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Aegilops × *Secale* hybrids: the production and cytology of diploid hybrids

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SUMMARY

Hybrids between four diploid species of *Aegilops* and species of *Secale* were obtained by using embryo culture. There was a marked incompatibility in the crosses between *Secale* species and each of the four species in Section Sitopsis of *Aegilops* and *Ae. mutica*. It is suggested that this genetic incompatibility with *Secale* species is an additional similarity between these species of *Aegilops* and the diploid species of *Triticum*.

Most chromosomes of *Aegilops* (A) and *Secale* (S) are univalent during meta-anaphase of meiosis in these hybrids, but some appeared to associate and others to pair as apparently normal chiasmate bivalents. Analysis of non-chiasmate and chiasmate associations showed that the frequencies of autosyndetic (AA and SS) and allosyndetic (AS) associations fitted the 3 AA : 7 AS : 3 SS ratio expected if association and pairing is at random. Any deviations from random involved a deficiency rather than an excess of *Aegilops–Secale* pairing. There is no evidence that the chromosomes of *Secale* are homologous with those of *Ae. caudata*, *Ae. comosa* and *Ae. umbellulata*, and it is suggested that the genome of *Secale* species does not show any homology with the genomes of the genera *Aegilops*. This does not preclude the presence of homologous segments. It is suggested that the possibility of random association of chromosomes should be considered when occasional pairing in interspecific hybrids is analysed, and that identification of chromosomes and recognition of chiasmata are required. The possibilities of chiasmata between non-homologous chromosomes, of a genetic mechanism in rye which suppresses the pairing of homoeologous chromosomes, and of other factors causing asynapsis and pseudo-synapsis between genetically similar chromosomes are discussed.

1. INTRODUCTION

Many intergeneric hybrids combining species of *Aegilops* or *Secale* with species of *Triticum* have been used extensively in cytogenetic research, both for evolutionary studies and for plant breeding. Almost all the genomes present in species of *Aegilops* are at least partly homologous with the three genomes of *Triticum aestivum*. In contrast, extensive cytological studies have not produced any evidence of homology between the chromosomes of *Secale* and *Triticum* species (Riley & Kimber, 1966).

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Chromosome pairing in hybrids between some polyploid species of *Aegilops* and diploid *Secale cereale* has been reported, but the analysis of homology is made uncritical by the presence of two or more sets of *Aegilops* chromosomes. From meiotic analysis based on the identification of parental chromosomes in diploid hybrids, Melnyk & Unrau (1959) and Melnyk (1961) reported that *Secale* chromosomes associated and appeared to form bivalents and multivalents with those of each of two diploid species, *Ae. squarrosa* and *Ae. comosa*. They concluded that the homology between the chromosomes of *Ae. squarrosa* and *S. cereale* is extensive and is expressed freely in the absence of any genetic mechanism restricting pairing to fully homologous chromosomes.

If the reported intergeneric associations involve chiasmata and the hybrids produce any viable gametes, then recombination and transference of genetic material between *Aegilops* and *Secale* would seem to be possible. Further studies to clarify the true nature of these intergeneric chromosome associations are reported. Eight diploid species of *Aegilops* and three species of *Secale* were used in the hybridization, and a critical analysis of meiosis in three hybrids between *S. cereale* and *Ae. umbellulata*, *Ae. comosa* and *Ae. caudata* is presented. Autotetraploid forms of *Ae. longissima* and *Ae. squarrosa* were included, and meiotic studies of the triploid hybrid *Ae. squarrosa* (4x) × *S. cereale* are reported to support conclusions from the analysis of diploid hybrids.

2. MATERIALS AND METHODS

Single accessions of *Ae. caudata* (C), *Ae. uniaristata* (M^u), *Ae. mutica* (M^t), *Ae. umbellulata* (C^u) and of autotetraploid forms of *Ae. squarrosa* (D) and *Ae. longissima* (S^l) were obtained from Dr R. Riley, Plant Breeding Institute, Cambridge. All other species, *Ae. comosa* (M), *Ae. sharonensis* (S^l), *Ae. bicornis* (S^b) and *Ae. speltoides* (S^s) were in the collection of the Agricultural Botany Garden, University of Reading. Most crosses were made with *S. cereale* Spring rye (5SC6) from Svalöf, Sweden, other crosses being made with four accessions from Leningrad, *S. cereale* (5SC13), autotetraploid *S. cereale* (4SC1), *S. vavilovii* and *S. montanum*.

Controlled pollination was carried out under glasshouse conditions during the spring and summer of 1963 and 1964. Abortive seed development soon became apparent, and embryo culture was used as a routine procedure for the production of hybrids. The culture medium was similar to that used by Wagenaar (1959) and W. Lange (personal communication) to produce hybrids with *Hordeum* species, and consisted of Difco orchid agar (20·00 g), casein hydrolysate (1·00 g), yeast extract (0·04 g) and sucrose (10·00 g) per litre of distilled water.

Quantities of 10 ml of nutrient media were sterilized in 1 × 6 in. test-tubes. Embryos were excised under sterile conditions from caryopses surface-sterilized with 1:500 mercuric chloride solution, and placed below the surface of the culture medium. Excision and culturing of embryos proved to be successful when caryopses were left to develop for 21–24 days after pollination. The embryos were incubated in darkness at a temperature varying between 23 and 25 °C, and were transferred to light after germination, when precaution was taken against

excessive heating by partially immersing the tubes in water. Young seedlings were transplanted into compost after the shoot and root systems had developed.

For meiotic studies, spikes were fixed in acetic acid : ethyl alcohol, 1 : 3. Anthers were hydrolysed in 1 N hydrochloric acid at 60 °C for 8 min and stained in Feulgen's solution for 1–3 h. Observations were made on temporary squashes mounted in acetocarmine and on permanent preparations.

3. RESULTS

(i) *Production of hybrids*

Since attempts to use the self-incompatible *Secale* species as seed parents in intergeneric crosses have usually been unsuccessful, most crosses were made with the self-compatible *Aegilops* species as seed parents. A limited number of reciprocal crosses proved to be unsuccessful. The diploid species of *Aegilops* appear to form two groups with regard to the success of crosses with *Secale* pollen. Species in the first group showed seed development in at least 20% of the pollinated florets, while species in the second group gave little or no seed set. The number of crosses on to *Ae. uniaristata* is too small for the result to be meaningful.

Table 1. *Crossability of species of* Aegilops *with* Secale, *and the culture of hybrid embryos*

Combination	No. florets pollinated	Seed set (%)	No. embryos cultured	No. embryos germinated	No. plants obtained
Ae. umbellulata × *S. cereale* (5SC6)	224	27·7	28	5	1
Ae. caudata × *S. cereale* (5SC6)	444	33·8	78	42	14
Ae. caudata × *S. vavilovii*	48	52·1	11	9	2
Ae. comosa × *S. cereale* (5SC6)	483	30·2	20	1	1
Ae. comosa × *S. vavilovii*	310	23·2	36	1	0
Ae. uniaristata × *S. cereale*	28	3·6	—	—	—
Ae. squarrosa × *S. cereale* (5SC6)	46	2·2	—	—	—
Ae. squarrosa × *S. vavilovii*	148	31·1	22	9	2
Ae. squarrosa × *S. montanum*	64	25·0	11	1	0
Ae. squarrosa × *S. cereale* (4x, 4SC1)	52	7·7	—	—	—
Ae. squarrosa (4x) × *S. cereale* (5SC6)	57	40·4	7	5	2
Ae. squarrosa (4x) × *S. cereale* (4x, 4SC1)	44	25·0	5	2	0
Totals and mean crossability	1948	(557) 28·6	218	75	22
Crossability with *Sitopsis* species and *Ae. mutica*	1969	(96) 4·9	4	0	0

The first group included *Ae. umbellulata*, *Ae. comosa*, *Ae. caudata* and *Ae. squarrosa* (Table 1). Hybrid seeds obtained from each of these species were of normal size, and 218 embryos suitable for culturing were obtained from 344 seeds. Melnyk (1961) did not obtain any seed from 152 *Ae. caudata* × *Se. cereale* crosses,

but 177 seeds were obtained from 492 pollinated florets. Some of the differences between the success of *Ae. caudata* × *S. cereale* crosses could be due to timing of pollination, since it was observed that high seed set was obtained only when the *Ae. caudata* stigmas were pollinated before the glumes had opened. Comparable seed sets were obtained in crosses with *Ae. squarrosa* when either *S. montanum* (25 %) or *S. vavilovii* (31 %) was used, but crosses with *S. cereale* were much less successful. Melnyk (1961) reported 47·6 % seed set in 271 *Ae. squarrosa* × *S. cereale* crosses. The failure of this cross could have been caused by genetic cross-incompatibility between the two strains that were used, but since the number of crosses was small (46) it could equally be experimental error. The seed set (34 %) from autotetraploid *Ae. squarrosa* × *S. cereale* crosses was relatively high.

The four species in the Section Sitopsis and *Ae. mutica* were highly incompatible with *Secale* species. Seed development was stimulated in 41 of 433 florets of *Ae. mutica*, in 17 of 591 florets of *Ae. longissima* (2x) and in 38 of 549 florets of *Ae. bicornis*. Seed development was not stimulated in 68 florets of *Ae. sharonensis*, 192 florets of *Ae. speltoides* and 136 florets of *Ae. longissima* (4x). Altogether 96 seeds were obtained, but all were much smaller than normal seed and embryos were either absent or too small for culturing. Four embryos from *Ae. bicornis* were placed on the culture medium, but did not survive.

Although environmental differences might account for some of the variation in crossing results, there is evidence of genome-dependent genetic differences in the cross-compatibility of *Aegilops* species with *Secale* species, Sitopsis species with the S genome having less compatibility than other species with a C, D or M genome. There are indications also that genetic variation in both parents and the genetic consequences of autotetraploidy may influence the success of crosses; for example, between *Ae. squarrosa* and *Secale* species.

Altogether, 222 embryos were cultured of which 75 germinated, and many of these did not survive due to malformation of either the shoot or root system. Twenty-two hybrids were grown to maturity from cultured embryos, and at least one plant was obtained from six of the eight intergeneric hybrid combinations that were cultured. *Ae. caudata*, *Ae. comosa*, *Ae. umbellulata* and *Ae. squarrosa* (both 2x and 4x) were parents of viable intergeneric hybrids.

(ii) *Cytological analysis*

Critical analysis of the various meiotic associations depended on the ability to distinguish the parental chromosomes. Earlier workers, including von Berg (1931) and Kagawa & Chizaki (1934), assumed that the larger meiotic chromosomes in polyploid *Aegilops* × *Secale* hybrids belonged to the *Secale* complement. Melnyk (1961) verified this assumption indirectly by comparative karyotype analysis of somatic chromosomes of *Ae. squarrosa*, *Ae. comosa*, *Ae. cylindrica* and *Ae. crassa*, and the F_1 hybrids between each of these species and *S. cereale*. Direct evidence for the distinction between the parental chromosomes was obtained from measurements of chromosome lengths in ten pollen mother cells from both *Ae. umbellulata* × *S. cereale* and *Ae. caudata* × *S. cereale* hybrids.

The chromosomes had characteristic thickness and appearance, the thicker chromosomes resembling those of *Secale* (Plate 1, figs. 1, 2). The distribution of chromosome lengths was bimodal. In *Ae. caudata* × *S. cereale* cells the total length of the seven thinner chromosomes ranged from 17·5 to 24·0 μ, and of seven thicker chromosomes from 29·0 to 35·5 μ. All the thicker *Secale* chromosomes were usually longer than the longest *Aegilops* chromosomes, and when occasionally one and rarely two of the *Secale* chromosomes were not clearly distinguishable by their length they were still readily distinguishable by their thickness. Similar results were obtained from the *Ae. umbellulata* × *S. cereale* hybrid, although the total lengths per cell of the *Ae. umbellulata* chromosomes (29·5–38·0 μ) and of *S. cereale* (41·0–50·0 μ) exceeded those in the comparable *Ae. caudata* × *S. cereale* hybrid substantially. It seems unlikely that this difference was due solely to the measurements being taken at different stages of division.

As expected, this evidence confirmed that *Secale* chromosomes differ in size from those of *Aegilops* species in pollen mother cells as well as in somatic cells. A reliable classification of the different types of chromosome associations could therefore be made on a subjective basis. Two types of autosyndetic association either within the *Aegilops* (AA) or within the *Secale* (SS) complements, and one type of allosyndetic association between the *Aegilops* and the *Secale* chromosomes (AS) were scored. All autosyndetic associations were homomorphic and the allosyndetic associations were hetermorphic.

Owing to restricted association of chromosomes the distinction between metaphase I and anaphase I was not clear in many cells. Observations were confined to these undefined stages resembling the meta-anaphase stages reported in polyhaploids of wheat (Person, 1955). Some of the associated chromosomes were indistinguishable from rod bivalents or chain multivalents with terminal chiasmata. These associations, which were frequently near the equatorial region of the cell and appeared to be orientated, were scored and are referred to as chiasmate associations (Plate 2, Fig. 6). Other loose associations of univalents or attached univalents (Walters, 1954) were scattered throughout the cell together with the unassociated univalents. The associations were either side-by-side, end-to-end or side-to-end (Plate 1, Figs. 3, 4).

Several workers (Riley & Chapman, 1957) have shown that the frequency of side-by-side association is inversely correlated with the frequency of chiasmata, and these associations appear therefore to be an alternative product of prophase pairing and to indicate homology between chromosome segments. Riley & Chapman (1957) also concluded that end-to-end associations in *Triticum* polyhaploids were not a result of prophase pairing, but Wagenaar (1960) reported that both end-to-end and side-by-side associations in *Hordeum jubatum* × *H. bulbosum* hybrids were consequences of prophase pairing. Walters (1954) suggested that the form of chromosome associations in *Bromus* was determined by the location in the cell of prophase associations, associations located near to the equator becoming orientated to resemble bivalents and those outside this region remaining loosely associated; but Wagenaar (1959) did not accept this relationship for *Hordeum* hybrids.

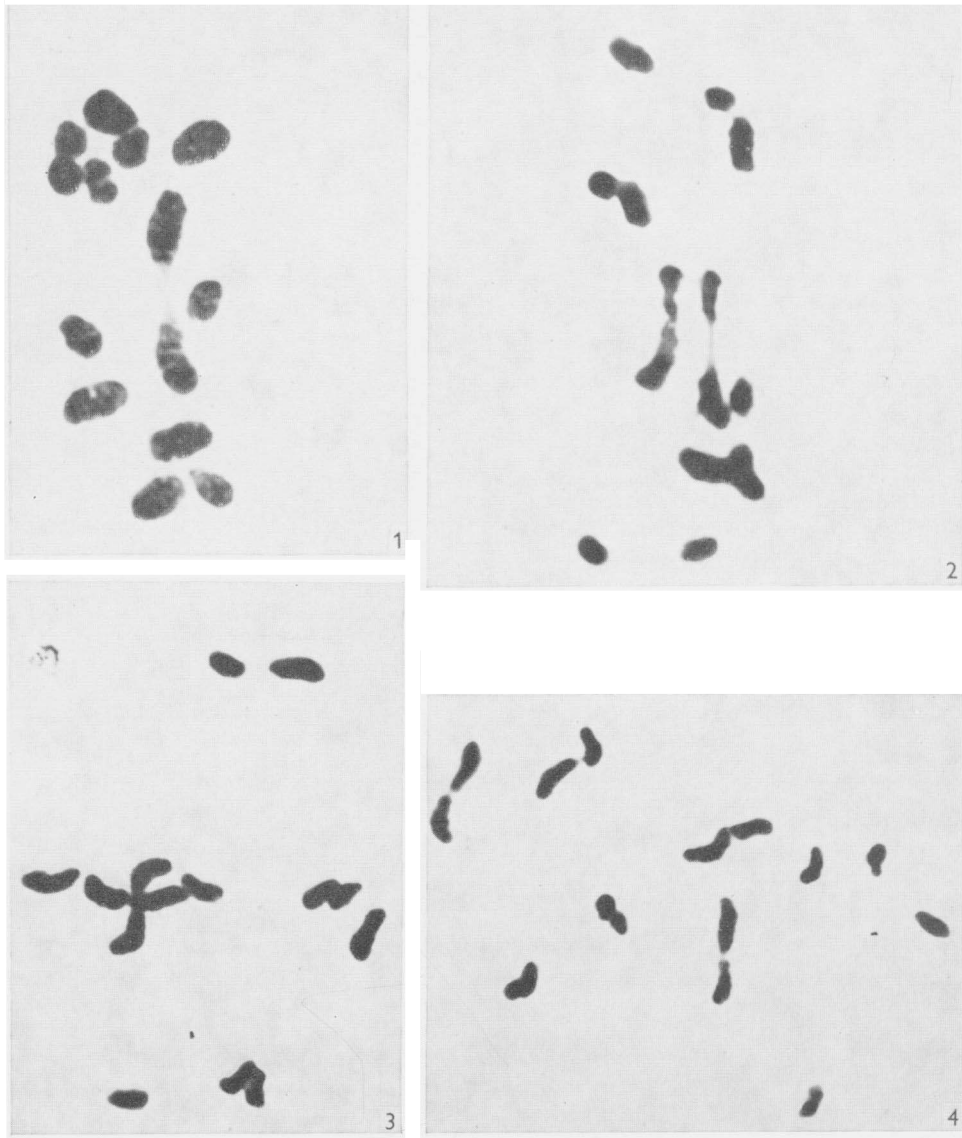
After considering the uncertain significance of the different types of univalent association, and the subjectiveness of some of the scoring of these associations, it was decided that chromosome associations should be divided into two categories, chiasmate associations and non-chiasmate associations. Chiasmate associations appeared to involve a terminal chiasma (Plate 2, Figs. 5–8), but it was not possible to determine whether the pairing was the result of chiasmata or of heterochromatic connexions (Riley & Chapman, 1957) or of stickiness. The term is used for convenience only, with no implication that chiasmata were present either in all or in any of the associations resembling bivalents and multivalents. Non-chiasmate associations were all loosely attached end-to-end, side-to-side and end-to-side figures which were not orientated at the equatorial region. The two categories of associations were scored from the same cell simultaneously.

Table 2. *Observed (O) frequencies of non-chiasmate associations of* Aegilops *(A) and* Secale *(S) chromosomes compared with the 3:7:3 ratio expected (E) if association is at random*

Hybrid	Plant no.		Type of association AA	AS	SS	χ^2 D.F. 2	No. cells scored
Ae. caudata × *S. cereale*	Ct/27	O	35	76	23	2·76	50
		E	30·9	72·2	30·9		
Ae. caudata × *S. cereale*	Ct/31	O	27	57	27	0·28	75
		E	25·6	59·8	25·6		
Ae. caudata × *S. vavilovii*	Cv/5	O	27	76	42	3·48	50
		E	33·5	78·1	33·5		
Ae. comosa × *S. cereale*	G/4	O	23	32	25	6·23*	66
		E	18·5	43·1	18·5		
Ae. umbellulata × *S. cereale*	U/15	O	40	99	58	4·57	100
		E	45·5	106·1	45·5		
Total	—	—	—	—	—	17·32, D.F. 10	—
All plants	—	O	152	340	175	3·94, D.F. 2	341
	—	E	153·9	359·2	153·9		
Heterogeneity between plants	—	—	—	—	—	13·38, D.F. 8	—

* $0{\cdot}01 < P < 0{\cdot}05$

If the *Secale* and *Aegilops* chromosomes have no specific homology, all of the associations will occur at random, each chromosome having an equal chance of forming an association with any other. It can be shown that in cells with seven *Secale* and seven *Aegilops* chromosomes this random association will give frequencies of 21 AA, 49 AS and 21 SS types; that is, in the proportions of 3:7:3, and this will occur independently of the total frequency of association. Similar proportions could be produced by homologous associations between specific chromosomes of *Aegilops* and *Secale*, but it is extremely unlikely that this would give the same ratio in each hybrid. Consistent significant deviations from this ratio will suggest that some or all of the associations were occurring between specific



Photomicrographs of pollen mother cells of diploid ($2n = 14$) hybrids of *Aegilops* (A) and *Secale* (S). The different thickness and length of *Secale* and *Aegilops* chromosomes, both associated and unassociated, is clear in most cells. The interpretation of orientation in some cells is arbitrary:

Figs. 1 and 2: *Ae. caudata* × *S. cereale*

Fig. 1. One homomorphic SS chiasmate association and two associations, AS and AA.

Fig. 2. Two heteromorphic AS chiasmate associations, two AS associations and an SS association.

Figs. 3 and 4: *Ae. umbellulata* × *S. cereale*

Fig. 3. One SS chiasmate association, one SSA and two AA associations.

Fig. 4. Two chiasmate associations, one homomorphic SS and the other heteromorphic AS, and two AS associations.

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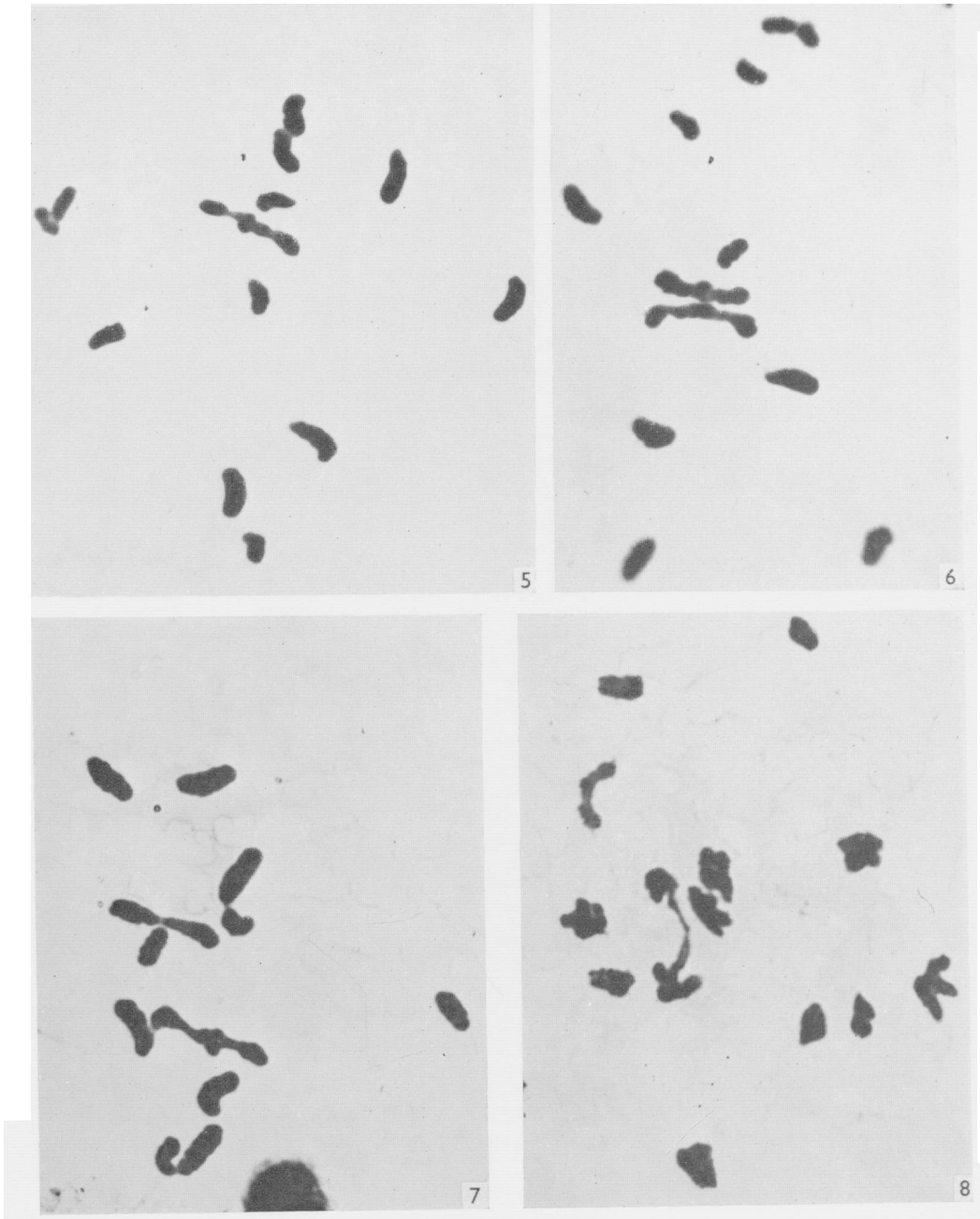

Photomicrographs of pollen mother cells in diploid ($2n = 14$) hybrids of *Aegilops* (A) and *Secale* (S) – *Ae. umbellulata* × *S. cereale*:

Fig. 5. One AS chiasmate association and two associations, AA and SS.

Fig. 6. Two AS chiasmate associations and an AA association.

Fig. 7. Two chiasmate associations, AS and AA, and two heteromorphic AS associations.

Fig. 8. Twelve univalents and an associated pair of chromosomes, showing a persistent bond between chromatids.

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chromosomes. Inspection of the frequencies should then indicate whether this specificity was between chromosomes of the same or different genomes.

The observed frequences of AA, AS and SS non-chiasmate associations and the frequencies expected if these associations occur at random are presented in Table 2. The deviation from the expected frequencies is not significant ($P > 0{\cdot}05$) in four of the hybrid plants. The chi-square for *Ae. comosa* × *S. cereale* (plant G/4) has a probability of between 0·05 and 0·02. Since the chi-squares for the overall deviation of all five hybrid plants and for heterogeneity between these plants both have a probability greater than 0·05, it seems reasonable to conclude that all of the results agree with the calculated ratios based on the random association of chromosomes. The apparent excess of SS associations in *Ae. umbellulata* × *S. cereale* and the deficiency of AS associations in *Ae. comosa* × *S. cereale* can be accepted as sampling error. Similar deviations in the frequencies of either AA or SS associations are present in the three *Ae. caudata* × *Secale* hybrids.

Table 3. *Observed* (*O*) *frequencies of chiasmate associations of* Aegilops (*A*) *and* Secale (*S*) *chromosomes compared with the 3:7:3 ratio expected* (*E*) *if association is at random*

Hybrid	Plant no.		Type of association AA	AS	SS	χ^2 D.F. 2	No. cells scored
Ae. caudata × *S. cereale*	Ct/27	O	2	12	6	2·03	50
		E	4·6	10·8	4·6		
Ae. caudata × *S. cereale*	Ct/31	O	8	22	9	0·16	75
		E	9	21	9		
Ae. caudata × *S. vavilovii*	Cv/5	O	6	25	4	4·66	50
		E	8·1	18·8	8·1		
Ae. comosa × *S. cereale*	G/4	O	10	16	10	1·30	66
		E	8·3	19·4	8·3		
Ae. umbellulata × *S. cereale*	U/15	O	14	19	7	3·32	100
		E	9·2	21·5	9·2		
Total	—	—	—	—	—	11·47, D.F. 10	—
All plants	—	O	40	94	36	0·35, D.F. 2	341
	—	E	39·2	91·5	39·2		
Heterogeneity between plants	—	—	—	—	—	11·12, D.F. 8	—

The frequencies of AA, AS and SS chiasmate associations in all five hybrids agree with a 3:7:3 ratio (Table 3). There appears to be a tendency in the *Ae. caudata* × *Secale* hybrids for the frequency of AS associations to be greater than expected, but the chi-square for the three hybrid plants (2·91, 2 D.F.) has a probability greater than 0·1. The data for all five hybrids are homogeneous and agree with ratios based on the random association of chromosomes.

The frequencies of chiasmate and non-chiasmate associations in each hybrid are compared in Table 4. The chi-squares for each hybrid and for all plants all have a

probability greater than 0·05, indicating that the distribution of AA, AS and SS is similar in the two classifications. Since both chiasmate and non-chiasmate associations occur at random, the frequencies are combined and analysed in Table 5. The frequencies in four of the hybrid plants agree with the expected ratio. The deviation in *Ae. comosa* × *S. cereale* is due to a deficiency of AS associations. Most of this deficiency is of non-chiasmate associations (see Table 2), although there is a similar but smaller and non-significant deficiency of chiasmate AS associations

Table 4. *Comparison between chiasmate* ($\times^{ta}$) *and non-chiasmate* (*non-* $\times^{ta}$) *chromosome association frequencies*

Hybrid	Plant no.	Type of association	AA	AS	SS	χ^2 D.F. 2	No. cells scored
Ae. caudata × *S. cereale*	Ct/27	$\times^{ta}$	2	12	6	3·44	50
		Non- $\times^{ta}$	35	76	23		
Ae. caudata × *S. cereale*	Ct/31	$\times^{ta}$	9	22	9	0·16	75
		Non- $\times^{ta}$	27	57	27		
Ae. caudata × *S. vavilovii*	Cv/5	$\times^{ta}$	6	25	4	5·24	50
		Non- $\times^{ta}$	27	76	42		
Ae. comosa × *S. cereale*	G/4	$\times^{ta}$	10	16	10	0·28	66
		Non- $\times^{ta}$	23	32	25		
Ae. umbellulata × *S. cereale*	U/15	$\times^{ta}$	14	19	7	4·97	100
		Non- $\times^{ta}$	40	99	58		
All plants	—	$\times^{ta}$	41	94	36	1·96, D.F. 2	341
		Non- $\times^{ta}$	152	340	175		

Table 5. *Pooled frequencies of associations* (*chiasmate and non-chiasmate*) *compared with the 3:7:3 random association ratio*

Hybrid	Plant no.	Type of association	AA	AS	SS	χ^2 D.F. 2	No. cells scored
Ae. caudata × *S. cereale*	Ct/27	O	37	88	29	1·56	50
		E	35·5	82·9	35·5		
Ae. caudata × *S. cereale*	Ct/31	O	35	79	36	0·10	75
		E	34·6	80·8	34·6		
Ae. caudata × *S. vavilovii*	Cv/5	O	33	101	46	2·40	50
		E	41·5	96·9	41·5		
Ae. comosa × *S. cereale*	G/4	O	33	48	35	7·30*	66
		E	26·8	62·4	26·8		
Ae. umbellulata × *S. cereale*	U/15	O	54	118	65	2·67	100
		E	54·7	127·6	54·7		
Total						14·03, D.F. 10	
All plants	—	O	192	434	211	2·27, D.F. 2	341
		E	193·2	450·7	193·2		
Heterogeneity between plants		—	—	—	—	11·76, D.F. 8	

* $0{\cdot}01 < P < 0{\cdot}05$

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in this hybrid (Table 3). The chi-squares for all plants and for heterogeneity between plants suggest that it is reasonable to ignore the deviation in this hybrid.

The associations between chromosomes in meiosis are usually expressed as mean frequencies of univalents, bivalents and multivalents per cell, as shown in Table 6. The mean frequencies per cell of AA, AS and SS follow a definite pattern, and in most hybrids the frequency of AS associations is about twice that of either AA or SS associations. A normal interpretation would be that there is some allosyndetic homology between the *Aegilops* and *Secale* chromosomes, and that this homology is greater than the exceptional autosyndetic homology within either the *Aegilops* or the *Secale* sets of chromosomes. There is, however, close agreement between the mean frequencies per cell of the three types of association and the 3:7:3 ratio expected if all association is random. The significance of all associations between *Aegilops* and *Secale* chromosomes must therefore be questioned.

Table 6. *Mean frequencies per cell of univalents and bivalents with chiasmate associations in diploid* Aegilops × Secale *hybrids*

Hybrid	Plant no.	Univalents		Bivalents			Multivalents	No. cells scored
		A	S	AA	AS	SS		
Ae. caudata × *S. cereale*	Ct/27	6·68	6·52	0·04	0·24	0·12	—	50
Ae. caudata × *S. cereale*	Ct/31	6·49	6·46	0·10	0·28	0·12	—	75
Ae. caudata × *S. vavilovii*	Cv/5	6·26	6·34	0·12	0·50	0·08	—	50
Ae. comosa × *S. cereale*	G/4	6·45	6·45	0·16	0·24	0·16	—	66
Ae. umbellulata × *S. cereale*	U/15	6·54	6·69	0·12	0·19	0·06	0·01 AAA	100
Overall means	—	6·49	6·51	0·12	0·28	0·11	—	—

The diploid hybrid *Ae. squarrosa* × *S. cereale* was not produced but an analysis of the relevant triploid hybrid *Ae. squarrosa* ($2n = 28$) × *S. cereale* showed a high AA bivalent mean (6·69) very close to the maximum number (7) possible. The complete absence of allosyndetic pairing between the *Ae. squarrosa* and *S. cereale* chromosomes in this triploid does not confirm the homology reported by Melnyk & Unrau (1959), although there is the possibility of preferential synapsis.

4. DISCUSSION

(i) *Crossability*

The success or failure of interspecific crosses is not necessarily a measure of genetic similarity or relationship. Both the consistency of the results and the techniques used must be considered. From the results reported by Melnyk (1961) and in the present paper, four species – *Ae. umbellulata* (C^u), *Ae. comosa* (M), *Ae. squarrosa* (D) and *Ae. caudata* (C) – give high frequencies of seed development in crosses with species of *Secale*, usually from 20 % to 50 %. Six other species – *Ae. uniaristata* (M^u), *Ae. mutica* (M^t) and the four species in the Sitopsis section (S, S^1, S^b) – give consistently low seed set, frequently with no seed development and almost always with less than 10 % seed set. The seed development in comparable

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crosses reported by Oehler (1934) are in some agreement with this difference, although the crosses were much less successful and, since embryos were not cultured, comparable hybrids were not obtained. Although negative results of interspecific crosses cannot be unequivocal, the consistency of the results is of interest.

The evolutionary significance of such differences in the compatibility is questionable since in all cases the crosses are not successful without the artificial culturing of embryos. Reproductive isolation between each of the diploid species of *Aegilops* and *Secale* species is apparently complete, although in some cases there is a tendency for fertilization to occur and consequently for reproductive potential to be reduced by seed breakdown. The low crossability of the species in Section Sitopsis of *Aegilops* with *Secale* species is comparable to that between the diploid species of *Triticum* and of *Secale*. Chennaveeraiah (1960) has drawn attention to morphological similarities between species in Section Sitopsis and the diploid members of *Triticum*, particularly to the the occurrence of a rudimentary keel on the glume. The pronounced cross-incompatibility of the *Sitopsis* species with *Secale* is an additional similarity. The overall genetic incompatibility between each of these species groups and *Secale* species may be significant evidence of common evolution.

(ii) *The implications of chromosome association*

Associations between the chromosomes of *Secale* and *Aegilops* are of special interest as they may provide evidence of intergeneric relationships, and indicate the possibility of recombination and gene transference in plant breeding. The usefulness and significance of such associations depends on the nature and origin of the chromosome pairing. The appearance of the homomorphic and heteromorphic associations at meta-anaphase was not conclusive evidence of the nature of the pairing, although a limited number of the bivalents were indistinguishable from, and would have been scored as, associations involving chiasmata if meiosis had been regular.

Statistical analysis casts doubts on the significance of the observed associations between *Aegilops* and *Secale* chromosomes. The frequencies of AA, AS and SS chiasmate and non-chiasmate associations in the five hybrids are in agreement with the 3:7:3 ratio derived by assuming that the chances of association are equal for each chromosome. Further, since the pooled data agree with the theoretical ratio, with one exception which can be validly disregarded, it is reasonable to conclude that chiasmate and non-chiasmate association are manifestations of the same phenomenon of random chromosome pairing. The randomness of chromosome association in these hybrids appears to be contrary to the results of Melnyk & Unrau (1959), who concluded that a high frequency of heteromorphic bivalents indicated considerable homology between *Ae. squarrosa* and *S. cereale*. Such a conclusion would have resulted from an inspection of mean frequences of associations per cell. A more critical analysis shows a definite pattern of associations in the absence of specific homology between chromosomes. It is of importance therefore that Melnyk's results for *Ae. squarrosa* × *S. cereale* do not deviate significantly from the 3:7:3 ratio, and that although the excess of AS associations in

Ae. comosa × *S. cereale* (Melnyk, 1961) is statistically significant, the number of pollen mother cells analysed was quite small.

The conclusion that there is no convincing evidence of particular homology between *Aegilops* and *Secale* chromosomes is supported by the meiotic analysis of the triploid hybrid between 4x *Ae. squarrosa* and *S. cereale*. No trivalents were observed in the triploid hybrid studied in the present work, and bivalent formation was restricted to *Ae. squarrosa* chromosomes, with the *Secale* chromosomes occurring as univalents. However, it would be unsound to place too much weight on the absence of trivalents, because any intergeneric pairing due to genetic correspondence between small segments of chromosomes could be masked completely by preferential pairing in such a hybrid. Heteromorphic trivalents would occur only if large segments of *Ae. squarrosa* and *S. cereale* chromosomes were homologous. Nevertheless, all the present evidence suggests that the homology of *Secale* chromosomes with the *Ae. squarrosa* genome is not greater than with other genomes in the genus *Aegilops*.

The evidence suggests that all, or almost all, the chromosome association in diploid *Aegilops* × *Secale* hybrids is of a random and unspecific nature. Such pairing could be similar to the distributive pairing suggested by Grell (1967) following a study of homologous and non-homologous pairing in female *Drosophila melanogaster*. Grell suggested that two types of pairing occur – exchange pairing and distributive pairing. Exchange pairing precedes exchange and occurs only between homologous regions of chromosomes. Distributive pairing occurs between chromosomes which have not been involved in exchange, and is not restricted to homologous regions. The two conditions for distributive pairing – that there is no synapsis or exchange with an independent homologue and that recognition is independent of homology – appear to be present in diploid *Aegilops* × *Secale* hybrids. In *Drosophila melanogaster* the distributive pairing does not occur at random and Grell suggested that recognition at distributive pairing is correlated with total size of chromosome. The random association of *Aegilops* and *Secale* chromosomes suggests that any differences in chromosome size do not affect the frequency of distributive pairing.

In the absence of a clear distinction between pseudochiasmate and true chiasmate association, the pairing data cannot be used with confidence to indicate any partial homologies or homoeologies between particular chromosomes of these genera, but this does not necessarily rule out the occurrence of homologous segments and of true chiasmata between them. It is generally assumed that chiasmata form between homologous segments only, and that the capacity of chromosomes to pair and therefore to produce the possibility of chiasmata is some measure of similarity in structure and of genetic equivalence. Kimber & Riley (1963*a*) accepted the relationship between homology and chiasma formation primarily because the evidence was inadequate to refute the hypothesis. Nevertheless, the conclusion of Levan (1942) that 'the possibility that chiasmata may be formed by non-homologous chromosomes cannot be entirely disregarded' may still be relevant to exceptional meiotic divisions. The possibility will be negligible in cells in which homologues are present, but be much greater when there are no homologous sets of

chromosomes. Since gametes of these plants will be inviable the genetic consequences will not be detected. Analysis of chromosomes in haploids and in some sterile hybrids can therefore provide critical cytological evidence on the specificity of chiasma formation, although this cannot be confirmed by genetic analysis.

Since the chiasmate associations in the diploid *Aegilops* × *Secale* hybrids occur at random it is unlikely that they are all formed between homologous segments. Riley & Chapman (1957) suggested that associations between chromosomes expected to be non-homologous were probably due to connexions between heterochromatin, but Levan (1942) and Dakar (1967) interpreted associations as chiasmata in haploids of *Secale cereale* and *Pelargonium* 'Kleine Liebling' respectively. They concluded that some interchromosomal homology was present within the genome, although there was no evidence that the bivalents were formed by particular chromosomes. Chiasmata may occur when duplicate and therefore homologous segments are adjacent during a random arrangement of chromosomes. Okamoto & Sears (1962) reported that four of the translocations obtained from haploids of *Triticum aestivum* involved chromosomes that were not homologous. They suggested that the translocations were produced by chiasmata between interstitial homologous segments in non-homologous chromosomes, but recognized that it is necessary to use 'cytologically identifiable chromosomes' to determine whether particular chromosomes have paired.

An alternative hypothesis is that chiasmata may occur automatically between associated chromosomes whether or not the segments are homologous. A critical study of chromosome associations and chiasmata in the earlier stages of meiosis, as reported for pachytene and diplotene in haploid rice (Chu, 1967) and in mice (Douglas, 1966), is required to confirm the occurrence of chiasmata between non-homologous chromosomes. These stages of meiosis were not analysed in the *Aegilops* × *Secale* hybrids, and the nature of the apparent chiasmata in metaphase bivalents has not been determined. But the randomness of the association demonstrates the possibility of misinterpreting the occasional association of chromosomes in interspecific hybrids and the importance of identifying chromosomes and of recognizing chiasmata.

The lack of convincing evidence for true homoeologous pairing between *Secale* and *Aegilops* chromosomes is an unexpected result since these related genera must have many genes in common. The chromosomes of the diploid species of *Triticum* show undoubted homoeology with those of a number of diploid species of *Aegilops*, as frequencies of bivalents in hybrids of the type A × B, B × D and A × D are high (Kimber & Riley, 1963*b*). There is considerable evidence that *Secale* chromosomes do not pair with those of *Triticum* species even when the restriction of 5B is not present, but Bielig & Driscoll (1970) reported the association in some cells between the long-arm telocentric chromosome 5 of rye and either one or two wheat chromosomes. It was presumed that this association was with homoeologous chromosomes of wheat, either 5A or 5D. Analysis of pairing in the subsequent generation did not demonstrate any wheat–rye recombination products.

Since chromosome substitution studies have shown specific genetic relationships

between some rye chromosomes and the homoeologous groups of wheat, it seems likely that causes other than complete absence of genetically identical segments on homoeologous chromosomes may determine lack of pairing in *Aegilops* × *Secale* and *Triticum* × *Secale* hybrids. For example, synapsis and recombination could be precluded by differences in size, but instances in which chromosomes that have different lengths in somatic cells have similar lengths and pair normally during meiosis (Tobgy, 1943) suggest that differences such as length *per se* do not necessarily reduce the pairing between homologous chromosomes.

It seems more probable that the absence of true homoeologous pairing between the *Aegilops* and *Secale* chromosomes is a consequence of genetic difference. Riley & Law (1965) reported greater pairing in a nulli-5B haploid of *T. aestivum* than between the same chromosomes in the hybrid between nulli-5B of *T. aestivum* and *S. cereale*, and suggested that the rye genotype has a suppressing influence on chromosome pairing similar to that of chromosome 5B. Shastry & Rao (1961) reported a comparable failure of meiotic pairing in the F_1 hybrid *Oryza sativa* × *O. australensis*, but the association between chromosomes was not at random. No allosyndetic *sativa–australensis* bivalents were observed, and almost all of the autosyndetic bivalents involved two *sativa* chromosomes. Shastry & Rao observed that the progress of the chromosome sets from the two species during meiosis was not synchronous. The different timing of the meiotic stages indicated that the two chromosome sets were at least partly autonomous during meiosis. The low frequency of pairing between the homologous chromosomes in the amphidiploid *Ae. umbellulata* × *Haynaldia villosa* (Sears, 1941) is an extreme example of an interaction between genotypes from different species which affects the pairing of homologues. The considerable non-homology of wheat and rye chromosomes could be a consequence of similar interaction.

Possibly the most critical stage in meiosis is the initiation of chromosome pairing. Sybenga (1966) has postulated that this is controlled by specific units or zygomeres, which, like centromeres and nucleolar organizers, become active at specific stages and locations. Pairing between chromosomes in hybrids occurs only when some zygomeres are common to both parental chromosomes. A genuine reduction of pairing between homoeologous chromosomes in hybrids in which the rye genotype is present may thus indicate the presence of factors similar to those on chromosome 5B of wheat which restrict pairing to chromosomes with very similar zygomeres. Evidence for such an influence of the rye genotype is not convincing, for autosyndetic pairing in hybrids between the polyploid species of *Aegilops* and *Secale* is apparently unaffected (Majisu & Jones, unpublished). It seems more likely that the zygomeric system which initiates chromosome pairing in rye is different from those of *Aegilops* or *Triticum*. Synapsis may be initiated near the nuclear membrane and preceded by the adjacent attachment of homologous chromosomes to the nuclear membrane (Moens, 1969). The absence of alignment of chromosomes in pre-meiotic nuclei, possibly due to inactivity of zygomeres, could be responsible for the absence of homologous, exchange pairing in the diploid *Aegilops* × *Secale* hybrids.

In conclusion, there is at present no suggestion that differential pairing with *Secale* chromosomes can be used as evidence of evolutionary relationships with other genera in the Triticinae. They do not show any specific homology with chromosomes of diploid *Aegilops* species, and indirect transfer of genetic factors between *Secale* and *Triticum* via meiotic recombination with *Aegilops* is not more practicable than direct transfer.

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