Theoretical and Applied Genetics 45, 104–109 (1974)

Further Discovery of Alien Cytoplasms Inducing Haploids and Twins in Common Wheat[1]

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Summary. In addition to the already known *Aegilops caudata* cytoplasm, the cytoplasms of five *Aegilops* species, all belonging to the section Polyeides, were found to induce haploids (11–56%) and twins (0.5–15%) in a common wheat, Salmon, at high frequencies. The great majority of the twin pairs were of the diplo-haplo type. The origin of both the haploids and twins was ascribed to the induction of parthenogenesis in Salmon by the alien cytoplasm. Pollen parents produced some differences in haploid frequency. The distribution of the parthenogenesis-inducing cytoplasms in the genus *Aegilops* is discussed in relation to the phylogeny of the donor species.

Introduction

Kihara and Tsunewaki (1962) first reported that the cytoplasm of *Aegilops caudata* induced haploidy in common wheat. Tsunewaki (1964) found that the frequency of haploid formation by this cytoplasm differed with the strain of wheat used: a common wheat derivative of 8 x *Triticale*, named Salmon, showed an extremely high frequency of haploids (about 30%) when the cytoplasm of *Ae. caudata* was introduced, while Tve, Macha and some other strains of common wheat with the same alien cytoplasm showed only a slightly increased frequency of haploids (a few percent). Furthermore, Tsunewaki *et al.* (1968) compared three alien cytoplasms, i.e. those of *Ae. caudata*, *Ae. ovata* and *Triticum timopheevi*, and found that only the *Ae. caudata* cytoplasm was effective in inducing haploids and twins in Salmon.

In the course of studying the genetic differentiation of the cytoplasm in *Triticum* (wheat genus) and *Aegilops*, we discovered that the cytoplasms of five other *Aegilops* species caused the relatively frequent occurrence of haploids and twins. Haploids are very useful in both theoretical and applied genetics, so our findings are reported here.

Materials and Methods

Salmon is a 6 x derivative of 8 x *Triticale*, whose genome constitution is almost the same as that of common wheat, *Triticum aestivum* (2*n* = 42, genome constitution AABBDD) (Tsunewaki 1964). However, Salmon's chromosome complement differs somewhat from that of common wheat in that a small part of chromosome 1B, including the nucleolus-organizing region, is replaced by a segment of the rye chromosome 1R (Zeller 1973), and chromosome 2B has lost the W_1 locus for wax production and the Rfu_2 locus for a fertility-restoring gene against *Ae. umbellulata* cytoplasm (Tsunewaki 1964, 1974).

[1] Contribution from the Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Japan, No. 383. This work was supported by a Grant-in-Aid (No. 848002) from the Ministry of Education.

The cytoplasms of five *Aegilops* species were studied, and compared with that of *Ae. caudata* (2*n* = 14, CC); they were *Ae. umbellulata* (2*n* = 14, C^uC^u), *Ae. triuncialis* (2*n* = 28, CCC^uC^u), *Ae. columnaris* (2*n* = 28, $C^uC^uM^cM^c$), *Ae. kotschyi* (2*n* = 28, $C^uC^uS^vS^v$), and *Ae. variabilis* (2*n* = 28, $C^uC^uS^vS^v$), all belonging to the section Polyeides of the genus *Aegilops*.

Based on the proposal of Tsunewaki (1969), the cytoplasm substitution lines of Salmon are designated by the name of the cytoplasm donor (in parentheses) and Salmon, with a hyphen between the two names. For example, (*caudata*)-Salmon indicates a cytoplasm substitution line of Salmon with the *Ae. caudata* cytoplasm.

Names of the common wheat strains used in the present investigation are abbreviated as follows:

Common wheat strains	Abbreviation
Triticum aestivum var. *erythrospermum*	Tve
Triticum aestivum cv. Chinese Spring	CS
Triticum aestivum cv. Jones Fife	JF
Triticum aestivum strain P168	P168
Triticum macha var. *subletschchumicum*	Macha

(*caudata*)-Salmon: In 1960, Salmon was first crossed as male parent to the (*caudata*)-Tve, produced by Kihara (1959). This hybrid has since been successively backcrossed with Salmon once a year, to produce a cytoplasm substitution line of Salmon with the *Ae. caudata* cytoplasm. In autumn 1973, this line reached the B_{13} generation, its pedigree being *Ae. caudata*/Tve[12]/Salmon[14]. This line was cytologically stable from the beginning of the backcross program, showing 21″ at MI of meiosis in most PMC's, and it is completely male sterile with normal female fertility.

(*umbellulata*)-Salmon: In 1966, Salmon was first crossed as male to (*umbellulata*)-CS, produced by Muramatsu (1965). The hybrid has been successively backcrossed with Salmon to produce the (*umbellulata*)-Salmon line, reaching the B_7 generation in autumn 1973. Its pedigree for this generation was *Ae. umbellulata*/CS[7]/Salmon[8]. This line was cytologically stable, with 2*n* = 42 from the beginning of the backcross program, and it is completely male sterile with normal female fertility.

(*triuncialis*)-Salmon: A B_1 plant, *Ae. triuncialis* × common wheat[2], was first crossed as female with Salmon in 1968. Salmon has since been successively backcrossed

as male to this hybrid. In autumn 1973, this (*triuncialis*)-Salmon line reached the B_5 generation. As yet, it contains a subterminal chromosome of *Ae. triuncialis*, as well as the 42 chromosomes of Salmon, and shows high female and complete male sterility. Its pedigree at present is *Ae. triuncialis*/spelta/CS/Salmon[6].

(*columnaris*)-Salmon: *Ae. columnaris* was first crossed as the female parent with JF in 1968, and has been successively backcrossed twice with the same pollen parent. This (*columnaris*)-JF^3 was crossed with Salmon in 1971, then backcrossed twice with Salmon by autumn 1973. Its pedigree is *Ae. columnaris*/JF^3/Salmon[3]. In its B_2 generation, (*columnaris*)-Salmon segregated plants having $2n = 40-43$ chromosomes, and showed complete male sterility with normal female fertility.

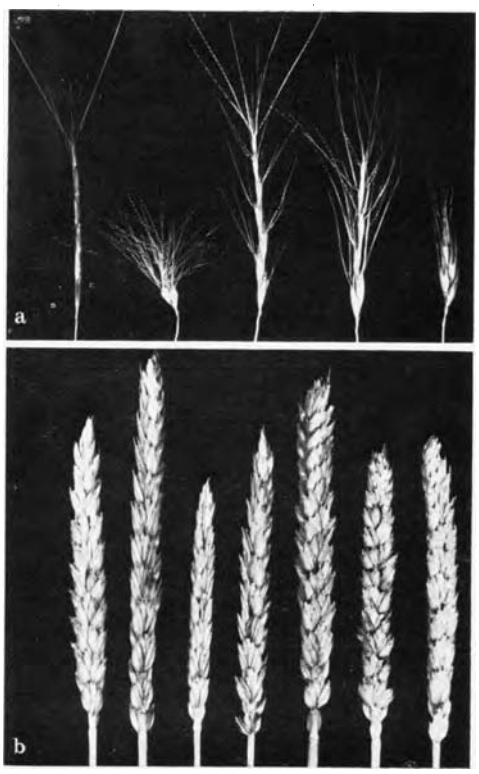

Fig. 1. Ears of Salmon and the cytoplasm donors to its cytoplasm substitution lines.
a. From left to right: ears of *Ae. caudata*, *Ae. umbellulata*, *Ae. triuncialis*, *Ae. columnaris* and *Ae. variabilis*
b. From left to right: ears of Salmon and its six cytoplasm substitution lines; (*caudata*)-, (*umbellulata*)-, (*triuncialis*)-, (*columnaris*)-, (*kotschyi*)-, and (*variabilis*)-Salmon

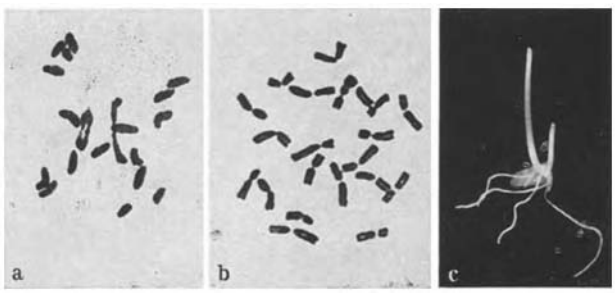

Fig. 2. Haploids and twins of the cytoplasm substitution lines.
a. MI of meiosis in a PMC of the haploid (*caudata*)-Salmon
b. Root-tip mitosis of the haploid (*triuncialis*)-Salmon
c. A twin seedling of (*variabilis*)-Salmon

(*kotschyi*)-Salmon: In the autumn of 1973, this line reached the B_2 generation. Its pedigree is *Ae. kotschyi*/CS^3/Salmon[3]. This line is already stable cytologically with normal female and partial male fertility.

(*variabilis*)-Salmon: This line also reached the B_2 generation in autumn 1973, with the pedigree *Ae. variabilis*/CS^3/Salmon[3]. At this stage of the backcrosses the line is still segregating two types of plants, i.e. $2n = 42$ and 43, and shows normal female fertility with almost complete male sterility.

Ears of all the cytoplasm donors and the six cytoplasm substitution lines of the most advanced backcross generations are shown in Fig. 1.

Except for the two cases described in "Results", all the haploids and diploids were cytologically confirmed by observing the chromosomes in root-tip mitosis or in PMC's meiosis, as shown in Fig. 2.

Some crosses leading to the production of these cytoplasm substitution lines were carried out by Dr. S. Sakamoto, Dr. T. Hori and Mr. T. Iwai, to whom we express our deepest gratitude.

Results

(*caudata*)-Salmon: Frequencies of twin pairs and haploids in the artificially pollinated offspring of (*caudata*)-Salmon in different backcross generations from B_2 to B_{13} are shown in the upper half of Table 1. Overall frequencies of twins and haploids were 8.7% (126 twin pairs out of 1,452 plants tested) and 26.0% (351 haploids out of 1,349 plants examined), respectively. These frequencies showed no big change during the 12 successive backcross generations.

The chromosome numbers of twins obtained in this line were investigated and results are shown on the top line of Table 2, including results obtained by Tsunewaki *et al.* (1968). The great majority (92.6%) of twin pairs was diplo-haplo ($2n + n$), and the remaining were the diplo-diplo (3.7%) or haplo-haplo (3.7%) type.

(*umbellulata*)-Salmon: Frequencies of twin pairs and haploids in different generations of this line, i.e. from the F_1 to B_6 generations, are shown in the lower half of Table 1. In 1970, this line was crossed as the female parent with Macha to screen haploids, because normal hybrids between Salmon and Macha become chlorotic due to complementary genes (Ch_1 in Macha and Ch_2 in Salmon), while the haploids remain normally green because they lack either gene. In this case the chromosome check was carried out only with green plants.

In 1971, (*umbellulata*)-Salmon was crossed as the female parent to CS monosomics × Salmon F_1's for monosomic analysis of the fertility-restoring genes for the *Ae. umbellulata* cytoplasm. In the offspring of this cross haploids were segregated among the diploids. The haploids were identified only by morphological observation, without the chromosome check. The criteria used were slender leaves and culms with non-waxy foliage, in addition to complete sterility. The occurrence of twins and haploids in this cross is shown in Table 3. Because of the small

Table 1. *Frequencies of twin pairs and haploids in artificially pollinated offspring of (caudata)- and (umbellulata)-Salmon in different backcross generations*

Line	Generation (♀ parent)	Year	♂ parent	Twins			Haploids		
				No. plants examined	No. twin pairs	%	No. plants examined	No. haploids	%
(*caudata*)-Salmon									
	B_2	1962	Salmon	107	9*	8.4	104	29	27.9
	B_5	1965	Salmon	31	3	9.7	19	5	26.3
	B_6	1966	Salmon	23	3	13.0	41	16	38.0
	B_7	1967	Salmon	132	20	15.2	192	39	20.3
	B_8	1968	Salmon	919	60	6.5	840	212	25.2
	B_9	1969	Salmon	16	2	12.5	—	—	—
	B_{10}	1970	Salmon	28	4	14.3	30	11	36.7
	B_{11}	1971	Salmon	20	2	10.0	12	4	33.3
	B_{12}	1972	Salmon	30	2	6.7	8	3	37.5
	B_{13}	1973	Salmon	146	21	14.4	103	32	31.1
	Total			1,452	126	8.7	1,349	351	26.0
(*umbellulata*)-Salmon									
	F_1	1967	Salmon	308	0	0.0	—	—	—
	B_3	1970	Macha	26	0	0.0	130	19	14.6
	B_4	1971	Salmon	15	0	0.0	15	0	0.0
	B_4	1971	CS × Salmon F_1	561	4	0.7	456	92	20.2
	B_5	1972	Salmon	18	1	5.6	15	0	0.0
	B_6	1973	Salmon	134	0	0.0	104	9	8.7
	Total			1,062	5	0.5	720	120	16.7

[1] Data on the B_2—B_8 generations of (*caudata*)-Salmon are taken from Tsunewaki *et al.* (1968).
[2] Data on the B_5 generation of (*umbellulata*)-Salmon crossed with CS × Salmon F_1 are taken from Table 3.
* One plant was triplet.

Table 2. *Classification of twin pairs regarding the chromosome number of the partners*

Line	Total no. pairs analyzed	Chromosome number of the partners		
		$2n + 2n$	$2n + n$	$n + n$
(*caudata*)-Salmon	81	3	75	3
(*triuncialis*)-Salmon	6	0	5	1
(*columnaris*)-Salmon	3	0	3	0
(*kotschyi*)-Salmon	1	0	1	0
(*variabilis*)-Salmon	10	0	9	1
Total	101	3	93	5

Data on 81 twin pairs of (*caudata*)-Salmon are taken from Tsunewaki *et al.* (1968).

number of offspring obtained in each monosomic line, no critical information could be obtained on the effect of the pollen parent of different monosomic types on haploid formation. The pooled data are included in Table 1.

Overall frequencies of twins and haploids in (*umbellulata*)-Salmon were 0.5% and 16.7%, respectively. Though the chromosome number of the twins was not determined, the *Ae. umbellulata* cytoplasm produced twins with very low frequency compared with all the alien cytoplasms tested.

(*triuncialis*)-Salmon: The occurrence of twins and haploids was investigated in offspring of the three backcross generations, B_2 to B_4. Results are shown at the top of Table 4. Overall frequencies of twins and haploids were 6.8% and 25.0%, respectively. As shown in Table 2, most twins (5 out of 6) were the diplo-haplo type.

Table 3. *Frequencies of twin pairs and haploids in the offspring of (umbellulata)-Salmon, crossed with CS monosomics × Salmon F_1's as the male parent*

Line (♂ parent)	No. progenies tested	Twins		Haploids		
		No. plants examined	No. twin pairs	No. plants examined	No. haploids	%
Disomic F_1	2	29	0	24	8	33
Mono-1A F_1	1	12	0	10	1	10
Mono-2A F_1	2	24	0	21	4	19
Mono-3A F_1	2	38	0	31	6	19
Mono-4A F_1	2	38	1	29	11	38
Mono-5A F_1	2	28	0	21	4	19
Mono-6A F_1	2	29	1	31	8	26
Mono-7A F_1	2	28	1	19	1	5
Mono-1B F_1	2	24	0	19	3	16
Mono-2B F_1	2	29	0	21	6	29
Mono-3B F_1	1	15	0	11	1	9
Mono-4B F_1	1	14	0	12	4	33
Mono-5B F_1	2	23	0	17	2	12
Mono-6B F_1	2	25	0	17	3	18
Mono-7B F_1	2	26	0	25	4	16
Mono-1D F_1	2	34	1	33	6	18
Mono-2D F_1	2	23	0	18	6	33
Mono-3D F_1	2	30	0	23	2	9
Mono-4D F_1	2	24	0	20	6	30
Mono-5D F_1	2	23	0	19	1	5
Mono-6D F_1	2	31	0	24	2	8
Mono-7D F_1	1	14	0	11	3	27
Total	40	561	4	456	92	20.2

Table 4. *Frequencies of twin pairs and haploids in artificially pollinated offspring of Salmon and its cytoplasm substitution lines, (triuncialis)-, (columnaris)-, (kotschyi)-, and (variabilis)-Salmon*

Line (♀ parent)	Generation (♀ parent)	Year	♂ parent	Twins			Haploids		
				No. plants examined	No. twin pairs	%	No. plants examined	No. haploids	%
(*triuncialis*)-Salmon									
	B_2	1971	Salmon	7	0	0.0	7	0	0.0
	B_3	1972	Salmon	11	0	0.0	11	5	45.5
	B_4	1973	Salmon	128	10*	7.8	102	25	24.5
	Total			146	10	6.8	120	30	25.0
(*columnaris*)-Salmon									
	B_1	1973	Salmon	33	5	15.2	28	3	10.7
(*kotschyi*)-Salmon									
	B_1	1973	Salmon	25	1	4.0	24	3	12.5
(*variabilis*)-Salmon									
	B_1	1973	Salmon	29	2	6.9	27	10	37.0
	B_1	1973	Tve	20	3	15.0	17	10	58.8
	B_1	1973	P168	12	1	8.3	11	10	90.9
	B_1	1973	JF	15	4	26.7	11	7	63.6
	Total			76	10	13.2	66	37	56.1
Normal Salmon									
	—	1962	Salmon	137	1	0.7	100	0	0.0
	—	1965	Salmon	63	0	0.0	—	—	—
	—	1966	Salmon	120	0	0.0	115	0	0.0
	—	1967	Salmon	415	0	0.0	118	0	0.0
	Total			735	1	0.1	333	0	0.0

Data on normal Salmon are cited from Tsunewaki *et al.* (1968).
* One plant was triplet

(*columnaris*)-Salmon: The occurrence of twins and haploids was investigated only in offspring of the B_1 generation. Table 4 shows that the frequencies of twins and haploids were 15.2% and 10.7%, respectively. All three twin pairs were the diplo-haplo type, as shown in Table 2.

(*kotschyi*)-Salmon: Only the offspring of the B_1 generation was investigated (Table 4). The frequencies of twins and haploids were 4.0% and 12.5%, respectively. A twin pair found in this line was also the diplo-haplo type (Table 2).

(*variabilis*)-Salmon: As Table 4 shows, the B_1 plants of this line were crossed as female with Salmon, Tve, P168 or JF. The overall frequencies of twins and haploids were 13.2% and 56.1%, respectively.

The frequency of haploids in the offspring of (*variabilis*)-Salmon B_1 × Salmon was 37%, while that of the three offspring, (*variabilis*)-Salmon B_1 × other wheats, was 69%; the latter was significantly higher than the former at the 1% level.

Of the ten twin pairs cytologically examined, nine were the diplo-haplo type, and the remaining one was the haplo-haplo type (Table 2).

Normal Salmon: The occurrence of twins and haploids in normal Salmon, i.e. Salmon with the common wheat cytoplasm, was investigated by Tsunewaki *et al.* (1968), whose results are cited in the last part of Table 4. The frequency of twins was only 0.1% (one out of 735), and that of haploids was 0.0% (none among the 333 plants examined).

Discussion

The occurrence of twins in relation to haploids: Tsunewaki *et al.* (1968) have clearly shown that in (*caudata*)-Salmon the egg cells have a high frequency (about 30%) of parthenogenesis, resulting in haploid embryo formation. In the embryo-sacs, about 24% of the synergids are fertilized after pollination, giving rise to diplo-haplo type twin pairs. In the present study, five new cytoplasms, from *Ae. umbellulata*, *Ae. triuncialis*, *Ae. columnaris*, *Ae. kotschyi*, and *Ae. variabilis*, were found to produce haploids and twins at high frequencies (11—56% and 0.5—15%, respectively), and the great majority of the twin pairs (92%) were of the diplo-haplo type. This result contrasts with the finding of Kawakami (1967), that the frequency of twins in normal strains of common wheat was only 0.05%, and the frequency of the diplo-haplo type among other twin types was 4.0%, the great majority (84%) being the diplo-diplo type. From this information, it can be postulated that all these cytoplasms induce parthenogenesis in the same way as the *Ae. caudata* cytoplasm.

Contribution of pollen parents to haploid induction: Chase (1949) demonstrated in corn that the pollen parent has a clear effect on parthenogenesis, though it does not contribute chromosomes to haploids. For example, two inbred lines, 38–11 and B112, produced about ten times higher frequencies of haploids (0.225% and 0.146%, respectively) than did another inbred, A385 (0.013%).

In the present investigation, (*variabilis*)-Salmon produced haploids at a frequency of 37% when pollinated by Salmon, while the frequency rose to 69% when crossed with three other pollen parents, the difference being highly significant. A similar trend was noted in (*umbellulata*)-Salmon, the frequency of haploids being 6.7% when it was pollinated by Salmon, but 14.6% and 20.2%, respectively, on pollination with Macha and CS × Salmon F_1's. Apparently the frequency of haploids becomes higher when pollen grains of wheat strains other than Salmon are used.

Distribution and origin of parthenogenesis-inducing cytoplasms in Aegilops: The cytoplasms of *Ae. umbellulata* (genome formula C^uC^u), *Ae. triuncialis* (CCC^uC^u), *Ae. columnaris* ($C^uC^uM^cM^c$), *Ae. kotschyi* ($C^uC^uS^vS^v$), and *Ae. variabilis* ($C^uC^uS^vS^v$) were found to induce haploid parthenogenesis in a common wheat, Salmon, at relatively high frequencies: 17% by *Ae. umbellulata*, 25% by *Ae. triuncialis*, 11% by *Ae. columnaris*, 13% by *Ae. kotschyi*, and 56% by *Ae. variabilis* cytoplasm. Donors of all these cytoplasms belong to the section Polyeides, having the C^u genome in common. Therefore, we concluded that the cytoplasms of the *Aegilops* species in the Polyeides generally induce haploid parthenogenesis in Salmon. There is at least one exception: Tsunewaki *et al.* (1968) reported that the cytoplasm of *Ae. ovata* ($C^uC^uM^oM^o$), of the same section, induced haploids very rarely in Salmon, the frequency being 0.6% (three out of 506 plants examined). Two other species, *Ae. triaristata* and *Ae. biuncialis*, also belong to this section but we have not yet incorporated their cytoplasms into Salmon to test for parthenogenesis induction.

Kihara and Tsunewaki (1962), and Tsunewaki *et al.* (1968), have reported that the cytoplasm of *Ae. caudata* (CC), one of the two species of the Cylindropyrum, induced a high frequency of haploid parthenogenesis. According to Kihara (1945), F_1 hybrids of *Ae. caudata* and *Ae. umbellulata* showed 3 to 6 bivalents, 5 as the mode. Apparently, the C^u genome of *Ae. umbellulata* is more closely related to the C genome of *Ae. caudata* than it is to any other genome in the diploid species of *Aegilops* and *Triticum*. We may, thus, conclude that the donors of parthenogenesis-inducing cytoplasms to Salmon are more or less related to one another in their genomic relationships.

From the fertility and general vigour of a set of cytoplasm substitution lines of common wheat, Tsunewaki (1973) tentatively concluded that the cytoplasm of *Ae. triuncialis* was derived from *Ae. umbellulata*, and that the cytoplasms of *Ae. columnaris* and *Ae. variabilis* (including *Ae. kotschyi*) came from the M^c and S^v genome donors, respectively, and not from *Ae. umbellulata*. According to Kihara (1945), diploid species with the M or modified M genome are found only in the Comopyrum section, and those with the S or modified S genome are included in the Sitopsis. One possible explanation of why a majority of the Polyeides species have parthenogenesis-inducing cytoplasm is that diploid species of Comopyrum and Sitopsis have parthenogenesis-inducing cytoplasm, like *Ae. caudata* of Cylindropyrum and *Ae. umbellulata* of Polyeides, and their cytoplasms were brought into the Polyeides when various types of amphidiploids were produced. Another possibility is that the cytoplasms of various amphidiploid species of the Polyeides were genetically modified, probably through the selection of mutant plasmagenes, so as to have some common characteristics which coordinate with the C^u genome; one of which is incidentally expressed as parthenogenesis induction when Salmon's nucleus is introduced into them. Future investigation of the cytoplasms in the diploid species of Comopyrum and Sitopsis should provide definitive information as to the correct explanation.

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Received March 22, 1974

Communicated by H. Kihara

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