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Chromosome Pairing and Fertility in Hybrids and Amphidiploids in the *Triticinae*

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INTRODUCTION

The recent discovery of efficient agents for inducing polyploidy in plants has provided the geneticist with a valuable new tool, by means of which new species can, within limits, be made to order. Little difficulty should now be experienced in converting a desirable hybrid, once it has been obtained, into an amphidiploid. Considerable difficulty may be encountered, however, in obtaining a suitable hybrid, whose amphidiploid derivative will have the characteristics desired. Aside from the uncertainties of the actual hybridizing, there is the problem, hitherto solved largely by trial and error, of selecting the proper parent species. Although the morphological characteristics of hybrids can be predicted beforehand with considerable success, no basis has yet been provided for the accurate prediction of the fertility and constancy of amphidiploids, characteristics which are of primary importance.

Darlington (1929) was perhaps the first to suggest that the fertility and constancy of an amphidiploid might be predicted from the degree of homology which exists among the chromosomes in the undoubled hybrid, and which can presumably be measured by studies of meiosis in this hybrid. Such predictions, while not ordinarily possible from the parent species alone, would nevertheless be of considerable value, particularly since cytological data are already available for many F_1 hybrids. Darlington's suggestion was based on the fact that tetraploids from pure species are ordinarily less fertile than those from hybrids, and that this difference is chiefly attributable to chromosomal irregularities caused by multivalent associations of the chromosomes in the autotetraploids. At that time very few experimental amphidiploids were available for study. Subsequently numerous amphi-

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diploids have been produced, but these form for the most part a heterogeneous group, differing widely among themselves in taxonomic position and in chromosome constitution. Data are needed for a group of amphidiploids involving various combinations of a few parental species, all of which preferably have the basic chromosome number of the family. Such a group are the 18 amphidiploids in the *Triticinae* whose production is reported in a companion publication (Sears, 1941). These involve the following species and varieties of *Triticum Aegilops,* and *Haynaldia,* all with seven pairs of chromosomes. Additional taxonomic data are given in the publication just referred to, along with information concerning sources of the material.

Triticum monococcum L.
T. aegilopoides Forsk. var. *baidaricum* Flaksb.
Aegilops caudata L. var. *polyathera* Boiss.
Ae. comosa Sibth. and Smith ssp. *Heldreichii* (Boiss.) Eig var. *subventricosa* Boiss.
Ae. sharonensis Eig var. *Typica* Eig.
Ae. speltoides Tausch var. *ligustica* (Savign.) Fiori.
Ae. speltoides Tausch var. *Aucheri* (Boiss.) Bornm.
Ae. squarrosa L. ssp. *eusquarrosa* Eig var. *typica* Eig.
Ae. umbellulata Zhuk.
Ae. uniaristata Vis.
Haynaldia villosa (L.) Schur.

The 10 different species involved are represented from one to seven times each in the 18 amphidiploids. The information available includes cytological observations on the undoubled hybrids and on the corresponding amphidiploids, and data on male and female fertility of the hybrids and amphidiploids and on the constancy of the amphidiploids.

CYTOLOGY OF UNDOUBLED HYBRIDS

Cytological observations were made from aceto-carmine smears of pollen mother cells. Previous to preparation of the smears, whole spikes were fixed for about two days in Carnoy's solution (6 parts of 95% ethyl alcohol : 3 parts chloroform : 1 part glacial acetic acid). Although fresh preparations were used for the most part, a few data were obtained from permanent slides made in the following way: The cover slip was soaked off by immersing the slide in 1 part 95% ethyl alcohol : 1 part glacial acetic acid. (Addition of a few cc of tertiary butyl alcohol hastened the process if the slide had been sealed.) Slide and cover slip were then transferred to 1 part 95% ethyl : 1 part tertiary butyl for a few minutes and from there to pure tertiary

butyl for a similar length of time. After this a drop of balsam was placed on the slide and the cover slip replaced.

In Table 1 cytological data on the synaptic behavior of the chromosomes at first metaphase are given for a number of F_1 hybrids, including the 19 different combinations from which amphidiploids have been obtained. For the most part the data were secured from a single cytological preparation from each hybrid. Where more than one preparation from the same or from different plants were used, however, reasonable agreement was observed in the amount and kind of pairing. Fair agreement was found, also, in data from hybrids involving the same parents but made in different years. *Aegilops speltoides* var. *ligustica* I x *Ae. caudata* (and reciprocal) showed very similar cytological behavior in 1937 and 1938. *Ae. caudata* x *Ae. umbellulata* in 1939 had fewer univalents and more trivalents than in 1938, while *Ae. speltoides* var. *ligustica* II x *Triticum monococcum* in 1939 showed some increase in bivalents over 1938 at the expense of univalents.

The two strains of *Ae. speltoides* var. *ligustica* used are practically indistinguishable morphologically, but strain II showed considerably less pairing in all of its hybrids than did strain I. Strain II was quite similar in pairing to *Ae. speltoides* var. *Aucheri* in hybrids with *Ae. caudata*, the only species with which *Aucheri* was crossed.

Data for three hybrids, *T. monococcum* x *Ae. uniaristata, Ae. caudata* x *Ae. umbellulata* (1938), and *Ae. speltoides* var. *ligustica* I x *Ae. umbellulata* are taken from a previous publication (Sears, 1939; table II). Eleven hybrids, including two of the above, have been studied cytologically by previous investigators. As far as can be determined from the different ways in which the data have been recorded, the previous results are largely confirmed by the present data. The 11 hybrids are listed below, together with notes on conflicting results, where such occurred.

T. aegilopoides x *Ae. squarrosa* (Kihara and Lilienfeld, 1934).

T. aegilopoides x *H. villosa* (Sando, 1935; Kihara, 1937). Present results agree approximately with those of Sando, who found up to five bivalents per cell. Kihara found more pairing— one to six configurations (bivalents and trivalents), including up to two closed bivalents.

Ae. caudata x *Ae. speltoides* var. *ligustica* (Kihara and Lilienfeld, 1936) and reciprocal (Kihara, 1937). Slightly less pairing is indicated in their data than was found in the present study for hybrids involving strain II of *ligustica*, and much less than for those involving strain I.

Ae. caudata x *Ae. squarrosa* (Kihara, 1940).

TABLE 1.—CHROMOSOME PAIRING IN F_1 HYBRIDS IN THE SEVEN-CHROMOSOME *Triticinae*.

Hybrid	Year Studied	No. Cells Examined	Univalents per Cell Range	Univalents per Cell Av.	Open Bivalents per Cell Range	Open Bivalents per Cell Av.	Closed Bivalents per Cell Range	Closed Bivalents per Cell Av.	Trivalents per Cell Range	Trivalents per Cell Av.	Quadrivalents per Cell Range	Quadrivalents per Cell Av.
Triticum monococcum x *Aegilops caudata* ..	1937	75	0-8	4.80	1-7	3.69	0-1	.03	0-2	.59	0	--
T. monococcum x *Ae. comosa*	1938	50	2-14	7.44	0-6	3.00	0-1	.22	0-1	.04	0	--
T. aegilopoides x *Ae. comosa*	1939	50	1-14	7.06	0-5	2.48	0-1	.08	0-2	.42	0-1	.14
**T. monococcum*† x *Ae. squarrosa*	1939	50	0-10	3.36	0-6	3.28	0-4	1.58	0-1	.20	0-1	.08
**T. aegilopoides* x *Ae. squarrosa*	1938	50	0-12	6.56	0-7	2.88	0-1	.28	0-3	.34	0-1	.02
**T. aegilopoides* x *Ae. umbellulata*	1938	50	1-12	5.78	0-6	2.78	0-1	.10	0-2	.82	0	--
**T. monococcum* x *Ae. uniaristata*	1938	100	5-14	10.15	0-4	1.86	0-1	.02	0-1	.03	0	--
**T. aegilopoides* x *Ae. uniaristata*	1938	50	4-12	7.66	1-5	2.98	0	--	0-1	.10	0-1	.02
T. monococcum† x *Haynaldia villosa*	1939	50	6-14	9.92	0-4	1.78	0-1	.14	0-1	.08	0	--
T. aegilopoides x *H. villosa*	1938	50	5-14	11.56	0-4	1.14	0-1	.02	0-1	.04	0	--
Ae. caudata x *Ae. speltoides ligustica* I ..	1938	50	0-5	2.74	1-5	3.26	0-2	.48	0-2	1.26	0	--
Ae. caudata x *Ae. speltoides Aucheri*	1938	50	2-12	6.82	0-6	3.14	0-1	.12	0-2	.22	0	--
**Ae. caudata* x *Ae. speltoides ligustica* II ..	1938	50	2-12	6.70	1-6	2.78	0-2	.12	0-2	.50	0	--
**Ae. caudata* x *Ae. squarrosa*	1939	50	1-8	3.46	1-5	3.00	0-2	.60	0-3	1.06	0-1	.04
**Ae. caudata* x *Ae. umbellulata*	1938	50	3-10	5.64	1-5	3.46	0-1	.12	0-2	.40	0	--
**Ae. caudata* x *Ae. umbellulata*	1939	50	0-8	3.28	1-6	3.40	0-3	.28	0-3	1.12	0	--
**Ae. caudata* x *Ae. uniaristata*	1939	50	1-10	4.50	1-6	3.50	0-2	.44	0-2	.46	0-1	.06
**Ae. comosa* x *Ae. uniaristata*	1938	50	1-8	2.88	2-6	3.98	0-2	.56	0-1	.68	0	--
**Ae. sharonensis* x *Ae. caudata*	1938	50	3-12	6.34	1-5	3.26	0-1	.12	0-2	.30	0	--
**Ae. sharonensis* x *Ae. umbellulata*	1938	50	2-14	6.74	0-6	2.78	0	--	0-2	.54	0-1	.02
**Ae. sharonensis* x *Ae. uniaristata*	1938	50	1-10	4.88	1-6	3.70	0-2	.38	0-1	.34	0	--
Ae. speltoides lig. I x *T. monococcum*	1937	50	0-4	.82	0-6	3.84	0-5	2.44	0-2	.18	0-1	.02
Ae. speltoides lig. II x *T. monococcum* ...	1938	150	2-14	8.42	0-6	2.34	0-2	.44	0-2	.15	0	--
**Ae. speltoides lig.* II x *T. monococcum* ...	1939	50	1-12	6.04	0-6	3.28	0-3	.38	0-1	.16	0-1	.04
Ae. speltoides lig. I x *Ae. caudata*	1937	50	0-8	3.30	1-6	3.10	0-2	.42	0-2	1.22	0	--
Ae. speltoides lig. I x *Ae. caudata*	1938	50	1-7	2.94	1-6	3.20	0-2	.44	0-3	1.26	0	--
Ae. speltoides lig. I x *Ae. comosa*	1938	50	0-8	2.68	1-6	3.80	0-4	1-12	0-2	.44	0-1	.04
Ae. speltoides lig. I x *Ae. sharonensis*	1938	50	0-2	.32	0-5	1.50	2-6	5.34	0	--	0	--
**Ae. speltoides lig.* II x *Ae. sharonensis* ...	1938	50	0-4	.68	1-6	3.00	1-6	3.66	0	--	0	--
**Ae. speltoides lig.* I x *Ae. umbellulata*	1938	50	1-7	3.22	1-6	2.98	0	--	0-3	1.58	0-1	.02
**Ae. speltoides lig.* II x *Ae. umbellulata* ...	1939	50	4-14	9.08	0-4	1.98	0	--	0-2	.32	0	--
**Ae. speltoides lig.* II x *Ae. uniaristata* ...	1938	50	0-12	5.54	1-7	3.70	0-2	.26	0-1	.18	0	--
Ae. squarrosa x *Ae. uniaristata*	1939	50	3-14	5.94	0-5	3.62	0-1	.20	0-1	.14	0	--
**Ae. umbellulata* x *Ae. uniaristata*	1938	50	4-14	8.30	0-5	2.70	0	--	0-1	.10	0	--
**Ae. umbellulata* x *H. villosa*	1939	100	8-14	12.66	0-3	.64	0	--	0-1	.02	0	--

*Data available for corresponding amphidiploid (tables 2-5).
†Derivative of *T. monococcum* x *T. aegilopoides*.

Ae. caudata x *Ae. umbellulata* (Kihara, 1937; Sorokina, 1937). Pairing in their material was similar to results recorded here for 1938, when the frequency of trivalents and closed bivalents was lower than in the 1939 material.

Ae. caudata x *Ae. uniaristata* (Kihara, 1937).

Ae. comosa ssp. *eucomosa* x *Ae. uniaristata* (Kihara, 1937) and the reciprocal, involving *Ae. comosa* ssp. *Heldreichii* (Percival, 1932). Although *Heldreichii* was the subspecies used in the present investigation, the cytological data resemble those of Kihara rather than Percival. Percival found five to seven bivalents per cell, including two to three ring bivalents.

Ae. speltoides var. *ligustica* x *T. monococcum* (Chizaki, 1932; Kihara and Lilienfeld, 1932). Their results are similar to those obtained here with var. *ligustica* II.

Ae. speltoides var. *ligustica* x *Ae. umbellulata* (Kihara, 1937). Pairing in Kihara's hybrid was similar to present results with *ligustica* II. His hybrid showed considerable fertility in F_1, however.

Ae. umbellulata x *Ae. uniaristata* (von Berg, 1937) and reciprocal (Percival, 1932; Kihara, 1937). Present results are similar to those of Percival, who reported none to four bivalents. Kihara and von Berg (particularly the latter) found considerably more pairing. Von Berg's data show ranges and average frequencies of 2-12 (5.85) univalents, 0-6 (3.32) open bivalents, 0-2 (.13) closed bivalents, 0-2 (.41) trivalents, and 0-1 (.01) quadrivalents.

Ae. umbellulata x *H. villosa* (von Berg, 1937). Whereas von Berg found no pairing whatever, up to three bivalents were observed in the present material (Fig. 1a). Rare trivalents were also found.

The data in Table 1, which include analyses of several hybrids not previously reported, tend to confirm the findings of taxonomists and cytologists as to the close relationships between the genera *Triticum* and *Aegilops*. Hybrids of *Triticum* with *Aegilops* showed as much chromosome pairing as interspecific hybrids within the genus *Aegilops*. Particularly high in pairing was *Ae. speltoides* var. *ligustica* I x *T. monococcum* (Fig. 1b), with more chromosome association than any other hybrid except *Ae. speltoides* x *Ae. sharonensis*. The close relationship of *Triticum* to *Ae. speltoides* has frequently been pointed out, but so much homology of chromosomes has not been indicated before. L. Smith (unpublished), however, had previously observed similarly good pairing in this hybrid, using the same strain I of var. *ligustica*. Hybrids of *T. monococcum* and *T. aegilopoides* with other species of *Aegilops* showed approximate agreement between the amount of chromosome pairing and the extent of taxonomic divergence be-

tween the parents, with the exception of *T. aegilopoides* x *Ae. umbellulata*, which had more pairing than hybrids involving *Ae. comosa* or *Ae. uniaristata*, although *Ae. umbellulata* is considered more widely differentiated from *Triticum*.

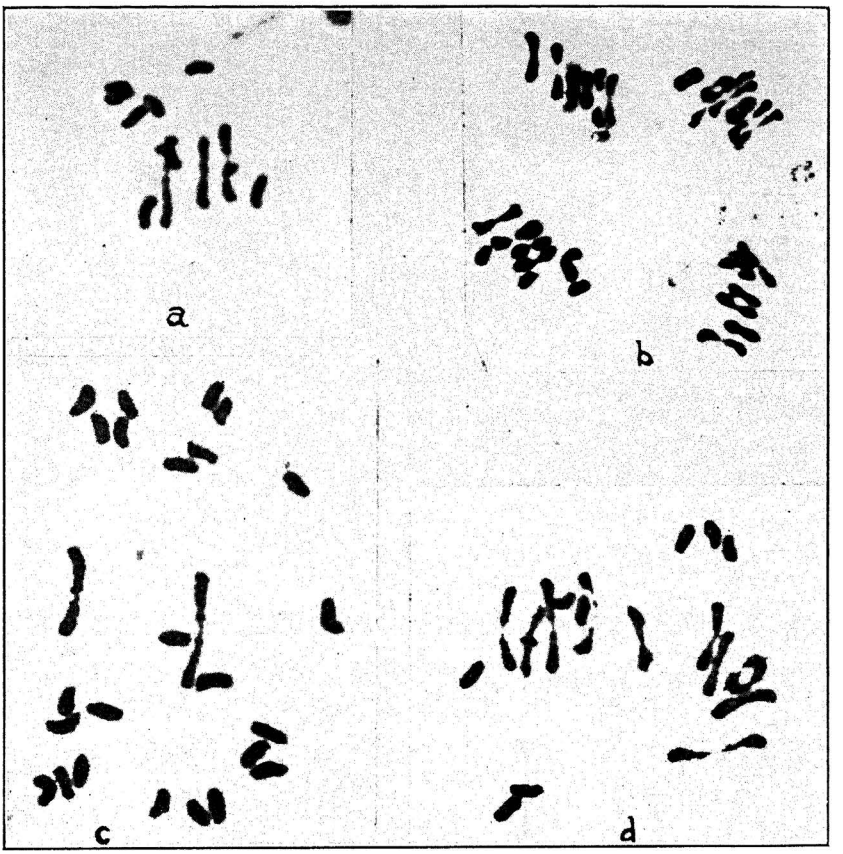

Fig. 1.—First metaphase in PMC of (a) 2n *Aegilops umbellulata* x *Haynaldia villosa*, showing 3II 8I; (b) 2n *Ae. speltoides* var. *ligustica* I x *Triticum monococcum*, showing 7II (upper two cells), 5II 1IV (lower right), and 6II 2I (lower left); (c, d) 4n *Ae. umbellulata* x *H. villosa*, with 2II 24I and 10II 8I, respectively. All x 915.

Ae. speltoides x *Ae. sharonensis* had more pairing than any other hybrid, which is in agreement with the close taxonomic relationship of the two species. Of the two, *Ae. sharonensis* is considered by Eig (1929) to be more closely related to *Triticum* (and consequently less closely related to other species of *Aegilops*). This conclusion is borne out by the occurrence of less pairing in hybrids of *Ae. sharonensis* with other *Aegilops* species than in hybrids of *Ae. speltoides* (strain I) with

the same species. However, Eig also considers *Ae. bicornis* to be closer to *Triticum* than is *Ae. speltoides*, yet observations of Kihara (1937) and unpublished data of the present writer show that hybrids of *T. aegilopoides* and *T. monococcum* with *Ae. bicornis* are comparatively low in pairing. No success has attended efforts to cross *T. monococcum* and *T. aegilopoides* with *Ae. sharonensis*.

FERTILITY OF HYBRIDS AND AMPHIDIPLOIDS

No undoubled hybrid of the 19 which were studied had more than five per cent of pollen which was judged microscopically to be viable, except *Aegilops speltoides* var. *ligustica* II x *Ae. sharonensis*, which had about 12 per cent. No hybrid set any seeds, but this does not prove that all were completely female sterile, since there was little opportunity for pollination to occur.

Data on the fertility of the amphidiploids are summarized in Table 2. The amphidiploids are listed in reverse order according to the amount of chromosome pairing observed in the F_1 hybrids from which they were produced. As a measure of F_1 pairing, the average frequency of univalent chromosomes is used. High univalent frequency corresponds to a low degree of pairing.

TABLE 2.—FERTILITY OF AMPHIDIPLOIDS IN THE SEVEN-CHROMOSOME *Triticinae*.

Amphidiploid	Univalents per 2n Microsporocyte (from table 1)	% Non-Aborted Pollen In 4n Sectors 1938-39	% Non-Aborted Pollen In Offspring 1939-40	% Seed-Set in Offspring 1939-40
Aegilops umbellulata x *Haynaldia villosa* ...	12.66	31	13	0
Triticum monococcum x *Ae. uniaristata*	10.15	95*	92	94
Ae. speltoides lig. II x *Ae. umbellulata*	9.08	--	--	27
Ae. umbellulata x *Ae. uniaristata*	8.30	--	93	81
T. aegilopoides x *Ae. uniaristata*	7.66	96	95	47
Ae. sharonensis x *Ae. umbellulata*	6.74	88	95	40
Ae. caudata x *Ae. speltoides lig.* II	6.70	84	72	17
T. aegilopoides x *Ae. squarrosa*	6.56	--	92	76
Ae. sharonensis x *Ae. caudata*	6.34	95	90	29
Ae. speltoides lig. II x *T. monococcum*	6.04	73	79	25
T. aegilopoides x *Ae. umbellulata*	5.78	84	98	86
Ae. caudata x *Ae. umbellulata* (1938)	5.64	80*	--	49**
Ae. speltoides lig. II x *Ae. uniaristata*	5.54	95	74	13
Ae. sharonensis x *Ae. uniaristata*	4.88	98	93	44
Ae. caudata x *Ae. uniaristata*	4.50	89	72	52
Ae. caudata x *Ae. squarrosa*	3.46	93	84	48
T. monococcum† x *Ae. squarrosa*	3.36	--	95	81
Ae. caudata x *Ae. umbellulata* (1939)	3.28	--	83	54
Ae. speltoides lig. I x *Ae. umbellulata*	3.22	70*	--	39**
Ae. comosa x *Ae. uniaristata*	2.88	79	54	78
Ae. speltoides lig. II x *Ae. sharonensis*	.68	94	94	25

*Approximate values, obtained in 1937-38.
**Data from the 1938-39 season.
†Derivative of *T. monococcum* x *T. aegilopoides*.

As an index to male fertility, the percentage of non-aborted pollen was used. Although subject to some error, in that not all normal-appearing pollen grains may be capable of functioning properly, this

index is commonly used and is presumably reliable for the present purpose, namely, a comparison of the amphidiploids among themselves. Determinations of pollen condition were made from 4n sectors on the colchicine-treated hybrids and again the following year from the amphidiploid plants grown from seed on the 4n sectors. About 500 pollen grains were classified for each determination. Since the percentage of pollen abortion in many of the amphidiploids was evidently influenced by unfavorable conditions of environment, additional pollen counts, often distributed through a period of several weeks, were made wherever there was reason to suspect that the first sample was abnormally high in aborted grains. When a constant value was reached, this was assumed to represent the true amount of abortion. Even with these precautions to avoid inaccuracies due to temporary environmental fluctuations, some amphidiploids showed differences between the two seasons. Presumably there were persistent differences in the seasons which had some effect on the general level of pollen abortion. Also, it is possible that some of the amphidiploid offspring differed from their parents in chromosome constitution. Only those offspring were scored for fertility which were capable of forming 14 pairs of chromosomes (except possibly *Aegilops umbellulata* x *Haynaldia villosa*), but small deficiencies and duplications, such as might result from allosyndesis in an amphidiploid, could have escaped detection.

The second index to fertility, the percentage of seed set, is more comprehensive, since it depends upon the success of both the male and the female sexual processes, and also upon the viability of the various zygotic combinations. Seed set was determined from two or more spikes from each of two or more plants, except for *Aegilops speltoides* var. *ligustica* II x *Ae. umbellulata,* where all the spikes came from a single plant. For the most part, those spikes produced first by the plant were used. Some amphidiploids, however, were not in a healthy condition at the onset of flowering, so it was necessary to select later spikes. Only the primary floret was counted in each spikelet. The total number of florets from which seed set was determined varied from 31 in *Ae. sharonensis* x *Ae. caudata* to 232 in *Ae. speltoides* var. *ligustica* II x *T. monococcum*, the average being 74. The rather small number of florets used introduces an appreciable source of error. Probably much more important, however, are errors due to environmental conditions being below the optimum for seed setting. There is little doubt that seed sets for some amphidiploids were lower than could have been obtained under more favorable conditions. Aside from the *a priori* improbability that 18 amphidiploids with widely

different growth habits and times of maturity should all respond satisfactorily when subjected to the same cultural conditions, there is the fact that the parental species, which are presumably capable of high seed sets under suitable conditions, were of considerably reduced fertility in some instances. *Triticum monococcum* set 92%, *T. aegilopoides* 71%, *Aegilops caudata* 65%, *Ae. comosa* 97%, Ae. *sharonensis* 76%, *Ae. speltoides* 88%, *Ae. squarrosa* 73%, *Ae. umbellulata* 70%, and *Ae. uniaristata* 90%. *Haynaldia villosa,* being abnormally sterile because of self-incompatibility, was not scored for seed set. Another factor suspected of reducing fertility in some amphidiploids and possibly in certain parent species was their inability to pollinate themselves adequately under greenhouse conditions.

It has been suggested that the chromosome homologies responsible for F_1 pairing determine the fertility (and constancy) of the tetraploid. Under these circumstances the amphidiploids listed in table 2, which have been placed in reverse order according to the extent of pairing in their respective F_1's, should show increasingly greater fertility toward the top of the table. There are several exceptions which apparently cannot be explained by experimental error or by the adverse effect of unfavorable environment on fertility.

Aegilops umbellulata x *Haynaldia villosa* was least fertile of all the amphidiploids, although it had less pairing in F_1 than any other hybrid. In the 1938-39 season very few seeds were obtained on the tetraploid sectors, in spite of careful handling of the plants and repeated hand pollinations. In 1939-40, two plants with the normal number of chromosomes were available, but neither of these set a seed. At least three other amphidiploids, *Ae. speltoides* var. *ligustica* II x *Ae. umbellulata, Ae. caudata* x *Ae. speltoides* var. *ligustica* II, and *Ae. speltoides* var. *ligustica* II x *Triticum monococcum*, may also be cited as being of unexpectedly low fertility.

Four amphidiploids disagreed more or less from expectation in the opposite manner; that is, 4n fertility was higher than expected from the amount of 2n pairing. *Triticum aegilopoides* x *Aegilops umbellulata* and *T. monococcum* x *Ae. squarrosa* were both relatively high in seed set and in percentage of non-aborted pollen, while *Ae. comosa* x *Ae. uniaristata* was high in seed set, and *Ae. speltoides* var. *ligustica* II x *Ae. sharonensis* was high in non-aborted pollen. Although the differences from expectation here are not as great as in those amphidiploids with low fertility, they are perhaps as significant, since experimental errors tended to reduce fertility rather than to raise it.

CYTOLOGY OF AMPHIDIPLOIDS

The expectation of a close relationship between 2n chromosome pairing and 4n fertility is based upon two assumptions, that the frequency of multivalents in the amphidiploid will be determined by the amount of pairing which occurs in the undoubled hybrid, and that amphidiploid fertility will in turn depend upon multivalent frequency. It is therefore of interest to examine the cytological data obtained from the amphidiploids. These data are presented in Table 3.

Cytological observations were usually recorded in detail from but a single preparation from each amphidiploid, but additional preparations from other plants of nearly every amphidiploid were inspected to ascertain that fair values had been obtained.

In Table 3 the amphidiploids are listed according to the amount of pairing in the respective undoubled hybrids, those with least pairing (that is, most univalents) being given first. On the assumption that the chromosome homologies responsible for F_1 pairing determine the frequency of multivalents in the tetraploid, the amphidiploids should show increasingly more multivalents toward the bottom of the table. In general this appears to be true, but there are certain obvious exceptions. *Aegilops caudata* x *Ae. speltoides* var. *ligustica* II and *Ae. speltoides* var. *ligustica* II x *Triticum monococcum*, both of which were low in fertility, had unusually high frequencies of multivalents for the amount of 2n pairing they exhibited. Likewise, *Ae. speltoides* var. *ligustica* II x *Ae. umbellulata* had more multivalents (approximately the same frequency as in *Ae. speltoides* var. *ligustica* I x *Ae. umbellulata*) than would have been predicted from F_1 pairing data. It was also of unexpectedly low fertility (Table 2).

Two of the amphidiploids mentioned as unexpectedly high in fertility, *T. aegilopoides* x *Ae. umbellulata* and *T. monococcum* x *Ae. squarrosa*, are seen to be relatively low in multivalent frequency.

In Table 4 significant data on 4n pairing and fertility are repeated, with the amphidiploids rearranged according to the frequency of multivalents. On the assumption that amphidiploid fertility depends upon multivalent frequency, fertility should be greater toward the bottom of the table. Although this is true in general, there are several notable exceptions which are not attributable to reductions in fertility caused by unfavorable environmental conditions. *Aegilops umbellulata* x *Haynaldia villosa* is of extremely low fertility, in spite of a very low frequency of multivalents. Its infertility is evidently due to an extraordinarily high frequency of univalents.

Two amphidiploids, *Ae. comosa* x *Ae. uniaristata* and *Ae. speltoides* var. *ligustica* II x *Ae. sharonensis*, were higher in fertility than was

TABLE 3.—CHROMOSOME PAIRING IN AMPHIDIPLOIDS IN THE SEVEN-CHROMOSOME *Triticinae*.

Amphidiploid	Univalents per 2n Microsporocyte (from table 1)	No. 4n Cells Examined	Univalents per Cell		Bivalents per Cell		Trivalents per Cell		Quadrivalents per Cell		Other Multivalents per Cell		Av. No. Chromosomes per Cell in all Multivalents
			Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	
Aegilops umbellulata x *Haynaldia villosa*	12.66	50	6-26	16.60	1-11	5.48	0-1	.08	0-2	.05	0	--	.44
Triticum monococcum x *Ae. uniaristata* ...	10.15	50	0-4	.12	11-14	13.82	0	--	0-1	.06	0	--	.24
Ae. umbellulata x *Ae. uniaristata*	8.30	50	0-8	.74	10-14	13.26	0-1	.10	0-1	.08	0-1VI	.02	.74
T. aegilopoides x *Ae. uniaristata*	7.66	50	0-2	.30	10-14	13.00	0-1	.08	0-2	.34	0-1V	.02	1.70
Ae. sharonensis x *Ae. umbellulata*	6.74	50	0-4	.74	10-14	12.88	0-1	.18	0-2	.24	0	--	1.50
Ae. caudata x *Ae. speltoides ligustica* II	6.70	50	0-16	3.98	4-14	9.88	0-3	.78	0-2	.48	0	--	4.26
T. aegilopoides x *Ae. squarrosa*	6.56	50	0-4	1.00	9-14	12.66	0-2	.16	0-2	.30	0	--	1.68
Ae. sharonensis x *Ae. caudata*	6.34	50	0-4	.52	10-14	12.82	0-1	.16	0-2	.34	0	--	1.84
Ae. speltoides lig. II x *T. monococcum*	6.04	25	0-8	1.60	7-14	10.84	0-1	.20	0-3	.96	0-1VII	.04	4.72
T. aegilopoides x *Ae. umbellulata*	5.78	50	0-5	.90	10-14	13.24	0-1	.18	0-1	.02	0	--	.62
Ae. caudata x *Ae. umbellulata* (1938)	5.64	15	0-5	1.13	8-14	10.53	0-2	.87	0-2	.80	0	--	5.81
Ae. speltoides lig. II x *Ae. uniaristata*	5.54	25	0-6	2.44	7-14	11.16	0-1	.44	0-2	.48	0	--	3.24
Ae. sharonensis x *Ae. uniaristata*	4.88	50	0-6	.70	9-14	13.24	0-1	.06	0-2	.16	0	--	.82
Ae. caudata x *Ae. uniaristata*	4.50	50	0-6	1.72	8-14	12.02	0-1	.24	0-2	.38	0	--	2.24
Ae. caudata x *Ae. squarrosa*	3.46	50	0-6	1.32	9-14	12.16	0-2	.28	0-2	.38	0	--	2.36
*T. monococcum** x *Ae. squarrosa*	3.36	25	0-5	.64	10-14	12.64	0-1	.16	0-1	.40	0	--	2.08
Ae. caudata x *Ae. umbellulata* (1939)	3.28	75	0-8	1.61	6-14	11.25	0-3	.52	0-3	.56	0-1VI	.01	3.86
Ae. speltoides lig. I x *Ae. umbellulata*	3.22	25	0-9	2.60	6-14	10.24	0-3	.68	0-3	.64	0-1VIII	.04	4.92
Ae. comosa x *Ae. uniaristata*	2.88	50	0-4	.60	7-14	10.38	0-2	.26	0-4	1.44	0-1V	.02	6.64
Ae. speltoides lig. II x *Ae. sharonensis*	.68	25	0-4	.72	6-14	10.08	0-1	.08	0-4	1.72	0	--	7.12

*Derivative of *T. monococcum* x *T. aegilopoides*.

commensurate with their multivant frequency. The former was relatively high in seed set, while the latter was high in non-aborted pollen.

TABLE 4.—CHROMOSOME PAIRING AND FERTILITY OF AMPHIDIPLOIDS IN THE SEVEN-CHROMOSOME *Triticinae*.

Amphidiploid	Av. No. Chromosomes per Cell in		% Non-Aborted Pollen		% Seed Set
	Multivalents	Univalents	1938-39	1939-40	1939-40
Triticum monococcum x *Aegilops uniaristata*	.24	.12	95	92	94
Ae. umbellulata x *Haynaldia villosa*	.44	16.60	31	13	0
T. aegilopoides x *Ae. umbellulata*	.62	.90	84	98	86
Ae. umbellulata x *Ae. uniaristata*	.74	.74	--	93	81
Ae. sharonensis x *Ae. uniaristata*	.82	.70	98	93	44
Ae. sharonensis x *Ae. umbellulata*	1.50	.74	88	95	40
T. aegilopoides x *Ae. squarrosa*	1.68	1.00	--	92	76
T. aegilopoides x *Ae. uniaristata*	1.70	.30	96	95	47
Ae. sharonensis x *Ae. caudata*	1.84	.52	95	90	29
*T. monococcum** x *Ae. squarrosa*	2.08	.64	--	95	81
Ae. caudata x *Ae. uniaristata*	2.24	1.72	89	72	52
Ae. caudata x *Ae. squarrosa*	2.36	1.32	93	84	48
Ae. speltoides lig. II x *Ae. uniaristata*	3.24	2.44	95	74	13
Ae. caudata x *Ae. umbellulata* (1939)	3.86	1.61	--	83	54
Ae. caudata x *Ae. speltoides lig.* II	4.26	3.98	84	72	17
Ae. speltoides lig. II x *T. monococcum*	4.72	1.60	73	79	25
Ae. speltoides lig. II x *Ae. umbellulata*	4.92	2.60	70	--	39
Ae. caudata x *Ae. umbellulata* (1938)	5.81	1.13	80	--	49
Ae. comosa x *Ae. uniaristata*	6.64	.60	79	54	78
Ae. speltoides lig. II x *Ae. sharonensis*	7.12	.72	94	94	25

*Derivative of *T. monococcum* x *T. aegilopoides*.

CONSTANCY OF AMPHIDIPLOIDS

Several amphidiploids give promise of maintaining a fairly constant number of chromosomes, although the progenies thus far grown have been rather small. In Table 5 the available data are summarized, the amphidiploids being listed in the same order as in Tables 2 and 3.

Triticum monococcum x *Aegilops uniaristata*, one of the most fertile amphidiploids, showed perfect constancy in the 17 plants examined. Several other progenies of up to 11 were all, or all but one, of normal constitution. Most of these were from amphidiploids reasonably high in fertility and low in multivalent frequency, but *Ae. caudata* x *Ae. umbellulata*, which was rather low in fertility and high in multivalents, produced 10 normal offspring out of 11 (lumping the data for the amphidiploids produced in 1938 and 1939); and *Ae. comosa* x *Ae. uniaristata* and *Ae. speltoides* var. *ligustica* II x *Ae. sharonensis*, both high in multivalents, produced four normals out of five and six out of seven, respectively. Even *Ae. umbellulata* x *Haynaldia villosa*, which was extremely low in fertility and high in frequency of univalent chromosomes, produced only one plant known to be abnormally constituted out of three (although one of the others had a heteromorphic bivalent, and none of the three was actually observed to form the maximum number of pairs). Those amphidiploids with off-

spring of conspicuously irregular chromosome constitution were all of the group which exhibited meiotic irregularities and low fertility.

TABLE 5.—CHROMOSOME CONSTITUTION OF OFFSPRING OF AMPHIDIPLOID PLANTS IN THE SEVEN-CHROMOSOME *Triticinae*.

Amphidiploid	14^{II}	$13^{II}\ 1^{I}$	$1^{III}\ 13^{II}$	$12^{II}\ 2^{I}$	$1^{III}\ 12^{II}\ 1^{I}$
Aegilops umbellulata x *Haynaldia villosa* ...	2	1	--	--	--
Triticum monococcum x *Ae. uniaristata*	17	--	--	--	--
Ae. speltoides lig. II x *Ae. umbellulata*	1	5	--	1	--
Ae. umbellulata x *Ae. uniaristata*	2	--	1	--	--
T. aegilopoides x *Ae. uniaristata*	6	--	--	--	--
Ae. sharonensis x *Ae. umbellulata*	4	--	--	--	1
Ae. caudata x *Ae. speltoides lig.* II	6	3	--	--	--
T. aegilopoides x *Ae. squarrosa*	4	1	--	--	--
Ae. sharonensis x *Ae. caudata*	2	1	--	--	--
Ae. speltoides lig. II x *T. monococcum*	2	--	--	3	--
T. aegilopoides x *Ae. umbellulata*	10	1	--	--	--
Ae. caudata x *Ae. umbellulata* (1938)	7	--	1	--	--
Ae. speltoides lig. II x *Ae. uniaristata*	3	2	--	--	--
Ae. sharonensis x *Ae. uniaristata*	6	1	--	--	--
Ae. caudata x *Ae. uniaristata*	6	1	--	--	--
Ae. caudata x *Ae. squarrosa*	5	1	--	--	--
*T. monococcum** x *Ae. squarrosa*	4	--	--	--	--
Ae. caudata x *Ae. umbellulata* (1939)	3	--	--	--	--
Ae. speltoides lig. I x *Ae. umbellulata*	11	5	2	--	--
Ae. comosa x *Ae. uniaristata*	4	1	--	--	--
Ae. speltoides lig. II x *Ae. sharonensis*	6	1	--	--	--

*Derivative of *T. monococcum* x *T. aegilopoides*.

Out of a total of 144 offspring of amphidiploid plants, 111 were of regular constitution. Of the remainder, 29 had one or more monosomes, and 5 had a trisome. The predominance of monosomes over trisomes finds a ready explanation in the tendency of univalents to be lost at meiosis. A monosomic plant results from the simple loss of one univalent chromosome, while the occurrence of a trisomic plant requires the inclusion in a single gamete of both members of a pair of univalents.

In the data on chromosome constitution of offspring from colchicine-treated hybrids, there is no evidence that other than exact duplication of the chromosomes occurred. Aneuploid types have been found in *Datura* following colchicine treatment (Blakeslee, 1941).

DISCUSSION

The possibility of predicting the fertility of an amphidiploid from cytological study of the diploid hybrid from which it is to be produced has been recognized for some time. Darlington in 1929 stated that "the constancy and relative fertility of the hybrid tetraploid depends on the degree of its hybridity, for, in so far as the corresponding chromosomes of the opposite species are capable of pairing in the tetraploid derivative, dissimilar gametes will be produced, both as a result of genetic segregation and abnormality in the division of quadrivalents. In both cases fertility is reduced."

Preliminary results with three hybrids in the seven-chromosome *Triticinae* (Sears, 1939) tended to confirm the existence of a close inverse relationship between 2n pairing and 4n fertility. The subsequent study of these and 15 additional amphidiploids, however, has shown that some 2n hybrids with little chromosome pairing may give rise to less fertile amphidiploids than other hybrids with much more pairing. Some of these exceptions may be attributed to the circumstances under which the data were collected. In particular, the environment may have been so unfavorable for certain amphidiploids that fertility was substantially reduced, especially in regard to seed setting. Also, the fact that the data concerning chromosome pairing in most of the 2n hybrids were obtained from single cytological preparations introduces a possible source of error. There is little reason to believe, however, that this error appreciably affects the conclusions which have been reached. With certain 2n hybrids samples have been taken from different plants and even in different years, and the variation has been slight compared to the deviation in various amphidiploids from their expected fertility and cytological behavior.

There are thus certain exceptions to the hypothesis concerning predictability of 4n fertility from 2n chromosome pairing which are evidently not attributable to peculiarities of the present experiment. There are apparently several inherent factors which affect 4n fertility besides the chromosome homologies indicated by the amount of 2n pairing.

The two assumptions basic to the theory that amphidiploid fertility depends upon the amount of chromosome pairing in the undoubled hybrid are that the homologies indicated by 2n pairing determine the frequency of multivalents in the tetraploid, and that this multivalent frequency determines tetraploid fertility. The exceptions to the theory concerning the relationship between 2n pairing and 4n fertility may thus be classified as to which of these basic assumptions they conflict with.

There were several amphidiploids in which the frequency of multivalents was not closely related to the extent of 2n pairing in the respective undoubled hybrids. Most of these exceptions may be attributed to failure of 2n pairing accurately to measure chromosome homologies. For example, all 2n hybrids involving strain II of *Aegilops speltoides* var. *ligustica* showed considerably less pairing than the same hybrids involving strain I, although there is no reason to believe that the chromosomes of strain II differ structurally from those of strain I. Amphidiploids involving strain II showed cytological behavior like that expected if strain I had been involved. Differences

were found in the amount of pairing in hybrids of several species of *Aegilops* with *Triticum*, depending on whether *T. aegilopoides*, *T. monococcum*, or derivatives of *T. monococcum* x *T. aegilopoides* were used. These differences can hardly indicate corresponding differences in chromosome homology, for Smith (1936) found a very close cytological relationship between the two species; furthermore, amphidiploids with *Ae. squarrosa* involving two different *Triticums* were cytologically very similar in behavior even though a large difference had been found in the amount of pairing in the respective undoubled hybrids. Another hybrid, *Ae. caudata* x *Ae. umbellulata*, had less pairing in plants grown in one year than in those grown the following year, but multivalent frequency was somewhat higher in the amphidiploid produced from the hybrid with less pairing. From these various data, it is obvious that 2n pairing does not always indicate the full amount of homology which exists among the chromosomes and which may lead to multivalent formation in the amphidiploid. Both environmental and genetic factors may decrease 2n pairing. Similar results have been obtained by other investigators. Levan (1941), for example, found that the same plants of *Allium Cepa* x *A. fistulosum* differed in pairing in two different years, particularly as regarded the frequency of trivalents.

Another factor not to be overlooked, although no definite evidence is available that it is of appreciable importance, is that the type of homology measured by the amount of 2n pairing may not always be such as to lead to proportionate frequencies of multivalents in the amphidiploid. Short homologous segments might bring about nearly as much pairing as longer segments in diploid hybrids, where competition in pairing is low, but in the corresponding tetraploids the longer segments would presumably result in multivalents more frequently than would the shorter segments. Perhaps this circumstance may account for the unexpectedly low frequency of multivalents in the tetraploids of *Triticum aegilopoides* x *Aegilops umbellulata*, *Ae. sharonensis* x *Ae. uniaristata*, and *T. monococcum* x *Ae. squarrosa*. It is possible that a different criterion for measuring 2n pairing, such as the average number of chiasmata per sporocyte, would give a clearer picture of chromosome homologies in some hybrids. However, the use of chiasma frequency would throw *T. monococcum* x *Ae. squarrosa* still more out of line, since closed bivalents were unusually frequent in this hybrid and chiasma frequency therefore relatively high. In few other hybrids would the situation be affected materially by use of chiasma frequency. Most hybrids had few closed bivalents,

and only infrequently did more than one chiasma occur per chromosome arm.

There were several amphidiploids which conflicted with the assumption that 4n fertility is determined primarily by the frequency of 4n multivalents. The most striking example was *Aegilops umbellulata* x *Haynaldia villosa*, where fertility was extremely low because of a very high frequency of univalent chromosomes. Some univalence was to be expected in amphidiploids with frequent multivalents, but in some amphidiploids there was evidently a tendency for univalents to occur in considerable excess of those resulting from multivalent homologies. In the extreme case of *Ae. umbellulata* x *H. villosa*, this tendency may best be explained as the result of some physiological upset, dependent on the extreme hybridity involved. Abnormalities of various sorts, such as dwarfness, frequently occur in wide hybrids (East, 1936), and there is no reason to suppose that some types of abnormality may not involve a reduction in chromosome pairing. Possibly the rather frequent univalents in certain other amphidiploids may be caused by similar, though less severe, upsets due to hybridity. *Ae. caudata* x *Ae. uniaristata*, *Ae. speltoides* var. *ligustica* II x *Ae. uniaristata*, and *Ae. caudata* x *Ae. speltoides* var. *ligustica* II may be cited as amphidiploids whose rather low fertility may be attributed, in part at least, to high univalent frequencies.

In addition to the amphidiploids with fertility disproportionately low for the multivalent frequencies concerned, there were at least two, *Ae. comosa* x *Ae. uniaristata* and *Ae. speltoides* var. *ligustica* II x *Ae. sharonensis*, which were somewhat higher in fertility than was expected from their high multivalent frequencies.

The relation between 2n pairing and 4n fertility was disturbed in at least one amphidiploid, *Ae. caudata* x *Ae. speltoides* var. *ligustica* II, by two different factors, in this case a reduction in 2n pairing and an increase in 4n univalent frequency, both having an adverse effect on fertility. Since factors exist which tend to increase fertility as well as to lower it, occasional amphidiploids may have had their expected fertility even though both the relation between 2n pairing and 4n multivalents and the relation between 4n multivalents and 4n fertility were disturbed. *Ae. sharonensis* x *Ae. uniaristata* was to some extent such an amphidiploid, for it had an unexpectedly low frequency of 4n multivalents but was still rather low in seed set.

As concerns this particular group of amphidiploids, it is obvious that 2n pairing data provide no satisfactory basis for the prediction of 4n fertility. There is some reason to believe that the constant and highly fertile amphidiploids will chiefly come from F_1 hybrids with

little chromosome pairing; but whether a particular F_1 with little pairing will give rise to a good amphidiploid, or whether it will even produce a better amphidiploid than another hybrid with considerably more pairing, evidently cannot be foretold with any worth-while degree of accuracy.

Within other plant groups (or even elsewhere in the *Triticinae*), it is quite possible that the variables found here will be absent or of greatly reduced importance, and it may be possible to predict amphidiploid fertility and constancy from F_1 pairing data. It is not unlikely, however, that in many groups difficulties similar to those recorded here will be encountered.

SUMMARY

Twenty-four different hybrids involving ten different species of *Triticum*, *Aegilops*, and *Haynaldia* with n=7 varied in chromosome pairing from an average frequency of 12.66 to 0.32 univalents per microsporocyte.

The amphidiploids obtained from 18 of these varied in fertility from nearly perfect to almost zero. There was no consistent relationship between 4n fertility and lack of 2n pairing. This is attributed chiefly to the following factors:

1. Data on chromosome pairing in the 2n hybrids did not always measure accurately the homologies responsible for multivalent formation in the amphidiploids, principally because some hybrids failed to show all the pairing of which they were capable.

2. Meiotic disturbances other than those due to multivalent homologies increased the frequency of univalents in some amphidiploids and thereby reduced fertility. The condition in *Ae. umbellulata* x *H. villosa* was an extreme example of this phenomenon.

3. Certain amphidiploids were relatively fertile in spite of high frequencies of multivalents.

4. Fertility (particularly seed set) of some amphidiploids was adversely affected by environmental conditions.

A total of 111 among 144 offspring of amphidiploid plants from all crosses were of regular chromosome constitution.

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