecific triploid hybrids was followed in the wheat (*Triticum-Ægilops*) group in two species combinations:

(i) *T. durum* $\times$ *T. bœoticum*; (ii) *T. durum* $\times$ *Æ. longissima*.

2. In each combination triploid F_1 hybrids are not completely sterile. When exposed to massive back pollination by the tetraploid parents, triploids set occasional seed.

3. Analysis of the ensuing F_2 and F_3 derivatives gave the following results:

- (i) When the diploid and the tetraploid species share one genome (*durum* $\times$ *bœoticum* AAB genomic combination) reproductive barriers are effectively crossed by occasional production in the F_1 triploids of balanced, roughly $n = 14$ AB types of female gametophytes.
- (ii) when three different genomes constitute the triploid hybrid (*durum* $\times$ *longissima* ABS^1 genomic combination) the disruptive effect of meiosis in the F_1 triploid is overcome by occasional formation of unreduced or almost unreduced ABS^1 eggs. But in comparison to the first combination introgression here is a slower process.

4. Data indicate the operation in the wheat group of diploid to tetraploid introgression in nature.

Acknowledgments.—Thanks are due to Miss Zohar Brick for the preparation of the triploid hybrids that started this study. The authors are greatly indebted to the Agricultural Research Service of the United States Department of Agriculture for a research grant (FG-1s-129) which supported this study.

7. REFERENCES

- FELDMAN, M. 1965. Further evidence for natural hybridization between tetraploid species of *Ægilops* section *Pleionathera*. *Evolution*, 19, 162-174.
- HARLAN, J. R. 1965. The possible role of weed races in the evolution of cultivated plants. *Euphytica*, 14, 173-176.
- KIHARA, H. 1954. Considerations on the evolution and distribution of *Ægilops* species based on the analyzer-method. *Cytologia*, 19, 336-357.
- PAZY, B., AND ZOHARY, D. 1965. The process of introgression between *Ægilops* polyploids: Natural hybridization between *A. variabilis*, *A. ovata* and *A. biuncialis*. *Evolution*, 19, 385-394.
- SEARS, E. R. 1959. Weizen I: The systematics, cytology and genetics of wheats. In *Handbuch der Pflanzenzüchtung* ed. H. Kappert, and W. Rudorf, Vol. II, 164-187. Parey, Berlin.
- ZOHARY, D. 1965. Colonizer species in the wheat group in *The Genetics of Colonizing Species* ed. H. G. Baker, and G. L. Stebbins, 404-420. Academic Press, New York.
- ZOHARY, D., AND FELDMAN, M. 1962. Hybridization between amphidiploids and the evolution of polyploids in the wheat (*Ægilops-Triticum*) group. *Evolution*, 16, 44-61.
- ZOHARY, D., AND NUR, U. 1959. Natural triploids in the Orchard Grass, *Dactylis glomerata* L. polyploid complex and their significance for gene flow from diploid to tetraploid levels. *Evolution*, 13, 311-317.

Note added in Proof

Since the completion of this paper we have grown additional generations from the $ABS^1 \text{ durum} \times \text{longissima}$ combination. Among F_4 and F_5 plants derived from the F_2 AABBS¹ pentaploids we already detected four $2n=42$ plants. These hexaploids were all fertile and showed more or less regular chromosome pairing in meiosis (most p.m.c.s had 21 bivalents). Thus in a matter of 2-3 generations F_2 pentaploids gave rise to stabilised AABBS¹S¹ amphidiploids, and the process of increasing chromosome number was completed.