Genome-plasmon interactions in wheat*

Koichiro TSUNEWAKI

Laboratory of Genetics, Faculty of Agriculture, Kyoto University,
Sakyo-ku, Kyoto 606-01, Japan

(Received 1 February 1993)

ABSTRACT

This article reviews our studies of plastome, chondriome and plasmon diversity among *Triticum* (wheat) and *Aegilops* species, and of interactions among wheat genomes and alien plasmons. Restriction fragment length polymorphisms (RFLPs) of chloroplast and mitochondrial DNAs of nearly all species of those genera were studied by means of restriction endonuclease analysis and Southern hybridization analysis. The results show that the plasmons of the two genera can be classified into 18 types, most of which appear to have diversified at the diploid level. The maternal lineages of most polyploid species, including emmer, timopheevi and common wheats, can be ascertained. These results have been supported by the observed differences in the effects of alien plasmons on various characters of 12 common wheats. The phenotypic outcomes of the interactions between wheat genomes and alien plasmons were, in some cases, novel; several may be of practical use. The genes involved in these interactions were analyzed using various aneuploids of a common wheat Chinese Spring.

1. DIVERSITY OF ORGANELLAR GENOMES AMONG *TRITICUM* AND *AEGILOPS* SPECIES

(A) Chloroplast genome (plastome) diversity

Intraspecific variation

So far, intraspecific variation of chloroplast (ct) DNA has been investigated on a large scale with four species, *T. dicoccoides* (wild emmer wheat), *T. araraticum* (wild timopheevi wheat) (Mori et al., 1988), *Ae. squarrosa* (D genome donor to common wheat) (Terachi et al., 1985), and *Ae. triuncialis* (Murai and Tsunewaki, 1986). In the comparative studies, four six-base cutters (*Bam*HI, *Hind*III, *Sma*I and *Xho*I) were used. Limited information is also available for *Ae. speltoides* (Ogihara and Tsunewaki, 1988).

In the restriction analysis of ctDNAs, of 27 *T. dicoccoides* accessions, all but three showed the same restriction fragment pattern after their ctDNAs had been digested with each endonuclease. Two of the three exceptional accessions, one

* Records of the Kihara Prize Lecture of the Genetics Society of Japan (1992). Dedicated to my Professor, the late Dr. H. Kihara for his enormous contributions to wheat genetics. Contribution No. 530 from the Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Japan. This work was supported in part by Grants-in-Aid (Nos. 02404001, 04305009 and 04304012) from the Ministry of Education, Science and Culture, Japan.

Fig. 1. *Hind*III-fragment patterns of ctDNAs from 18 accessions of *T. dicoccoides* collected at different localities within the areas of distribution (Mori et al., 1988). *: unique fragment, o: one copy lost.

each from Iraq and Israel, had the same 50 bp insertion, whereas the third one, from Israel, possessed an additional *Hind*III site that cleaved one of the 8.2 kb *Hind*III fragments in two fragments (4.2 and 4.0 kb) (Fig. 1). Similarly, all 27 accessions of *T. araraticum* showed identical restriction patterns, with all four restriction enzymes. Among 16 accessions of *Ae. squarrosa*, only two revealed different restriction patterns, and these only after *Bam*HI digestion.

The results of *Ae. triuncialis* ctDNA analyses were different. Among 26 accessions of this species, three ctDNA types were found. One was identical to ctDNA of *Ae. caudata*, and was found in 13 accessions collected in different localities from the Iberian Peninsula to Afghanistan. The second was identical to *Ae. umbellulata*, and was found in eight accessions collected in different localities, from Greece to Afghanistan. The third differed from the above two, and was found in five accessions collected at a single locality in Azerbaijan, Transcaucasus. Because *Ae. triuncialis* is known to be an amphidiploid between *Ae. caudata* and *Ae. umbellulata* (Kihara and Kondo, 1943), our results indicate that *Ae. triuncialis* originated from reciprocal crosses between the two parental species. In addition, the third chloroplast genome type appears to have risen in Transcaucasus, by mutations, from *Ae. caudata* or *Ae. umbellulata*.

Ae. speltooides (including *Ae. aucheri*) is a second example showing great intraspecific variation of ctDNA (Ogihara and Tsunewaki, 1988). Two clearly

different ctDNAs were found among four accessions of this species. They differ from one another by at least two mutations, one a length mutation, and one a base substitution.

Fig. 2. *Bam*HI-fragment patterns of ctDNAs from 40 accessions of *Triticum* and *Aegilops* species (Ogihara and Tsunewaki, 1988). For their code numbers, refer to Table 1. o & *: Fragment lost and gained, respectively, compared with the pattern of *T. boeoticum* (lane 01).

Table 1. *Triticum* and *Aegilops* species used, and their nuclear and cytoplasmic genomes

Species	Code No.	Ploidy (2n)	Nuclear genome (n) ^a	Plasma type ^b	Ct genome		Mt genome	
					Previous ^c	Revised	Previous ^d	Revised
<i>T. monococcum</i>	16	2x	A	A	1a	1	—	I
<i>T. boeoticum</i>	01	2x	A	A	—	—	—	—
<i>T. urartu</i>	—	2x	A	—	1a	1	—	—
<i>Ae. caudata</i>	02	2x	C	C	2	2	—	II
<i>Ae. triuncialis</i>	38	4x	C ^u C	C	2	2	—	—
<i>Syn-triuncialis</i>	27	4x	C ^u C	C	2	2	—	—
<i>Ae. umbellulata</i>	03	2x	C ^u	C ^u	3	3	IIIa	IIIa
<i>Ae. triuncialis</i>	26	4x	C ^u C	C ^u	3	3	IIIb	IIIb
<i>Ae. biuncialis</i>	29	4x	C ^u M ^b	C ^u	3	3	IIIb	IIIb
<i>Ae. triaristata</i> 4x	32, 37	4x	C ^u M ^t	C ^u	3	3	IIIc	IIIc
<i>Ae. triaristata</i> 6x	54, 57	6x	C ^u M ^t M ^{t2}	C ^u	3	3	IIIc	IIIc
<i>Ae. columnaris</i>	30	4x	C ^u M ^c	C ^{u2}	3	3	IIId	IIId
<i>Ae. mutica</i> M	13	2x	Mt	Mt	4	4	IVa	IVa
<i>Ae. mutica</i> P	14	2x	Mt	Mt ²	4	4	IVb	IVb
<i>Ae. ovata</i>	31	4x	C ^u M ^o	M ^o	6	5	—	V
<i>Ae. speltoides</i>	09, 15	2x	S	G	5	6	Va	VIa
<i>T. araraticum</i>	23, 24	4x	AG	G	5	6	Vb	VIb
<i>T. timopheevi</i>	25	4x	AG	G	5	6	Va	VIa
<i>T. zhukovskyi</i>	51	6x	AAG	G	5	6	Va	VIa
<i>T. dicoccoides</i>	21	4x	AB	B	7	7	VIIb	VIIb
<i>T. dicoccum</i>	22	4x	AB	B	7	7	VIIa	VIIa
<i>T. aestivum</i>	52, 58	6x	ABD	B	7	7	VIIa	VIIa
<i>T. spelta</i>	52c	6x	ABD	B	7	7	VIIb	VIIb
<i>Ae. speltoides</i>	08, 17	2x	S	S	8	8	VIII	VIII
<i>Ae. squarrosa</i>	04, 19	2x	D	D	9a	9a	—	IX
<i>Ae. ventricosa</i>	36	4x	DM ^v	D	9a	9a	—	—
<i>Ae. cylindrica</i>	29	4x	CD	D	9b	9b	—	—
<i>Ae. uniaristata</i>	07	2x	M ^u	M ^u	10	10	—	X
<i>Ae. comosa</i>	05	2x	M	M	11a	11	—	XI
<i>Ae. heldreichii</i>	06	2x	M	M ^h	11b	12	—	XII
<i>Ae. sharonensis</i>	10	2x	S ^l	S ^l	1c	13	Ic	XIII
<i>Ae. bicornis</i>	12	2x	S ^b	S ^b	1b	14a	Ib1	XIVa
<i>Ae. searsii</i>	18	2x	S ^s	S ^v	1b	14b	—	XIVb
<i>Ae. kotschy</i>	33	4x	C ^u S ^v	S ^v	1b	14b	Ib2	XIVb
<i>Ae. variabilis</i>	34	4x	C ^u S ^v	S ^v	1b	14b	Ib2	XIVb
<i>Ae. longissima</i>	20	2x	S ^l	—	—	—	—	—
<i>Ae. crassa</i> 4x	35	4x	DM ^{cr}	D ²	1d	16	—	XVI
<i>Ae. crassa</i> 6x	55	6x	DD ² M ^{cr}	D ²	1d	16	—	—
<i>Ae. juvenalis</i>	53	6x	C ^u DM ^j	D ²	1d	16	—	—
<i>Ae. vavilovii</i>	56	6x	DM ^{cr} SP	D ²	1d	16	—	—

^a) After Kihara (ref. Liliensfeld, 1951) and Kihara and Tanaka (1970).

^b) After Tsunewaki (1980, 1989).

^c) After Ogiwara and Tsunewaki (1982, 1988).

^d) After Terachi and Tsunewaki (1986, 1992) and Terachi et al.(1990). Note) —: Not tested.

In conclusion, intraspecific variation of ctDNA is rare in *Triticum* and *Aegilops*, with the exceptions of *Ae. triuncialis* and *Ae. speltoides*.

Interspecific diversity

Restriction fragment patterns of ctDNAs isolated from 33 *Triticum* and *Aegilops* species (42 accessions in total), representing 28 nuclear genomes, were analyzed, using 13 restriction endonucleases, *Bam*HI, *Bgl*II, *Hind*III, *Kpn*I, *Pst*I, *Pvu*I, *Pvu*II, *Sal*I, *Sca*I, *Sma*I, *Sst*I, *Xba*I and *Xho*I, all being six-base cutters (Ogihara and Tsunewaki, 1982, 1988). As an example, *Bam*HI-fragment patterns are shown in Fig. 2. From these restriction fragment patterns, 17 different plastomes can be identified.

Comparisons of differences among restriction fragment patterns of common wheat and other species to the physical maps of common wheat ctDNA, showing cleavage sites of all 13 endonucleases, made it possible to identify differences, by length mutations and base substitutions, between ctDNAs of common wheat and other species (Ogihara and Tsunewaki, 1982, 1988). The portion of ctDNA examined for mutations has been estimated to be about 1.3% (imprecise because of the length mutations) of the entire ctDNA molecule. That is, about 285 restriction sites, each consisting of six bases, correspond to about 1.7 kb, 1.3% of 135.5 kb, the total genome size. Even though 1.7 kb is a small fraction of the total, it is

Fig. 3. A dendrogram showing phylogenetic relationships between 17 plastomes identified in 30 *Triticum* and *Aegilops* species, constructed by cluster analysis based on the numbers of mutations detected between the ctDNAs (Ogihara and Tsunewaki, 1988).

sufficient to identify different chloroplast genome types, each with at least two mutational differences between them. Based on these criteria, the different plastome types shown in Table 1, have been assigned. Some of the symbols in Table 1 have been modified from those used previously (Ogihara and Tsunewaki, 1982, 1988). This is because the previous 1a, 1b, 1c and 1d genomes, which gave identical restriction fragment patterns with four endonucleases (Ogihara and Tsunewaki, 1982), showed about the same magnitude of differences among them as between them and other chloroplast genomes studied later, using nine additional endonucleases. The same was true for 11a and 11b genomes.

Based on the numbers of mutations disclosed between all pairs of the 17 plastomes, a dendrogram indicating their phylogenetic relationships was constructed by cluster analysis using the UPGMA method (Fig. 3) (Sneath and Sokal, 1973).

(B) Mitochondrial genome (chondriome) diversity

Restriction endonuclease analysis of closely related species

Restriction fragment patterns of mtDNAs from five species (six accessions) having type 3 plastome, two accessions of *Ae. mutica* having type 4 plastome, ten species (12 accessions) having type 6, 7 or 8 plastome, and five species having plastome, 13, 14a or 14b, were studied using five six-base cutters, *Bam*HI, *Hind*III, *Pst*I, *Pvu*II and *Xho*I (Terachi and Tsunewaki, 1986; Terachi et al., 1990; Siregar et al., 1988). The six accessions having type 3 plastome were classified into four different, mtDNA groups (Fig. 4). Two accessions of *Ae. mutica* also had different mtDNAs. Among four species (five accessions) having

Fig. 4. Dendrograms showing phylogenetic relationships among chondriomes of closely related species, constructed by cluster analyses based on the percentages of restriction fragment differences observed among their mtDNAs (Terachi and Tsunewaki, 1986; Terachi et al., 1990).

type 6 plastome, *T. araraticum* (two accessions) had mtDNA slightly different from *Ae. speltoides*, *T. timopheevi* and *T. zhukovskyi*, all of which had identical mtDNA. Six species with type 7 plastome were classified into two groups; i.e., the mtDNA of *T. dicoccum*, *T. durum*, *T. aestivum* and *T. compactum* was slightly different from that of *T. dicoccoides* and *T. spelta*. Among the five species having type 13, 14a or 14b plastome, *Ae. kotschyi* and *Ae. variabilis* had identical mtDNA, whereas *Ae. searsii*, *Ae. bicornis* and *Ae. sharonensis* differed from one another, and from the former two species. Based on the percentages of differential fragments over total fragments observed, between mtDNAs of different sources, phylogenetic trees of their chondriomes were drawn (Fig. 4) by the UPGMA method. The results clearly demonstrate that chondriome diversity is much greater than plastome diversity, indicating a faster rate of evolutionary change.

Southern hybridization analysis of 17 plastomes

RFLP analysis of mtDNAs from 19 species, representing all 17 plastomes, was carried out using the following four mtDNA clones as probes: 1.5 kb and 1.9 kb

Fig. 5. Southern hybridization patterns of *Bam*HI-digests of mtDNAs from 19 accessions of *Triticum* and *Aegilops* species, representing 17 plastomes (Terachi and Tsunewaki, 1992). A 1.5 kb mtDNA fragment containing the gene, *atpA*, was used as a probe. Mnc: *T. monococcum*. Code or code numbers of other accessions are given in Table 1.

fragments containing the *atpA* and *coxII* genes of peas, and 5.1 kb and 3.2 kb fragments containing the 26S rRNA, and 18S and 5S rRNA genes of wheat (Terachi and Tsunewaki, 1992). Two endonucleases, *Bam*HI and *Hind*III were used for mtDNA digestion. An example of the Southern hybridization patterns is shown in Fig. 5.

Southern hybridization patterns obtained from the eight probe-enzyme combinations were analyzed. The total, and the differential numbers of hybrid fragments between all 19 species are scored, based on which genetic distances between chondriomes were estimated after Nei (1987), and a phylogenetic tree was constructed, by the UPGMA method, based on these distances (Fig. 6).

Fig. 6. A dendrogram showing phylogenetic relationships among the chondriomes of *Triticum* and *Aegilops* species representing all 17 plastomes (given in parentheses), constructed by cluster analysis, using Nei's genetic distances (Terachi and Tsunewaki, 1992).

From the results of the restriction and Southern analyses, chondriomes of *Triticum* and *Aegilops* species can be classified and designated as shown in Table 1. Some modifications of previously proposed designations (Terachi and Tsunewaki, 1986; Terachi et al., 1990) have been made so as to match with the preceding changes in plastome designation.

(C) Diversity and lineages of cytoplasmic genomes (plasmons) in *Triticum* and *Aegilops*

The phylogenetic trees of plastomes (Fig. 3) and chondriomes (Fig. 6) are in agreement in many respects, indicating a parallel evolutionary divergence of these two organellar genomes. First, the four major branches of both trees consist of (i) einkorn wheat, (ii) *Ae. speltoides*, the timopheevi wheat group, and common wheat group (emmer wheat group, inclusively), (iii) diploid species of the section

When nuclear and cytoplasmic genomes of polyploid species were compared to those of diploid species, it was possible to distinguish between the maternal and pollen parents of most tetraploids and all hexaploids (Fig. 7). Among the four plasmons unique to the tetraploids, B and M^o appear to have originated from *Ae. speltoides* and *Ae. mutica*, respectively (ref. Figs. 3 and 6). It is likely that the C^{u2} plasmon originated from a C^u plasmon at the diploid or tetraploid level. But few clues of the origin of D², found in a tetraploid, *Ae. crassa*, were observed, understandably because all other plasmons found in diploid and tetraploid species were only distantly related to D².

Fig. 7. Genome (nuclear; inner circle) and plasmon (outer circle) relationships between *Triticum* and *Aegilops* species, indicating the maternal and paternal lineages of polyploid species (Tsunewaki, 1989; partly modified). Different plasmons are depicted in different patterns.

2. EXPRESSION OF ALIEN PLASMONS IN COMMON WHEAT

(A) Persistence of alien plasmons in common wheat

To study expression of alien plasmons in common wheat, 12 common wheats, shown in Table 2, were used as the recurrent pollen parent, i.e., as the recipient of alien plasmons. All other species in Table 1 were used as the female parent, i.e., as the cytoplasm donor, in the initial crosses leading to the production of alloplasmic lines of common wheat. The backcross generations reached in 1992 by all alloplasmic lines, are shown in Table 3.

The symbolism used here is as follows: nuclei are identified by a single upper-case, alphabetical letter (Table 2), and cytoplasm by a two-digit code number (Table 1). When preferred, cytoplasm is identified by abbreviated

Table 2. Twelve common wheats used as recipients of alien cytoplasm in the production of alloplasmic wheat lines*

Species and lower taxon	Code	Abbreviation
<i>T. aestivum</i> var. <i>erythrospermum</i>	A	Tve
<i>T. aestivum</i> strain P168	B	P168
<i>T. aestivum</i> cv. Chinese Spring	C	CS
<i>T. aestivum</i> cv. Norin 26	D	N26
<i>T. aestivum</i> strain Salmon	E	Slm
<i>T. aestivum</i> cv. Jones Fife	F	JF
<i>T. aestivum</i> cv. Selkirk	G	Sk
<i>T. aestivum</i> cv. S-615	H	S615
<i>T. sphaerococcum</i> var. <i>rotundatum</i>	I	Sphr
<i>T. compactum</i> cv. No. 44	J	Cmp
<i>T. spelta</i> var. <i>duhamelianum</i>	K	Splt
<i>T. macha</i> var. <i>subletschchumicum</i>	L	Mch

* Note: All wheats used are hexaploid ($2n=42$, nuclear genome constitution AABBDD).

Table 3. Numbers of alloplasmic lines of 12 common wheats per set of five backcross generations, up to 1992

Backcross generation ^a	Recipient common wheat												Total	%
	Tve	P168	CS	N26	Slm	JF	Sk	S615	Sphr	Cmp	Splt	Mch		
0-4	1	1	1	1	0	1	0	1	0	1	1	2	10	1.8
5-9	6	5	3	7	5	5	5	6	8	4	4	5	63	11.4
10-14	37	40	30	35	37	37	34	39	35	37	37	37	435	78.8
15-19	1	0	8	2	3	2	5	0	3	3	3	2	32	5.8
20-40	1	0	4	1	1	1	2	0	0	1	1	0	12	2.2
Total	46	46	46	46	46	46	46	46	46	46	46	46	552	100.0

^a) 0: F₁ generation, 1-40: B₁ to B₄₀ generations.

species or accession names. Nucleus-cytoplasm (NC) hybrids, or alloplasmic lines of wheat having a wheat nucleus and an alien cytoplasm, are identified by a combined code of nuclear and cytoplasmic components, like A01, or by the species names of the nucleus and cytoplasm donors, for example, (*boeoticum*)-Tve, an NC hybrid having the nucleus of *T. aestivum* accession Tve, and the cytoplasm of *T. boeoticum*. Of the alloplasmic lines 86.8% reached B₁₀ or beyond, at which time backcrossing was stopped, unless necessary for the seed renewal.

The persistence of alien plasmons in wheat has been thoroughly investigated, and was first proved by Fukasawa (1959). In the present investigation, the alloplasmic line, (*caudata*)-Tve, reached the B₄₂ generation in 1992—a record number of backcrosses and years of viability among nucleus-cytoplasm hybrids of higher plants. The (*luteum*)-*hirsutum* line of *Epilobium* reached the B₂₉ generation during World War II, but is now lost (Michaelis, 1954). Alloplasmic (*caudata*)-Tve was produced by Kihara (1951, 1959), and has expressed complete male sterility since the B₄ generation. Since 1968 (B₁₈ generation), backcrossing this line has been continued by the author. The variation in seed fertility of the backcrosses (with normal pollen) and selfing at successive generations, is shown in Fig. 8. In short, the female fertility of (*caudata*)-Tve has remained almost normal, whereas male fertility was lost after B₄. The male sterility-inducing property of *Ae. caudata* cytoplasm has showed no signs of change, even after 43 generations of crosses with normal wheat pollen.

Kondo (1941) produced an amphidiploid between *Ae. caudata* (female parent) and *Ae. umbellulata*. This synthetic *Ae. triuncialis* line (Syn-*triuncialis* in Table 1) was used as a cytoplasm donor to 12 common wheats. The fertility

Fig. 8. Selfed and backcrossed seed fertility of (*caudata*)-Tve from the F₁ to the B₄₀ generation. Normal Tve is the recurrent pollen parent.

spectrum (described later) of this cytoplasm was essentially identical to that of *Ae. caudata* cytoplasm (Fig. 9). Furthermore, alloplasmic lines of CS having this cytoplasm (e.g., line C27) showed restriction fragment patterns of ctDNA identical to those of *Ae. caudata*, with all 13 restriction endonucleases (ref. Fig. 2, Ogiwara and Tsunewaki, 1988). These facts indicate that the plasmon of *Ae. caudata* did not change in the presence of nuclear genomes, C^u of *Ae. umbellulata*, or A, B, and D of common wheat, or after repeated pollination with normal wheat pollen for more than 18 generations.

These facts demonstrated that alien plasmons are transmitted only maternally, and persist for many years in common wheat without hereditary change.

Fig. 9. Fertility spectra of the cytoplasm of *Ae. caudata*, *Ae. umbellulata*, syn-*triuncialis*, and two *Ae. triuncialis* accessions (code nos. 26 and 38). Twelve wheat nuclei are given in their codes, A-L (ref. Table 2).

(B) Effects of alien plasmons on wheat characters

The most commonly observed effect of alien cytoplasm is male sterility. In Fig. 10, the average backcrossed and selfed seed fertilities of all alloplasmic lines are shown. The cytoplasm is grouped into 18 plasmons, though C^{u2} and M^h plasmons are included with C^u and M plasmons, respectively. The seed fertility of plasmon B was used as a control. Backcrossed seed fertility is a good parameter of female fertility, and selfed fertility reveals both female and male fertility. The range of backcrossed seed fertility, of 17 alloplasmons, was from 65% to 120% of the control. None of the alien plasmons seriously reduced female fertility of common wheats. However, its slight reduction was caused by A, C and S^1 plasmons. Contrary to their slight influence upon female fertility, alloplasmons exert a drastic effect upon male fertility, and as a consequence, on selfed seed fertility (Fig. 10).

Fig. 10. Selfed and backcrossed seed fertility of common wheats, as influenced by alien plasmons. In ordinate seed fertilities are given in percent as the average of 12 common wheats. Plasmons of the same plasma type are combined, a number of which are shown under the plasma type name. C^{u2} and M^h plasmons are included in C^u and M , respectively.

Twelve common wheats showed different responses to alien plasmons. A "fertility spectrum" of a cytoplasm combined with 12 wheat nuclei takes the form of a histographic curve. Based on observed selfed seed fertility, of the five most advanced generations, the fertility spectra of all plasmons were generated (Fig. 11). In this figure, plasmons with similar fertility spectra were combined.

Fig. 11. Fertility spectra of all plasmons of 45 accessions of *Triticum* and *Aegilops* species, based on records of the five most advanced backcross generations. Plasmons showing similar spectra are combined, and their average is given. The spectra are classified into ten types, I to VIII, plus I' and II'. Plasma types and the numbers of cytoplasm (in parentheses) classified into each spectrum type are also shown.

From these plasmon interactions with 12 common wheat genotypes, 18 plasmons were classified into ten groups.

In 1982, alloplasmic lines of 12 common wheats having 40 different cytoplasm (39 alien cytoplasm and one self cytoplasm) were grown in a split plot design, with four replications and 12 nuclei arranged in the main plots, and 40 cytoplasm in subplots (Tsunewaki and Tsujimoto, 1983). Two plants of each line were grown in each subplot. Observations were made on the following 16 characters; variegation in winter, dry matter weight, flag leaf length and width, number of internodes longer than 3 cm, culm diameter at the middle part of the second internode, lengths between first and third internodes from the top, plant height, ear length, number of ears per plant, number of spikelets per ear, awn length, heading date, and selfed seed fertility. Four other characters, namely, anther degeneration, pistillody and malformations other than pistillody, and pollen fertility were observed in one plot with no replication. Analyses of variance were carried out on the data of all characters except the last four, which were observed only in a single plot. The effects of cytoplasm, and interactions between nuclei and cytoplasm, were all significant at the 1% level on all 16 characters. It was concluded that all characters of common wheat are affected by alien plasmons, and that different wheat genotypes respond to alien cytoplasm in different ways. Such specific interactions between wheat genotypes and alien plasmons are clearly

Fig. 12. Effects of alloplasmons on heading of 12 common wheats, A-L. Differences between allo- and eu-plasmic lines, in number of days, are shown. Plasmons showing similar effects are grouped, and the histograms are drawn, based on group averages. Plasma types and numbers of cytoplasm (in parentheses) classified into each group are also shown. Data from the A plasmon are not available for some nuclei (open column) because the plants with this plasmon died before heading.

seen in the fertility spectra shown in Fig. 11. Similar interactions are also clear on heading date and dry matter, as shown in Figs. 12 and 13.

For further analysis, normalization of the data was made by dividing the original data by the square root of the error mean square for subplots. Using the normalized data of 480 nucleus-cytoplasm combinations, correlation coefficients between all pairs of 20 characters were calculated. A dendrogram showing the genetic relatedness among these characters was obtained by cluster analysis. From the results, 20 characters were clustered to four major groups, three of which are represented by selfed seed fertility, heading date and dry matter weight, respectively. The patterns of effects from alien cytoplasm on these three characters of common wheat, shown in Figs. 11, 12, and 13, present an over-all picture of the genetic effects of all plasmons upon wheat phenotypes.

To clarify the overview of the genetic relatedness between plasmons, based on their effects upon wheat phenotypes, correlation coefficients between plasmons were calculated from the normalized data of 240 nucleus-character combinations.

Fig. 13. Effects of alloplasmons on dry matter of 12 common wheats, A-L. The differences in percent, in dry matter between alloplasmic and euplasmic lines are given, as calculated by the formula, $(\text{alloplasmics} - \text{euplasmics}) / \text{euplasmics} \times 100$. Plasmons showing similar effects are grouped, and the histograms are based on their averages.

Based on these coefficients, the 40 plasmons were clustered, by the UPGMA method, as shown in Fig. 14. Plasmons differing in their plastomes and chondriomes fell into different clusters, except in the cases of B, S, and D plasmons, and S^v and M^u plasmons. From these observations it seems clear that functional divergence among plasmons was paralleled by molecular divergence of their plastomes and chondriomes.

Fig. 14. A dendrogram comparing genetic relatedness among 40 plasmons of *Triticum* and *Aegilops*, based on the correlation coefficients which were calculated from their normalized effects on 20 characters of 12 common wheats (Tsunewaki and Tsujimoto, 1983). The plasmons are identified by code number and plasma type (ref. Table 1). The fertility spectrum type and effect on growth vigor or heading are also given.

(C) Novel characters expressed by alien plasmons in common wheat

Various novel characters were expressed by different alien plasmons in certain common wheats (Table 4). One of these was cytoplasmic male sterility expressed in various nucleus-cytoplasm combinations (Fig. 11). Others were pistillody, germless grain formation, premature sprouting, haploid and twin seedling formation, variegation under low temperature, depressed growth vigor, and delayed heading.

Under field condition in Kyoto (36°N), pistillody was expressed by C and G cytoplasm, most frequently in CS, Sphr and Splt. In the case of D² cytoplasm, strong pistillody was induced in N26 and some other cultivars by long day treatment (15 h or longer light period) (Murai et al., 1988). Pistillated stamens

Table 4. Characteristic effects of alien plasmons on common wheat characters (Tsunewaki, 1980, 1989)

Effect	Plasma type														
	B,D S	S ^b	D ²	M ^a	S ^v	Mt	S ^l	C ^u C ^{u2}	M ^o	C	G	Mt ²	M ^h	M	A
Fert. spectrum	I	I	I'	II	II	II'	III	IV	V	VI	VII	VIII	VIII	VIII	VIII
Pistillody	—	—	+	—	—	—	—	—	—	+	+	—	—	—	—
Germless grain	—	—	—	+	+	—	+	+	—	+	—	—	—	—	—
Premat. sprout.	—	—	—	—	—	—	—	—	+	—	+	—	—	—	—
Parthenogenesis	—	—	—	+	+	+	+	+	—	+	—	+	—	—	—
Variegation	—	+ ^a	—	—	+ ^a	—	+ ^a	+	—	—	—	—	—	—	++
Growth depress.	—	—	—	—	—	—	+	+	—	—	—	—	+	++	++
Heading delay	—	—	—	—	—	++	+	+	++	—	—	—	+	+	++

—, + and ++: Not expressed, expressed, and strongly expressed, respectively.

^a) Variegation expressed in synthetic hexaploids not having the D genome.

have cavities in their bases, and in some cases they contain ovules never with embryo-sacs (Kihara and Tsunewaki, 1961; Murai, 1992); therefore, they do not set seed.

Germless grains were found most frequently in Tve and Slm possessing alien cytoplasm that induce haploid parthenogenesis in Slm; the frequency was relatively low, about 27% at most (Tsunewaki, 1980). CS and some other common wheats with the same plasmons rarely produce germless grains. The formation of germless grains is probably related to parthenogenesis of egg cells, and is suspected to be caused by the deaths of parthenogenetic embryos after endosperm development. The genetic and developmental mechanisms of germless grain formation have not yet been studied.

Premature sprouting is a function of seed germination before full maturity. This occurred frequently in Tve, Slm and S615 carrying G or M^o cytoplasm. The maintenance of these lines is difficult since most seeds are dead at the time of harvest. The rare, viable seeds are extremely shrivelled, indicating a voracious consumption of starch during seed filling, probably due to the precocious production of malt isozymes of alpha-amylase (Nishikawa, 1989). Neither the molecular biology (of plasmagenes) nor the metabolic phenomena of starch digestion are known, nor is it clear what roles nuclear genes play in this interesting but complex process.

Under natural conditions, variegation is caused, in winter, by A, C^u, and C^{u2} cytoplasm in nine of the 12 common wheats used. The A cytoplasm showed the most severe variegation, followed by the C^u cytoplasm. The cause of the variegation is a reduction (about 50% in the case of C^u cytoplasm) in both chlorophyll a and b. Variegation disappears during spring time, with raising temperatures (Mukai and Tsunewaki, 1976). JF, Splt and Mch carry a suppres-

sor gene(s) of these kinds of variegation.

Growth depression, typically expressed as reduced dry matter (ref. Fig. 13), is caused by many alien plasmons. Cytoplasm C, G, M^o, Mt², S, S^b and S^v caused 10–20% reduction (average for the 12 common wheats), whereas C^u, C^{u2}, M^h and S^l cytoplasm resulted in 30–40% reduction in dry matter by the time of harvest. The reduction caused by A and M cytoplasm was greater than 60%. Growth depression caused by cytoplasm A, C^u and C^{u2} was undoubtedly due in part to the variegation expressed in winter. Contrary to this, Mt cytoplasm increased dry matter about 6%. B, D, D², and M^u cytoplasm did not alter the dry matter in any nuclear genotype.

The interactions, expressed on dry matter, between wheat genotypes and alien cytoplasm were different, in particular the interactions involving Mt, M^o and M^h cytoplasm. With Mt cytoplasm and Tve, CS and S615 nuclear genotypes there was a 15–30% increase of dry matter, whereas with P168 and JF, more than a 5% decrease was observed. M^o cytoplasm resulted in a 10% increase of dry matter in CS and Sphr, but a 10–40% decrease in most of the other wheats. M^h cytoplasm resulted in 30–60% decrease of dry matter in all wheats except Tve and CS, which were little affected by this cytoplasm. Undoubtedly, certain wheat genotypes have genes for recovering growth vigor, impaired by alien plasmons, but these genes have not been identified so far.

As would be predicted, depressed growth vigor affects many other characters, e.g., plant height, lengths of various internodes, number of internodes, size of flag leaf (both length and width), number of ears per plant, and ear size (ear length and number of spikelets per ear).

A, C^u, C^{u2}, M, M^h, M^o, Mt, and S^l cytoplasm caused some delay in heading, whereas the other cytoplasm did not (ref. Fig. 12). C^u, C^{u2} and S^l cytoplasm resulted in 4–5 day, C cytoplasm 7 day, and M^o and Mt cytoplasm 10 day delays, and A cytoplasm a 17 day delay, the greatest of all. There were few deviations of heading time with other nucleus-plasmon combinations. The S^l cytoplasm-JF nuclear combination showed extreme delay, but S^l with Tve and Sk showed less delay. C^u and C^{u2} cytoplasm caused much more heading delay in Slm and S615 than in the other wheats; M and M^h cytoplasm in P168 and JF showed greater delay than they did in other wheats, but no delay in CS. Mt and M^o cytoplasm caused twice as much delay in Slm, Splt, JF, Cmp and Tve than in S615. Again, analyses of the relevant nuclear genes are yet to be done.

3. ANEUPLOID ANALYSES OF THE NUCLEAR FACTORS INVOLVED IN WHEAT GENOME-ALIEN PLASMON INTERACTIONS

(A) Male sterility-fertility restoration

Cytoplasm giving rise to the type I fertility spectrum did not cause male sterility in any of the 12 common wheats studied. At the other extreme,

cytoplasms with the type VIII spectrum caused complete sterility in all 12 wheat genotypes. For obvious reasons, genetical analyses of male sterility-fertility restoration can not be carried out using these cytoplasms.

From each of the other fertility spectra, except type III, at least one pair of male sterile and fertile wheats were subjected to aneuploid analyses using Sears' materials (Sears, 1954, 1964; Sears and Sears, 1977). The male sterility-inducing cytoplasm will be indicated here by its plasma type. Unidentified fertility-restoring gene(s) is symbolized by "*Rf*". In all cases, male fertility was estimated from selfed seed fertility, in that none of the male-sterile cytoplasms studied caused severe female sterility (ref. Fig. 10).

Type VII fertility spectrum: Among the 12 common wheats used, only Splt and Mch carried *Rf* gene(s), which with G cytoplasm gave rise to a type VII fertility spectrum. Many common wheat cultivars were rendered completely male-sterile in company with a G cytoplasm. Results of conventional and monosomic analyses of the *Rf* gene in Splt (Table 5a) indicated that this wheat carried a single dominant gene, designated *Rf3*, located on chromosome 1B (Tahir and Tsunewaki, 1969). A G cytoplasm-*Rf3* gene interaction allowed transmission of *Rf3* and *rf3* alleles, through both female and male gametes, of about 50%. Functional pollen formation was determined by the genotype of the sporophyte, thereby eliminating preferential fertilization that might arise from the genotype of the pollen.

Type VI fertility spectrum: In the presence of a C cytoplasm with a type VI fertility spectrum, Cmp, P168 and S615 exhibited restored fertility, whereas CS and the other wheats became completely or almost completely male sterile. The results of monosomic analysis carried out using (C)-Cmp and CS monosomics (Table 5b; Tsunewaki, 1974), indicated that Cmp carried two *Rf* genes, one each on chromosomes 6B and 1D, designated as *Rfc2* and *Rfc3*, respectively. At least three doses of these *Rf* genes were necessary for restoring more than 5% fertility. In this case, no critical test was carried out to determine whether fertility restoration was sporophytic or gametophytic.

Table 5. Conventional and aneuploid analyses of fertility-restoring (*Rf*) genes for *T. timopheevi* (G), *Ae. caudata* (C), *Ae. ovata* (M^o), *Ae. umbellulata* (C^u), *Ae. kotschyi* and *Ae. variabilis* (both S^v) cytoplasm (Tahir and Tsunewaki, 1969, 1971; Tsunewaki, 1974, unpubl.; Mukai and Tsunewaki, 1979; Tsujimoto and Tsunewaki, 1984)

Cross combination ^a	No. offspring		% Fertiles (Ratio tested)
	Fertile	Sterile	
a) <i>T. timopheevi</i> cytoplasm (G type)			
(G)- <i>Rfrf</i> × <i>rf rf</i>	935	994	48.5 ^{ns} (1:1)
(G)- <i>Rfrf</i> × (G)- <i>Rfrf</i>	214	64	77.0 ^{ns} (3:1)
(G)- <i>rf rf</i> × (CS mono-1B × Splt) mono F ₁	71	7	91.0** (1:1)

(G)- <i>rfrf</i> × (CS other monos. × Splt) mono F ₁ 's	975	963	50.3 ^{ns} (1:1)
b) <i>Ae. caudata</i> cytoplasm (C type)			
(C)-Cmp × (CS mono-6B × Cmp) mono F ₁	61	6	91.0** (3:1)
(C)-Cmp × (CS mono-1D × Cmp) mono F ₁	40	2	95.2** (3:1)
(C)-Cmp × (CS other monos. × Cmp) mono F ₁ s	881	291	75.2 ^{ns} (3:1)
c) <i>Ae. ovata</i> cytoplasm (M ^o type)			
(M ^o)- <i>Rfrf</i> × <i>rfrf</i>	857	850	50.2 ^{ns} (1:1)
(M ^o)- <i>Rfrf</i> × (M ^o)- <i>Rfrf</i>	342	26	92.9** (3:1)
(M ^o)- <i>rfrf</i> × (CS mono-1D × P168) mono F ₁	108	6	94.7** (1:1)
(M ^o)- <i>rfrf</i> × (CS other monos. × P168) mono F ₁ s	2105	605	78.2** (1:1)
d) <i>Ae. umbellulata</i> cytoplasm (C ^u type)			
(C ^u)-Slm × (CS mono-1B × Slm) mono F ₁	0	16	0.0* (1:3)
(C ^u)-Slm × (CS mono-2B × Slm) mono F ₁	0	15	0.0(*) (1:3)
(C ^u)-Slm × (CS other monos. × Slm) mono F ₁ s	76	257	22.8 ^{ns} (1:3)
e) <i>Ae. kotschy</i> and <i>Ae. variabilis</i> cytoplasm (S ^v type)			
(S ^v)-Splt × CS	10	0	100.0 ^{ns} (1:0)
(S ^v)-Splt × CS dt-1BS	10	0	100.0 ^{ns} (1:0)
(S ^v)-Splt × CS dt-1BL	0	10	0.0 ^{ns} (0:1)
[(S ^v)-Splt × CS] F ₁ × (Splt × CS) F ₁	170	62	73.3 ^{ns} (3:1)
[(S ^v)-Splt × CS] F ₁ × [(S ^v)-Splt × CS] F ₁	239	40	85.7** (3:1)
f) <i>Ae. uniaristata</i> cytoplasm (M ^u type)			
(M ^u)-Slm: 1B/1BL-1RS	0	20	0.0 ^{ns} (0:1)
(M ^u)-Cmp: 1B/1B	20	0	100.0 ^{ns} (1:0)
(M ^u)-Slm × Cmp: 1B/1B	5	0	100.0 ^{ns} (1:0)
(M ^u)-Slm × (Slm × Cmp) F ₁	18	13	58.1 ^{ns} (1:1)
(M ^u)-Slm × [(M ^u)-Slm × Cmp] F ₁	34	0	100.0 ^{ns} (1:1)
[(M ^u)-Slm × Cmp] F ₁ × Slm	7	186	3.6** (1:1)
(M ^u)-CS: 1B/1B	20	0	100.0 ^{ns} (1:0)
(M ^u)-CS dt-1BL: 1BL/1BL	0	5	0.0 ^{ns} (0:1)
g) <i>Ae. mutica</i> M cytoplasm (Mt type)			
(Mt)-CS: 1B/1B	20	0	100.0 ^{ns} (1:0)
(Mt)-Slm: 1B/1BL-1RS	0	20	0.0 ^{ns} (0:1)
[(Mt)-Slm × Cmp] F ₁ × Slm	8	154	4.9** (0:1)
(Mt)-Slm × [(Mt)-Slm × Cmp] F ₁	108	32	77.1** (1:0)
(Mt)-Slm × CS: 1B/1B	10	0	100.0 ^{ns} (1:0)
(Mt)-Slm × CS dt-1BL: 1B/1BL	0	5	0.0 ^{ns} (0:1)

^{ns}, *, and **. Statistically non-significant, and significant at 5% and 1% level, respectively, against the ratios given in parentheses.

^{a)} Mono; monosomic line, dt; ditelosomic line. The cytoplasm of each alloplasmic line is indicated by its plasma type. All lines not marked by plasma type have common wheat cytoplasm.

Type V fertility spectrum: With M^o cytoplasm of *Ae. ovata* showing type V fertility spectrum, only P168, CS and S615 were shown to carry one or more *Rf* genes. Genes involved with male sterility-fertility restoration were analyzed in two crosses, CS $\times$ P168, and N26 $\times$ CS (Tahir and Tsunewaki, 1971; Tsunewaki, 1982). The *Rf* genes of CS and S615 are recessive, as shown by the fact that F_1 hybrids between (M^o)-N26 and CS or S615 were almost completely male-sterile. An *Rf* gene in P168 is dominant, in that (M^o)-N26 $\times$ P168 F_1 's were highly fertile. From the segregation ratio of fertile and sterile plants in the B_1 generation, (M^o)-*Rfrf* $\times$ (B)-*rfrf*, it was clear that P168 carried one dominant *Rf* allele, designated as *Rfc1* (Table 5c). By monosomic analysis, this gene was located on chromosome 1D of P168. In fact, the 1D chromosome of P168 is a translocation chromosome consisting of parts of chromosome 1D of common wheat and chromosome 1C of *Ae. caudata* (Muramatsu, 1959; Mukai et al., 1985). When (M^o)-*Rfrf* was the pollen parent, *rf* pollen participated in fertilization, indicating sporophytic fertility restoration. However, fertilization by the *Rf* pollen was about 15 times frequent than that by the *rf* pollen. Preferential fertilization by *Rf* pollen was also seen in (B)-*Rfrf*, but the magnitude of preference was only 3.6 times that of *rf* pollen.

Type IV fertility spectrum: CS showed the highest level of fertility restora-

Fig. 15. Frequency distributions (frequency in percent) of different classes of selfed seed fertility within segregating populations from different crosses between cytoplasmic male sterile, restorer and/or maintainer lines of common wheat, for three alloplasmons. A) *Ae. umbellulata* (C^u type), and B) *Ae. uniaristata* (M^n), and C) *Ae. crassa* (D^2 type) cytoplasm.

tion, whereas Tve, N26, Slm, Sphr, Splt and Mch exhibited complete or almost complete sterility in combination with C^u and C^{u2} plasmons. Monosomic analysis of (C^u) -Slm and CS revealed that chromosomes 1B and 2B of CS carry two complementary genes which restored fertility more than 10%, if both genes are present (Table 5d; Tsunewaki, 1974). These genes are designated as *Rfu1* and *Rfu2*. The frequency distribution of different fertility classes in three hybrid populations, between JF and Splt, are shown in Fig. 15A. From the cross, (C^u) -Splt $\times$ [(C^u) -Splt $\times$ JF], a few completely male sterile plants were recovered, indicating a sporophytic fertility restoration. In the cross, (C^u) -Splt $\times$ [(B)-Splt $\times$ JF], the proportion of fertile plants was slightly higher than the expected 1/4. Apparently, some minor genes contributed to fertility restoration. The ratio of fertile:sterile plants deviated drastically from 1:3, in favor of the fertile class, when [(C^u)-Splt $\times$ JF] F_1 was the pollen parent. It was clear from the shift of the distribution curve, in comparing the cross, (C^u) -Splt $\times$ [(C^u)-Splt $\times$ JF] F_1 , with that of (C^u) -Splt $\times$ [(B)-Splt $\times$ JF] F_1 , that the advantage of *Rf* over *rf* pollen widened 2.4 times in C^u cytoplasm compared to B cytoplasm. Only one fertile plant was found among 178 progeny plants, from the cross, [(C^u)-Splt $\times$ JF] F_1 $\times$ Splt, suggesting that egg cells carrying the *Rf* genes of JF, in the presence of C^u cytoplasm, are infertile.

Type II fertility spectrum: In conjunction with S^v cytoplasm, Slm, Splt and Mch, are completely male sterile, whereas the other wheats are fully fertile. The male sterility-fertility restoration system against this plasmon was analyzed, using a CS $\times$ Splt cross combination (Mukai and Tsunewaki, 1979). Ditelosomic analysis revealed that the 1BS arm of CS carries a dominant *Rf* gene, designated *Rfv1* (Table 5e). The F_2 ratio supported this finding. Upon selfing the F_1 hybrid, [(S^v)-Splt $\times$ CS], completely male sterile plants were recovered, indicating that fertility restoration is sporophytic. From Table 5e, it was concluded that *Rf* and *rf* pollen were equally fertile in the presence of B cytoplasm, whereas *Rf* pollen was 1.6 times more effective in the presence of S^v cytoplasm.

Like S^v cytoplasm, *Ae. uniaristata* cytoplasm caused almost complete male-sterility in three of the 12 common wheats, Slm, Splt and Mch, but leads to normal fertility in the others. The factorial basis of male sterility-fertility restoration was studied in the cross, Slm $\times$ Cmp (Tsunewaki, unpubl.), whereas aneuploid analyses were made with CS (Mukai, 1983a). Results of these analyses, shown in Table 5f, indicated that an *Rf* gene located on the 1BS arm of CS plays a major role in restoring fertility. This *Rf* factor is designated *Rfn1* (Mukai, 1983a). Frequency distributions of different fertility classes, in three cross combinations between Slm and Cmp are shown in Fig. 15B. A special remark is needed for interpreting these results. (M^u)-Slm maintains a heterozygosity for the normal 1B chromosome of CS and the 1BL-1RS translocation chromosome of Slm, in spite of repeated backcrosses using Slm (homozygous for the translocation chromosome) as the recurrent pollen parent. Heterozygosity was maintained by haploid

formation from practically all egg cells carrying the translocated chromosome in the presence of M^u cytoplasm, as described in a later section. Therefore, all F_1 plants from the $(M^u)\text{-Slm} \times \text{Cmp}$ cross are homozygous for the normal 1B chromosome, and hence show normal fertility. $(M^u)\text{-Slm} \times [(B)\text{-Slm} \times \text{Cmp}]$ progeny were expected to segregate 1B homozygotes and 1B/1BL-1RS heterozygotes in a 1:1 ratio, and this was supported by actual data. All plants from the cross, $(M^u)\text{-Slm} \times [(M^u)\text{-Slm} \times \text{Cmp}]$, were highly fertile, as expected from the heterozygosity of $(M^u)\text{-Slm}$ for 1B and 1BL-1RS chromosomes, and exclusive fertilization of egg cells carrying normal 1B chromosomes. On the same bases, only sterile plants were expected from the cross, $[(M^u)\text{-Slm} \times \text{Cmp}] F_1 \times \text{Slm}$, and, in fact, 96.4% of the plants were sterile. The rare occurrence (7 out of 193 plants) of fertile plants, however, can not be explained using the same assumptions.

Type II' fertility spectrum: Of the 12 common wheats, Slm, S615, Splt, Mch and Tve were completely to nearly completely sterile, whereas the other common wheats were fertile in the presence of *Ae. mutica* cytoplasm (code no. 13). The results of conventional and aneuploid analyses using male-sterile Slm and fertile CS are summarized in Table 5g (Tsujiimoto and Tsunewaki, 1984; Tsunewaki, unpubl.). Like $(M^u)\text{-Slm}$, $(Mt)\text{-Slm}$ is a permanent heterozygote for the 1B and 1BL-1RS chromosomes. Aneuploid analyses identified a major *Rf* gene on the 1BS arm of CS, and this gene was designated *Rfm1* (Tsujiimoto and Tsunewaki, 1984). In the factorial analysis, two crosses were carried out (Table 5g), but a third, critical cross, $(Mt)\text{-Slm} \times [(B)\text{-Slm} \times \text{Cmp}] F_1$, was not made. In the presence of Mt cytoplasm, *Rf* pollen is favored in fertilization, three to one over *rf* pollen. The cross, $[(Mt)\text{-Slm} \times \text{Cmp}] F_1 \times \text{Slm}$, was expected to give only sterile plants, but, in fact, more than 95% of the progeny were sterile. However, plants that were more than 30% fertile appeared at a frequency of about 5%. These plants can not be explained by the present hypothesis.

Type I' fertility spectrum: In N26 having a D^2 cytoplasm, pistillody results in deep male sterility (Fig. 11). Conventional and aneuploid analyses were carried out to determine the number of genes involved in pistillody induction and their chromosomal locations (Murai et al., 1990b). Frequency distributions of different fertility classes in three segregating populations, $(D^2)\text{-N26} \times \text{CS}$ F_2 , $(D^2)\text{-N26} \times [(B)\text{-N26} \times \text{CS}] F_1$, and $(D^2)\text{-N26} \times [(D^2)\text{-N26} \times \text{CS}] F_1$, are shown in Fig. 15C. The frequency distributions were continuous, ranging from a low of zero, to a high of almost 80%. If 20% fertility was designated as the break point between sterile and fertile, the F_2 population showed a 3:1 ratio of fertile:sterile. The ratio was 1:1 in both B_1 populations. These results indicated that a major dominant gene and some minor genes were involved in fertility restoration by CS against a D^2 cytoplasm, and that fertility restoration (or suppression of pistillody) was determined sporophytically. Two types of pollen, i.e., carriers and non-carriers, showed mild competition.

To determine the chromosomal location of the major gene, $(D^2)\text{-N26}$ was crossed

as female parent to ditelosomics and nullitetrasomics of CS. Selfed seed fertility of the F_1 plants grown under long day condition (15 h day length) was examined. The results suggested that the major gene was located on chromosome arm 7BL, and that the minor genes resided on several other chromosomes of CS (Murai et al., 1990b). The major gene was designated *Rfd1*.

Chromosomal locations and the characteristics of the *Rf* genes described above are summarized in Table 6. *Rf* genes for different plasmons, such as *Rf3*, *Rfu1*, *Rfv1*, *Rfn1* and *Rfm1*, are all located on the 1BS arm. Tests for allelism show that *Rf3* was not allelic to *Rfv1* (Hamawaki and Mukai, 1980), and that *Rfv1* and *Rfn1* were the same genes (Tsunewaki, unpubl.). It was not established whether these genes were allelic to the other two genes. Clearly, the male sterility-fertility restoration observed has the same genetic basis in some wheat genotype-alien plasmon combinations, and a different genetic base in other combinations.

Table 6. Chromosomal location and characteristics of *Rf* genes identified

Male sterile plasma	Fert. spectrum	Restorer/maintainer	Carrier chromosome					Nature of restor. ^a	Certation ^b	
			1B	2B	6B	7B	1D		Sterile plasma	Wheat plasma
G	VII	Splt/CS	<i>Rf3</i>					S	No	No
C	VI	Cmp/CS			<i>Rfc2</i>		<i>Rfc3</i>	—	—	No
M ^o	V	P168/CS					<i>Rfc1</i>	S	Do	Do
C ^u	IV	CS/Slm	<i>Rfu1</i>	<i>Rfu2</i>				S	Do	No
S ^v	II	CS/Splt	<i>Rfv1</i>					S	Do	No
M ^u	II	Cmp/Slm	<i>Rfn1</i>					—	—	No
Mt	II'	CS/Slm	<i>Rfm1</i>					—	—	—
D ²	I'	CS/N26				<i>Rfd1</i>		—	Do	—

^{b)} S for sporophytic nature of fertility restoration.

^{b)} Certation between *Rf* and *rf* type pollen grains.

Note 1) —: not tested.

Note 2) *Rfc2* and *Rfc3* are duplicated genes, whereas *Rfu1* and *Rfu2* are recessive complementary genes.

Two other points must be mentioned. First, pollen fertility was determined sporophytically in all wheat genotype-alien plasmon combinations so far tested. Secondly, the *Rf* pollen was favored in fertilization over *rf* pollen, to different degrees, in the presence of most male-sterile cytoplasms. The *Rf3* gene of Splt, in G cytoplasm, was the only exception to this. The *Rfc1* gene from *Ae. caudata* leads to preferential fertilization, even in common wheat cytoplasm.

(B) Haploid parthenogenesis

A common wheat Slm has an interesting history of its origination (Tsunewaki, 1964). Certain alloplasmic lines of this wheat frequently produce haploids and

Table 7. Haploid and twin seedling formation in alloplasmic lines of Salmon (Tsunewaki et al., 1974, 1976; Kobayashi and Tsunewaki, 1980b; Mukai, 1981; Siregar et al., 1987; Tsunewaki, unpubl.)

Cytoplasm		No. plants tested	Haploid		Twin pair	
Donor species (code)	Plasma type		No.	%	No.	%
<i>T. boeoticum</i> (01)	A	75	1	1.3	0	0.0
<i>T. monococcum</i> (16)	„	13	0	0.0	0	0.0
<i>T. dicoccoides</i> (21)	B	592	0	0.0	0	0.0
<i>T. dicoccum</i> (22)	„	27	0	0.0	0	0.0
<i>T. aestivum</i> (52)	„	510	0	0.0	0	0.0
<i>Ae. caudata</i> (02)	C	500	113	22.6	69	13.8
<i>Syn-triuncialis</i> (27)	„	51	24	47.1	4	7.8
<i>Ae. triuncialis</i> (38)	„	37	2	5.4	1	2.7
<i>Ae. umbellulata</i> (03)	C ^u	298	26	8.7	0	0.0
<i>Ae. triuncialis</i> (26)	„	308	92	29.9	15	5.1
<i>Ae. biuncialis</i> (29)	„	174	70	40.2	8	4.6
<i>Ae. triaristata</i> 4x (32, 37)	„	186	27	14.5	9	4.8
<i>Ae. triaristata</i> 6x (54, 57)	„	187	32	17.1	0	0.0
<i>Ae. columnaris</i> (30)	C ^{u2}	401	54	13.5	17	4.4
<i>Ae. squarrosa</i> (04)	D	277	0	0.0	0	0.0
<i>Ae. cylindrica</i> (28)	„	217	0	0.0	0	0.0
<i>Ae. ventricosa</i> (36)	„	110	0	0.0	0	0.0
<i>Ae. crassa</i> 4x (35)	D ²	168	0	0.0	0	0.0
<i>Ae. crassa</i> 6x (55)	„	262	0	0.0	2	0.8
<i>Ae. juvenalis</i> (53)	„	121	0	0.0	0	0.0
<i>Ae. vavilovii</i> (56)	„	164	0	0.0	0	0.0
<i>Ae. speltoides</i> (09)	G	103	0	0.0	1	1.0
<i>T. araraticum</i> (23, 24)	„	215	0	0.0	0	0.0
<i>T. timopheevi</i> (25)	„	197	0	0.0	0	0.0
<i>T. zhukovskyi</i> (51)	„	135	0	0.0	0	0.0
<i>Ae. comosa</i> (05)	M	145	0	0.0	1	0.7
<i>Ae. heldreichii</i> (06)	M ^h	202	0	0.0	0	0.0
<i>Ae. ovata</i> (31)	M ^o	236	0	0.0	0	0.0
<i>Ae. uniaristata</i> (07)	M ^u	215	29	13.5	1	0.5
<i>Ae. mutica</i> (13)	Mt	257	46	17.9	10	3.9
<i>Ae. mutica</i> (14)	Mt ²	143	29	20.3	6	4.2
<i>Ae. speltoides</i> (08, 17)	S	593	0	0.0	1	0.2
<i>Ae. bicornis</i> (12)	Sb	205	0	0.0	0	0.0
<i>Ae. sharonensis</i> (10)	S ¹	92	23	25.0	6	6.5
<i>Ae. longissima</i> (20)	S ¹²	14	4	28.6	1	7.1
<i>Ae. searsii</i> (18)	S ^v	442	111	25.1	9	2.0
<i>Ae. kotschyi</i> (33)	„	443	96	21.7	31	7.0
<i>Ae. variabilis</i> (34)	„	257	55	21.4	21	9.0

twin seedlings. As described below, this is due to haploid parthenogenesis induction by way of interactions between the nuclear genome of Slm and some *Aegilops* cytoplasms. The frequencies of haploid and twin seedlings in successive backcross generations of (*caudata*)-Slm remained almost constant for 20 generations, indicating that parthenogenesis induction was a stable, heritable trait (Tsunewaki and Mukai, 1990). Similarly, certain alloplasmic lines of Mch produced haploids (Kobayashi and Tsunewaki, 1980b). Due to hybrid chlorosis that occurred in F₁ hybrids between Mch and other common wheats, including CS, the mechanisms responsible for haploid production by alloplasmic Mch could not be studied.

Aegilops cytoplasms inducing haploid parthenogenesis in Salmon: Table 7 gives the frequencies of haploid and twin seedlings observed in alloplasmic lines of Slm. Cytoplasms tested were classified as inducers and non-inducers of haploid parthenogenesis. All cytoplasms belonging to the nine plasma types, C, C^u, C^{u2}, M^u, Mt, Mt², S^l, S^{l2} and S^v, induced haploid parthenogenesis, whereas those of ten plasma types, A, B, D, D², G, M, M^h, M^o, S and S^b, produced practically no haploid and twin seedlings. No *Triticum* cytoplasm induced parthenogenesis. Its inducers are scattered among different branches of the phylogenetic trees of both chloroplast and mitochondrial genomes (ref. Figs. 3 and 6), suggesting that the plasmagene mutated recurrently toward parthenogenesis induction during speciation in *Aegilops*.

Developmental mechanisms of haploid and twin formation: When emasculated flowers remain unpollinated, (*caudata*)-Slm showed parthenogenesis of egg cells in about half of the ovules (Tsunewaki et al., 1968). This implied that almost all egg cells carrying the 1BL-1RS translocated chromosome underwent parthe-

Table 8. Embryo and endosperm development in unpollinated and pollinated ovules of (*caudata*)-Salmon (Tsunewaki et al., 1968)

Time (day)	No. ovules observed	Ovule type (%)					
		Embryo Endosperm	— —	+	— +	+	Twin embryos
a) Unpollinated ^a							
1	99		99	1	0	0	0
4	172		81	19	0	0	0
7	154		58	42	0	0	0
b) Pollinated ^b							
1	134		12	11	2	75	14
4	122		2	28	0	70	5
7	76		1	26	0	72	0

^{a)} Date of fixation after flowering.

^{b)} Fixed 3 days after pollination made at the indicated date.

nogenesis, since (*caudata*)-Slm is a permanent heterozygote for chromosome 1B and the 1BL-1RS translocated chromosome. In unpollinated ovules, no twin embryos were formed, nor did polar nuclei divide to form endosperm (Table 8). In the embryo-sacs containing parthenogenetic embryos, one of the synergids became large, resembling a normal egg cell, whereas the polar nuclei remain undivided. Therefore, unpollinated flowers, in which ovules bear a parthenogenetic embryo, never develop to mature seed because of the lack of endosperm. If the flowers were pollinated, both embryo and endosperm developed in most ovules, although some contained an embryo only, and lacked endosperm (Table 8). In some ovules, twin embryos were formed. These observations indicated the following; (i) egg cells have a strong tendency to develop into an embryo parthenogenetically, (ii) twin embryos are formed only when pollination occurs, and (iii) in embryo-sacs carrying the parthenogenetic embryo, one synergid occasionally develops into a cell resembling an egg cell.

Chromosome counts revealed that more than 90% of the twins were haplo-diplo types (Table 9). At the time of germination, most twins were different in size. In 80% of the haplo-diplo twins, the haploid twin was larger than the diploid twin. This indicates that the haploid embryo begins development earlier than its diploid twin in the same ovule. These observations suggest the following developmental mechanisms for haploid and twin formation in alloplasmic Slm; (i) some egg cells begin haploid parthenogenesis prior to fertilization, (ii) when polar nuclei are fertilized, the haploid embryo develops normally and produces a haploid seedling, owing to normal endosperm development, and (iii) occasionally, a synergid in the embryo-sac carrying the parthenogenetic embryo is fertilized, resulting in haplo-diplo twin embryos. The primary cause of haplo-diplo twin formation is haploid parthenogenesis of the egg cell. Thus, the alien cytoplasms which induce haploid and twin seedlings in Slm are regarded as the parthenogenesis-inducing cytoplasm.

If the above mechanism is correct, then twins from the same seed should receive the same chromosome (and gene) complement from the female parent,

Table 9. Classification of twin seedlings of (*caudata*)-Salmon by ploidy and size of the twins (Tsunewaki et al., 1968)

Size	Ploidy			Total no. (%)
	n-2n	2n-2n	n-n	
Large-large	3	0	0	3 (3.7)
Large-small	51	3	2	70 (86.4)
Small-large	14			
Small-small	7	0	1	8 (9.9)
Total (%)	75 (92.5)	3 (3.7)	3 (3.7)	81 (100.0)

since all the egg cell and synergids are produced through mitotic divisions of a single tetrad. To prove this, a genetic test was made, using the following three marker genes: *Hg* for glume hairiness on chromosome arm 1AS, ca. 50 map units from the centromere; *Hp* for peduncle hairiness on 4BS (following the new designation), ca. 30 map units from the centromere; and *C* for compact ear on 2DL, ca. 2 map units from the centromere. (*Caudata*)- or (*kotschyi*)-*Slm* was crossed as female parent to a wheat strain with genotype, *HgHgHpHpCC*, where *Slm* was triple recessive, *hghghpcc*. The F_1 hybrids, heterozygous for all three allele pairs, were testcrossed with pollen from the triple recessive. Among the testcross progeny, 22 twin pairs were obtained. All of the expected eight phenotypes were observed among these twin pairs, but twins from the same seed always showed the same phenotype (Fig. 16). The fact that the 22 twin pairs exhibited identical phenotypes, and the fact that their ploidy was haplo-diplo in the majority of cases, verified that the twins derived from two different cells of the same embryo-sac.

Fig. 16. Selected twin pairs showing eight possible phenotypes for three segregating allele pairs, *Hg/hg*, *Hp/hp*, and *C/c*. Note both members of all twin pairs have identical phenotypes (Tsunewaki and Mukai, 1990).

Genetic mechanism for parthenogenesis induction: The involvement of the 1BL-1RS translocation in *Slm* as the major nuclear factor for parthenogenesis induction was first proven by Kobayashi and Tsunewaki (1980a), using complete

Table 10. Haploid and twin seedling formation in various aneuploids from the crosses, (*kotschyi*)-Salmon x CS aneuploids (Mukai and Nakanishi, 1982)

Line ^a	2n	Offspring		Frequency (%)		
		Category	No. plants	Diploid	Haploid	Twins
(<i>kot</i>)-20'' + T''	42	—	125	10	54	37
(<i>kot</i>)-20'' + T'	41	{ with T	37	57	24	19
		{ without T	95	66	14	20
(<i>kot</i>)-20'' + 1BL''	42	—	41	100	0	0
(<i>kot</i>)-20'' + 1BS''	42	—	43	100	0	0
(<i>kot</i>)-20'' + T'' + 1BS''	44	{ with 1BS	78	100	0	0
		{ without 1BS	6	0	50	50
(<i>kot</i>)-20'' + T'' + 1BS'	43	{ with 1BS	59	100	0	0
		{ without 1BS	95	3	56	41

^a) T: 1BL-1RS translocation chromosome, L and S: long and short chromosome arm, respectively.

linkage between the translocation chromosome and the *rfv1* gene for male sterility induction by S^v cytoplasm. An important question was whether the deletion of the 1BS arm or the presence of the 1RS arm, or both, are necessary for induction of parthenogenesis. To clarify this and other points, Mukai and Nakanishi (1982) produced various aneuploids by crossing (*kotschyi*)-Slm to CS aneuploids, and examined haploid formation in the progeny plants (Table 10). The results indicate the following: (i) the 1RS arm carries a gene for parthenogenesis induction, sporophytically determined, and (ii) the 1BS arm carries a gene that suppresses the parthenogenesis induced by the gene on the 1RS arm; suppression is gametophytically determined. The gene for haploid parthenogenesis induction on the 1RS arm is designated *Ptg*, for parthenogenesis, whereas its suppressor on the 1BS arm is named *Spg*, for suppression of parthenogenesis. These results indicate that haploid parthenogenesis resulting in haploid and haplo-diplo twin formation depends on (i) the presence of a parthenogenesis-inducing cytoplasm, (ii) the nuclear gene, *Ptg*, located on the 1RS arm, and (iii) the absence of another nuclear gene, *Spg*, on the 1BS arm.

Conversion of a common wheat cultivar to haploid-producer: These results also indicate that any common wheat cultivar can be converted to a haploid producer by cotransfer of a parthenogenesis-inducing cytoplasm and the 1BL-1RS translocation chromosome of Slm. As a model case, CS has been converted to a haploid producer using (*kotschyi*)-Slm. First, (*kotschyi*)-Slm was crossed to CS, and n-2n twin seedlings were selected in the F₁ generation. The 2n twins were backcrossed, with CS pollen, and n-2n twin seedlings were selected in the B₁ generation; the 2n twins were backcrossed again with CS pollen. The n-2n type twinning is used as a marker for the 1BL-1RS chromosome. These steps, namely, selection of n-2n twin seedlings, and backcrosses of 2n twins with CS

Table 11. Frequency of haploids in the backcross generations, (*kotschy*)-Salmon $\times$ Chinese Springⁿ

Generation ^a	No. of plants		% Haploids
	Total	Haploid ^b	
F ₁	1209	304	25.1
B ₁	1314	50	3.8
B ₄	912	39	4.3
B ₅	717	67	9.3
Control	790	0	0.0

^a) Control is (*kotschy*)-CS.

^b) Includes haplo-diplo twins.

Note) In backcrosses, diploids of n-2n twins were used as the female parent.

pollen were repeated until the B₅ generation. The frequencies of haploids produced in the different backcross generations are given in Table 11.

The results demonstrate that the parthenogenesis-inducing *Aegilops* cytoplasm and the 1BL-1RS translocation chromosome of SIm can be used, efficiently to produce haploid common wheat. This method does not rely on tissue cultures, and should, therefore, be exploited for haploid breeding in common wheat.

Acknowledgements: The present works have been achieved with faithful collaboration of many coworkers besides those mentioned in the reference. Particular thanks are due to late Dr. Y. Nakai, Mr. R. Matsuo and Miss N. Fujita for their immense help in different phases of the present investigation. The author wishes to express his greatest appreciation to Dr. Val W. Woodward, University of Minnesota, USA, for his continuous encouragement and kind review of the present paper with valuable advice on its improvement. The works have been performed with research grants from various sources; from the Ministry of Education, Science and Culture, the Ministry of Agriculture, Forestry and Fishery, the Japan Society for the Promotion of Science, the Foundation for Genetics Promotion, Hamamatsu, the Food Life Research Foundation, Nisshin Milling Co., Zen-no, and Hokuren, to all of which the author is highly indebted.

REFERENCES

- Fukasawa, H. (1959). Nucleus substitution and restoration by means of successive backcrosses in wheat and its related genus *Aegilops*. *Jpn. J. Bot.* **17**, 55–91.
- Hamawaki, H. and Mukai, Y. (1980). Telocentric mapping of the fertility-restoring gene *Rfv1* against *Aegilops variabilis* cytoplasm in wheat. *Jpn. J. Genet.* **55**, 453.
- Kihara, H. (1951). Substitution of nucleus and its effects on genome manifestations. *Cytologia* **16**, 177–193.
- Kihara, H. (1959). Fertility and morphological variation in the substitution backcrosses of the hybrid *Triticum vulgare* $\times$ *Aegilops caudata*. *Proc. X Int. Congr. Genet.* **1**, 142–171.
- Kihara, H. and Kondo, N. (1943). Studies on amphidiploids of *Aegilops caudata* $\times$ *Ae. umbellulata* induced by colchicine (in Japanese). *Seiken Ziho* **2**, 24–42.

- Kihara, H. and Tanaka, M. (1970). Addendum to the classification of the genus *Aegilops* by means of genome-analysis. *Wheat Inform. Serv.* **30**, 1-2.
- Kihara, H. and Tsunewaki, K. (1961). Pistillody of *Triticum durum* induced by an alien cytoplasm. *Seiken Ziho* **12**, 1-10.
- Kobayashi, M. and Tsunewaki, K. (1980a). Haploid induction and its genetic mechanism in alloplasmic common wheat. *J. Heredity* **71**, 9-14.
- Kobayashi, M. and Tsunewaki, K. (1980b). Production of an effective haploid inducer utilizing the *kotschy* and *variabilis* cytoplasm. In: *Genetic diversity of the cytoplasm in Triticum and Aegilops* (ed.: K. Tsunewaki), pp. 250-265. Japan Soc. Promot. Sci., Tokyo.
- Kondo, N. (1941). Chromosome doubling in *Secale*, *Haynaldia* and *Aegilops* by colchicine treatment (in Japanese). *Jpn. J. Genet.* **17**, 46-54.
- Lilienfeld, F. A. (1951). H. Kihara: Genome-analysis in *Triticum* and *Aegilops*. X. Concluding review. *Cytologia* **16**, 101-123.
- Michaelis, P. (1954). Cytoplasmic inheritance in *Epilobium* and its theoretical significance. *Adv. Genet.* **6**, 288-401.
- Mori, N., Terachi, T. and Tsunewaki, K. (1988). Organellar genome differentiation in wild tetraploid wheats, *Triticum dicoccoides* and *T. araraticum*. *Proc. VII Int. Wheat Genet. Symp. Cambridge* **1**, 109-114.
- Mukai, Y. (1981). Genetic studies of the wheat haploids induced by alien cytoplasm. *Mem. Osaka Kyoiku Univ. III* **30**, 31-55.
- Mukai, Y. (1983a). Determination of the chromosome arm carrying a male fertility-restoring gene against the cytoplasm of *Aegilops uniariastata* in wheat. *Mem. Osaka Kyoiku Univ. III* **32**, 43-53.
- Mukai, Y. (1983b). Interactions of *Aegilops kotschy* and *Ae. variabilis* cytoplasm with homoeologous group 1 chromosomes in common wheat. *Proc. VI Int. Wheat Genet. Symp. Kyoto*, pp. 517-527.
- Mukai, Y. (1985). Production of haploid plants by genetic methods (in Japanese). *Saibo* **17**, 150-155.
- Mukai, Y. and Nakanishi, S. (1982). Genetic mechanism of parthenogenesis in common wheat with an alien cytoplasm (Abstr.). *Jpn. J. Genet.* **57**, 665.
- Mukai, Y. and Tsunewaki, K. (1976). Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. IV. Distribution of the cytoplasm inducing variegation in common wheat. *Theor. Appl. Genet.* **48**, 9-16.
- Mukai, Y. and Tsunewaki, K. (1979). Basic studies on hybrid wheat breeding. VIII. A new male sterility-fertility restoration system in common wheat utilizing the cytoplasm of *Aegilops kotschy* and *Ae. variabilis*. *Theor. Appl. Genet.* **54**, 153-160.
- Mukai, Y., Kitahata, Y., Kobayashi, Y. and Ohtsuka, I. (1985). Polymorphism of sat-chromosomes derived from *Aegilops caudata* in common wheat (Abstr.). *Jpn. J. Genet.* **60**, 615.
- Murai, K. (1992). Basic studies on hybrid wheat breeding utilizing *Aegilops crassa* cytoplasm. Doctor Thesis, Kyoto University, pp. 102.
- Murai, K. and Tsunewaki, K. (1986). Molecular basis of genetic diversity among cytoplasm of *Triticum* and *Aegilops* species. IV. CtDNA variation in *Ae. triuncialis*. *Heredity* **57**, 335-339.
- Murai, K., Tsuji, S., Hirohara, H., Ohtsuka, I. and Tsunewaki, K. (1988). Breeding of hybrid wheat using photoperiod-sensitive cytoplasmic male sterility (PCMS). 1. Breeding system of hybrid wheat (in Japanese). *Jpn. J. Breed. Suppl.* **38**(2), 284-285.
- Murai, K., Nishikawa, A., Tsuji, S., Hirohara, H. and Tsunewaki, K. (1990). Genetic analysis on the fertility restoration by *Triticum aestivum* cv. Chinese Spring against *Aegilops crassa* cytoplasm (in Japanese). *Jpn. J. Genet.* **65**, 449-450.
- Muramatsu, M. (1959). Homology of chromosomes of *Aegilops caudata* with common wheat. *Wheat Inform. Serv.* **9-10**, 32-33.

- Nei, M. (1987). *Molecular evolutionary genetics*. Columbia Univ. Press, New York. pp. 106–107.
- Nishikawa, K. (1989). Genetic and breeding study on amylase isozymes in wheat and its relatives (in Japanese). *Jpn. J. Breed. Suppl.* **39**(1), 2–5.
- Ogihara, Y. and Tsunewaki, K. (1982). Molecular basis of the genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. I. Diversity of the chloroplast genome and its lineage revealed by the restriction pattern of ctDNAs. *Jpn. J. Genet.* **57**, 371–396.
- Ogihara, Y. and Tsunewaki, K. (1988). Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. *Theor. Appl. Genet.* **76**, 321–332.
- Sears, E. R. (1954). The aneuploids of common wheat. *Res. Bull. Mo. Agr. Exp. Stat.* No. 572, pp. 59.
- Sears, E. R. (1966). Nullisomic-tetrasomic combinations in hexaploid wheat. In: *Chromosome manipulations and plant genetics* (eds.: R. Riley and K. R. Lewis, pp. 29–45. Oliver & Boyd, Edinburgh.
- Sears, E. R. and Sears, L. M. S. (1978). The telocentric chromosomes of common wheat. *Proc. V Int. Wheat Genet. Symp. New Delhi* **1**, 389–407.
- Siregar, U. J., Ishii, T. and Tsunewaki, K. (1988). *Aegilops searsii* is a possible cytoplasm donor to *Ae. kotschyi* and *Ae. variabilis*. *Proc. VII Int. Wheat Genet. Symp. Cambridge* **1**, 145–151.
- Sneath, P. H. A. and Sokal, R. R. (1973). *Numerical taxonomy*. Freeman, San Francisco. pp. 573.
- Tahir, C. M. and Tsunewaki, K. (1969). Monosomic analysis of *Triticum spelta* var. *duhamelianum*, a fertility restorer for *T. timopheevi* cytoplasm. *Jpn. J. Genet.* **44**, 1–9.
- Tahir, C. M. and Tsunewaki, K. (1971). Monosomic analysis of fertility restoring genes in *Triticum aestivum* strain P168. *Can. J. Genet. Cytol.* **13**, 14–19.
- Terachi, T. and Tsunewaki, K. (1986). The molecular basis of genetic diversity among cytoplasm of *Triticum* and *Aegilops*. 5. Mitochondrial genome diversity among *Aegilops* species having identical chloroplast genomes. *Theor. Appl. Genet.* **73**, 175–181.
- Terachi, T. and Tsunewaki, K. (1992). The molecular basis of genetic diversity among cytoplasm of *Triticum* and *Aegilops*. VIII. Mitochondrial RFLP analyses using cloned genes as probes. *Molec. Biol. Evol.* **9**, 917–931.
- Terachi, T., Kataoka, J. and Tsunewaki, K. (1985). Intraspecific variation of organellar DNAs in *Aegilops squarrosa* (in Japanese). *Jpn. J. Breed. Suppl.* **35**(2), 194–195.
- Terachi, T., Ogihara, Y. and Tsunewaki, K. (1990). The molecular basis of genetic diversity among cytoplasm of *Triticum* and *Aegilops*. 7. Restriction endonuclease analysis of mitochondrial DNAs from polyploid wheats and their ancestral species. *Theor. Appl. Genet.* **80**, 366–373.
- Tsujimoto, H. and Tsunewaki, K. (1984). Chromosome location of a fertility-restoring gene of a common wheat Chinese Spring for the *Ae. mutica* cytoplasm. *Wheat Inform. Serv.* **58**, 4–8.
- Tsunewaki, K. (1964). Genetic studies of a 6x-derivative from an 8x triticales. *Can. J. Genet. Cytol.* **6**, 1–11.
- Tsunewaki, K. (1974). Monosomic analysis of two restorers to *Ae. caudata* and *Ae. umbellulata* cytoplasm. *Jpn. J. Genet.* **49**, 425–433.
- Tsunewaki, K. (ed.) (1980). *Genetic diversity of the cytoplasm in Triticum and Aegilops*. Japan Soc. Promot. Sci., Tokyo. pp. 290.
- Tsunewaki, K. (1982). Monosomic analysis on the fertility restoration by *Triticum aestivum* cv. Chinese Spring against *Aegilops ovata* cytoplasm. *Jpn. J. Genet.* **57**, 513–525.
- Tsunewaki, K. (1989). Plasmon diversity in *Triticum* and *Aegilops* and its implication in wheat evolution. *Genome* **31**, 143–154.
- Tsunewaki, K. and Mukai, Y. (1990). Wheat haploids through the Salmon method. In: *Biotechnology in agriculture and forestry*. Vol. 13 (ed.: Y. P. S. Bajaj), pp. 460–478. Springer-Verlag, Berlin.
- Tsunewaki, K. and Tsujimoto, H. (1983). Genetic diversity of the cytoplasm in *Triticum* and

- Aegilops*. *Proc. VI Int. Wheat Genet. Symp. Kyoto*, pp. 1139–1144.
- Tsunewaki, K., Noda, K. and Fujisawa, T. (1968). Haploid and twin formation in a wheat strain Salmon with alien cytoplasms. *Cytologia* **33**, 526–538.
- Tsunewaki, K., Endo, T. R. and Mukai, Y. (1974). Further discovery of alien cytoplasms inducing haploids and twins in common wheat. *Theor. Appl. Genet.* **45**, 104–109.
- Tsunewaki, K., Mukai, Y., Endo, T. R., Tsuji, S. and Murata, M. (1976). Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. VI. Distribution of the haploid-inducing cytoplasms. *Jpn. J. Genet.* **51**, 193–200.