REVIEW

Genome-plasmon interactions in wheat*

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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. araratium* (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 (*BamHI, HindIII, SmaI* and *XhoI*) 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

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each from Iraq and Israel, had the same 50 bp insertion, whereas the third one, from Israel, possessed an additional HindIII site that cleaved one of the 8.2 kb HindIII 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 BamHI 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, Transcaucasia. 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 Transcaucasia, by mutations, from Ae. caudata or Ae. umbellulata.

Ae. speltoides (including Ae. auecheri) 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

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<th>Species</th>
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<th>Ct genome</th>
<th>Mt genome</th>
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a) After Kihara (ref. Lilienfeld, 1951) and Kihara and Tanaka (1970).
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, *BamHI, BglII, HindIII, KpnI, PstI, PvuI, PvuII, SalI, SacI, SmaI, SstI, XbaI* and *XhoI*, all being six-base cutters (Ogihara and Tsunewaki, 1982, 1988). As an example, *BamHI*-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).](image-url)
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 (Oghihara and Tsunewaki, 1982, 1988). This is because the previous 1a, 1b, 1c and 1d genomes, which gave identical restriction fragment patterns with four endonucleases (Oghihara 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, BamHI, HindIII, PstI, PvuII and XhoI (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

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</table>

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. sharonenensis* 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

![Image of Southern hybridization patterns](image.png)

**Fig. 5.** Southern hybridization patterns of BamHI-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 \textit{atpA} and \textit{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, \textit{BamHI} and \textit{HindIII} 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 \textit{Triticum} and \textit{Aegilops} species representing all 17 plastomes (given in parentheses), constructed by cluster analysis, using Nei's genetic distances (Terachi and Tsunewaki, 1992).](image)

From the results of the restriction and Southern analyses, chondriomes of \textit{Triticum} and \textit{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 \textit{Triticum} and \textit{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) \textit{Ae. speltoides}, the timopheevi wheat group, and common wheat group (emmer wheat group, inclusively), (iii) diploid species of the section
Comopyrum of *Aegilops*, and (iv) all other species. However, many minor differences were found between the two phylogenetic trees, which were assumed to be due to a limited number of probe-enzyme combinations used in the study of chondriome differentiation, and, in part, to different rates of evolutionary changes between plastomes and chondriomes in some branches of the phylogenetic tree.

Considering the differences within plastomes and chondriomes, 18 types of plasmon (plasma type) were distinguished among *Triticum* and *Aegilops* species; these are shown in Table I. Among these 18 plasmons, 14 were found among diploid species. This fact supports the view that most plasmon divergence had taken place at the diploid level. Four plasmons, B, Cu², D², and M⁰, were found only among tetraploids, never among diploids. All of the plasmons found within hexaploid species also existed in tetraploids, suggesting that none of the plasmons had differentiated at the hexaploid level.

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 M⁰ appear to have originated from *Ae. speltoides* and *Ae. mutica*, respectively (ref. Figs. 3 and 6). It is likely that the Cu² plasmon originated from a Cu 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.

![Diagram showing genome-plasmon interactions in wheat](image-url)

**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 cytoplasms by a two-digit code number (Table 1). When preferred, cytoplasms are identified by abbreviated

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

<table>
<thead>
<tr>
<th>Species and lower taxon</th>
<th>Code</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. aestivum var. erythrospermum</td>
<td>A</td>
<td>Tve</td>
</tr>
<tr>
<td>T. aestivum strain P168</td>
<td>B</td>
<td>P168</td>
</tr>
<tr>
<td>T. aestivum cv. Chinese Spring</td>
<td>C</td>
<td>CS</td>
</tr>
<tr>
<td>T. aestivum cv. Norin 26</td>
<td>D</td>
<td>N26</td>
</tr>
<tr>
<td>T. aestivum strain Salmon</td>
<td>E</td>
<td>SIm</td>
</tr>
<tr>
<td>T. aestivum cv. Jones Fife</td>
<td>F</td>
<td>JF</td>
</tr>
<tr>
<td>T. aestivum cv. Selkirk</td>
<td>G</td>
<td>Sk</td>
</tr>
<tr>
<td>T. aestivum cv. S-615</td>
<td>H</td>
<td>S615</td>
</tr>
<tr>
<td>T. sphaerococcum var. rotundatum</td>
<td>I</td>
<td>Sphr</td>
</tr>
<tr>
<td>T. compactum cv. No. 44</td>
<td>J</td>
<td>Cmp</td>
</tr>
<tr>
<td>T. spelta var. dukamelianum</td>
<td>K</td>
<td>Splt</td>
</tr>
<tr>
<td>T. macha var. subtletschchumicum</td>
<td>L</td>
<td>Meth</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Backcross generation*</th>
<th>Tve</th>
<th>P168</th>
<th>CS</th>
<th>N26</th>
<th>SIm</th>
<th>JF</th>
<th>Sk</th>
<th>S615</th>
<th>Sphr</th>
<th>Cmp</th>
<th>Splt</th>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
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<td>0</td>
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<td>5–9</td>
<td>6</td>
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<td>3</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6</td>
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<td>4</td>
<td>4</td>
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<td>2</td>
<td>3</td>
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<td>5</td>
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<td>46</td>
<td>46</td>
<td>552</td>
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</tr>
</tbody>
</table>

*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, Ogihara and Tsunewaki, 1988). These facts indicate that the plasmon of *Ae. caudata* did not change in the presence of nuclear genomes, C° 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 cytoplasmas 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 cytoplasms is male sterility. In Fig. 10, the average backcrossed and selfed seed fertilities of all alloplasmic lines are shown. The cytoplasms are 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 $S'$ 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 cytoplasms (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 cytoplasms (39 alien cytoplasms and one self cytoplasm) were grown in a split plot design, with four replications and 12 nuclei arranged in the main plots, and 40 cytoplasms 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, pistil lody and malformations other than pistil lody, 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 cytoplasms, and interactions between nuclei and cytoplasms, 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 cytoplasms in different ways. Such specific interactions between wheat genotypes and alien plasmons are clearly
Nucleus

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 cytoplasms (in parentheses) classified into each group are also shown. Data from the A plasmom are not available for some nuclei (open column) because the plants with this plasmom 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 cytoplasms 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, (alloplasmics-euplasmics)/euplasmics×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 $M^p$ plasmons. From these observations it seems clear that functional divergence among plasmons was paralleled by molecular divergence of their plastomes and chondriomes.
(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 cytoplasms, 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
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 cytoplasms 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° cytoplasms. 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 plasmogenes) 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°, and C°a2 cytoplasms in nine of the 12 common wheats used. The A cytoplasm showed the most severe variegation, followed by the C° cytoplasm. The cause of the variegation is a reduction (about 50% in the case of C° cytoplasm) in both chlorophyll a and b. Variegation disappears during spring time, with raising temperatures (Mukai and Tsunewaki, 1976). JF, Spilt and Meh 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. Cytoplasms C, G, M₀, Mt₂, S, Sᵇ and Sʲ caused 10–20% reduction (average for the 12 common wheats), whereas Cⁿ, Cⁿᵈ, Mʰ and S¹ cytoplasms resulted in 30–40% reduction in dry matter by the time of harvest. The reduction caused by A and M cytoplasms was greater than 60%. Growth depression caused by cytoplasms A, Cⁿ and Cⁿᵈ 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 Mⁿ cytoplasms did not alter the dry matter in any nuclear genotype.

The interactions, expressed on dry matter, between wheat genotypes and alien cytoplasms were different, in particular the interactions involving Mt, M₀ and Mʰ cytoplasms. 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. Mⁿ cytoplasm resulted in a 10% increase of dry matter in CS and Sphr, but a 10–40% decrease in most of the other wheats. Mʰ 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, Cⁿ, Cⁿᵈ, M, Mʰ, M₀, Mt, and S¹ cytoplasms caused some delay in heading, whereas the other cytoplasms did not (ref. Fig. 12). Cⁿ, Cⁿᵈ and S¹ cytoplasms resulted in 4–5 day, C cytoplasms 7 day, and Mⁿ and Mt cytoplasms 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 S¹ cytoplasm-JF nuclear combination showed extreme delay, but S¹ with Tve and Sk showed less delay. Cⁿ and Cⁿᵈ cytoplasms caused much more heading delay in Slm and S615 than in the other wheats; M and Mʰ cytoplasms in P168 and JF showed greater delay than they did in other wheats, but no delay in CS. Mt and Mⁿ cytoplasms caused twice as much delay in Slm, Spīt, 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

Cytoplasms 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 Spit 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 Spilt (Table 5a) indicated that this wheat carried a single dominant gene, designated Rfβ, located on chromosome 1B (Tahir and Tsunewaki, 1969). A G cytoplasm-Rfβ gene interaction allowed transmission of Rfβ and rβ 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 monosomes (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'), *Ae. umbellulata* (C'), *Ae. kotschyi* and *Ae. variabilis* (both S') cytoplasm (Tahir and Tsunewaki, 1969, 1971; Tsunewaki, 1974, unpubl.; Mukai and Tsunewaki, 1979; Tsujimoto and Tsunewaki, 1984)

<table>
<thead>
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<th>Cross combination*</th>
<th>No. offspring</th>
<th>% Fertiles (Ratio tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fertile</td>
<td>Sterile</td>
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<tr>
<td>a) <em>T. timopheevi</em> cytoplasm (G type)</td>
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**Genome-plasmon interactions in wheat**

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</tr>
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<td>(C)-Cmp x (CS mono-1D x Cmp)mono F1</td>
<td>40</td>
<td>2</td>
</tr>
<tr>
<td>(C)-Cmp x (CS other monos. x Cmp)mono F1's</td>
<td>881</td>
<td>291</td>
</tr>
<tr>
<td><strong>c)</strong> <em>Ae. ovata</em> cytoplasm (M° type)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(M°)-Rreff x rreff</td>
<td>857</td>
<td>850</td>
</tr>
<tr>
<td>(M°)-Rreff x (M°)-Rreff</td>
<td>342</td>
<td>26</td>
</tr>
<tr>
<td>(M°)-rreff x (CS mono-1D x P168)mono F1</td>
<td>108</td>
<td>6</td>
</tr>
<tr>
<td>(M°)-rreff x (CS other monos. x P168)mono F1's</td>
<td>2105</td>
<td>605</td>
</tr>
<tr>
<td><strong>d)</strong> <em>Ae. umbellulata</em> cytoplasm (C° type)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C°)-Slm x (CS mono-1B x Slm)mono F1</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>(C°)-Slm x (CS mono-2B x Slm)mono F1</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>(C°)-Slm x (CS other monos. x Slm)mono F1's</td>
<td>76</td>
<td>257</td>
</tr>
<tr>
<td><strong>e)</strong> <em>Ae. kotschyi</em> and <em>Ae. variabilis</em> cytoplasmas (S° type)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S°)-Spltt x CS</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>(S°)-Spltt x CS dt-1BS</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>(S°)-Spltt x CS dt-1BL</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>[(S°)-Spltt x CS] F1 x (Spltt x CS) F1</td>
<td>170</td>
<td>62</td>
</tr>
<tr>
<td>[(S°)-Spltt x CS] F1 x [(S°)-Spltt x CS] F1</td>
<td>239</td>
<td>40</td>
</tr>
<tr>
<td><strong>f)</strong> <em>Ae. uniaristata</em> cytoplasm (M° type)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(M°)-Slm: 1B/1BL-1RS</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>(M°)-Cmp: 1B/1B</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>(M°)-Slm x Cmp: 1B/1B</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>(M°)-Slm x (Slm x Cmp) F1</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>(M°)-Slm x [(M°)-Slm x Cmp] F1</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>[(M°)-Slm x Cmp] F1 x Slm</td>
<td>7</td>
<td>186</td>
</tr>
<tr>
<td>(M°)-CS: 1B/1B</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>(M°)-CS dt-1BL: 1BL/1BL</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td><strong>g)</strong> <em>Ae. mutica</em> M cytoplasm (Mt type)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Mt)-CS: 1B/1B</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>(Mt)-Slm: 1B/1BL-1RS</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>[(Mt)-Slm x Cmp] F1 x Slm</td>
<td>8</td>
<td>154</td>
</tr>
<tr>
<td>(Mt)-Slm x [(Mt)-Slm x Cmp] F1</td>
<td>108</td>
<td>32</td>
</tr>
<tr>
<td>(Mt)-Slm x CS: 1B/1B</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>(Mt)-Slm x CS dt-1BL: 1B/1BL</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

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

a) Mono; monosomic line, dt; ditelesomic 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×P168, and N26×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-stereile. An \(Rf\) gene in P168 is dominant, in that (M\(^o\))-N26×P168 \(F_1\)'s were highly fertile. From the segregation ratio of fertile and sterile plants in the \(B_1\) generation, (M\(^o\))-Rfrf×(B)-rfrf, it was clear that P168 carried one dominant \(Rf\) allele, designated as \(Rfct\) (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\(^o\) type), and B) Ae. uniaristata (M\(^o\)), and C) Ae. crassa (D\(^o\) type) cytoplasm.](image-url)
tion, whereas Tve, N26, Slm, Sphr, Splt and Mch exhibited complete or almost complete sterility in combination with C\textsuperscript{u} and C\textsuperscript{u2} plasmons. Monosomic analysis of (C\textsuperscript{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 \textit{Rfu1} and \textit{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\textsuperscript{u})-Splt × [(C\textsuperscript{u})-Splt × JF], a few completely male sterile plants were recovered, indicating a sporophytic fertility restoration. In the cross, (C\textsuperscript{u})-Splt × [(B)-Splt × 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\textsuperscript{u})-Splt × JF] F\textsubscript{1} was the pollen parent. It was clear from the shift of the distribution curve, in comparing the cross, (C\textsuperscript{u})-Splt × [(C\textsuperscript{u})-Splt × JF] F\textsubscript{1}, with that of (C\textsuperscript{u})-Splt × [(B)-Splt × JF] F\textsubscript{1}, that the advantage of \textit{Rf} over \textit{rf} pollen widened 2.4 times in C\textsuperscript{u} cytoplasm compared to B cytoplasm. Only one fertile plant was found among 178 progeny plants, from the cross, [(C\textsuperscript{u})-Splt × JF] F\textsubscript{1} × Splt, suggesting that egg cells carrying the \textit{Rf} genes of JF, in the presence of C\textsuperscript{u} cytoplasm, are infertile.

\textit{Type II fertility spectrum:} In conjunction with S\textsuperscript{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 × Splt cross combination (Mukai and Tsunewaki, 1979). Ditelosomic analysis revealed that the 1BS arm of CS carries a dominant \textit{Rf} gene, designated \textit{Rfu1} (Table 5e). The F\textsubscript{2} ratio supported this finding. Upon selfing the F\textsubscript{1} hybrid, [(S\textsuperscript{v})-Splt × CS], completely male sterile plants were recovered, indicating that fertility restoration is sporophytic. From Table 5e, it was concluded that \textit{Rf} and \textit{rf} pollen were equally fertile in the presence of B cytoplasm, whereas \textit{Rf} pollen was 1.6 times more effective in the presence of S\textsuperscript{v} cytoplasm.

Like S\textsuperscript{v} cytoplasm, \textit{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 × Cmp (Tsunewaki, unpubl.), whereas aneuploid analyses were made with CS (Mukai, 1983a). Results of these analyses, shown in Table 5f, indicated that an \textit{Rf} gene located on the 1BS arm of CS plays a major role in restoring fertility. This \textit{Rf} factor is designated \textit{Rfu1} (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\textsuperscript{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\textsuperscript{a} cytoplasm, as described in a later section. Therefore, all F\textsubscript{1} plants from the (M\textsuperscript{a}-Slm × Cmp) cross are homozygous for the normal 1B chromosome, and hence show normal fertility. (M\textsuperscript{a})-Slm × [(B)-Slm × 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\textsuperscript{a})-Slm × [(M\textsuperscript{a})-Slm × Cmp], were highly fertile, as expected from the heterozygosity of (M\textsuperscript{a})-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\textsuperscript{a})-Slm × Cmp] F\textsubscript{1} × 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 I\textsuperscript{'} fertility spectrum:* Of the 12 common wheats, Slm, S615, Spilt, 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 (Tsujimoto and Tsunewaki, 1984; Tsunewaki, unpubl.). Like (M\textsuperscript{a})-Slm, (Mt)-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* (Tsujimoto and Tsunewaki, 1984). In the factorial analysis, two crosses were carried out (Table 5g), but a third, critical cross, (Mt)-Slm × [(B)-Slm × Cmp] F\textsubscript{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)-Slm × Cmp] F\textsubscript{1} × 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\textsuperscript{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\textsuperscript{2})-N26 × CS F\textsubscript{2}, (D\textsuperscript{2})-N26 × [(B)-N26 × CS]F\textsubscript{1}, and (D\textsuperscript{2})-N26 × [(D\textsuperscript{2})-N26 × CS] F\textsubscript{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\textsubscript{2} population showed a 3:1 ratio of fertile:sterile. The ratio was 1:1 in both B\textsubscript{1} populations. These results indicated that a major dominant gene and some minor genes were involved in fertility restoration by CS against a D\textsuperscript{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\textsuperscript{2})-N26 was crossed
as female parent to ditelosomics and nullitetrasomics of CS. Selfed seed fertility of the F₁ 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, Rfυ1, Rfu1 and Rfu1, are all located on the 1BS arm. Tests for allelism show that Rf3 was not allelic to Rfυ1 (Hamawaki and Mukai, 1980), and that Rfυ1 and Rfu1 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>
<thead>
<tr>
<th>Male sterile plasma</th>
<th>Fert. spectrum</th>
<th>Restorer/maintainer</th>
<th>Carrier chromosome</th>
<th>Nature of restor.</th>
<th>Certation a)</th>
<th>Sterile plasma</th>
<th>Wheat plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>VII</td>
<td>Splt/CS</td>
<td>Rf3</td>
<td>S</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>VI</td>
<td>Cmp/CS</td>
<td>Rfc2</td>
<td>—</td>
<td>—</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>M°</td>
<td>V</td>
<td>P168/CS</td>
<td>Rfc1</td>
<td>S</td>
<td>Do</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td>C'</td>
<td>IV</td>
<td>CS/Slm</td>
<td>Rfu1 Rfu2</td>
<td>S</td>
<td>Do</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>S'</td>
<td>II</td>
<td>CS/Splt</td>
<td>Rfu1</td>
<td>S</td>
<td>Do</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>M'</td>
<td>II</td>
<td>Cmp/Slm</td>
<td>Rfu1</td>
<td>—</td>
<td>—</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Mt</td>
<td>II'</td>
<td>CS/Slm</td>
<td>Rfu1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>D²</td>
<td>I'</td>
<td>CS/N26</td>
<td>Rfd1</td>
<td>—</td>
<td>Do</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

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 allopamnic lines of Salmon (Tsunewaki et al., 1974, 1976; Kobayashi and Tsunewaki, 1980b; Mukai, 1981; Siregar et al., 1987; Tsunewaki, unpubl.)

<table>
<thead>
<tr>
<th>Donor species (code)</th>
<th>Cytoplasm</th>
<th>Plasma type</th>
<th>No. plants tested</th>
<th>Haploid</th>
<th>Twin pair</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td><em>T. boeoticum</em> (01)</td>
<td>A</td>
<td>75</td>
<td>1</td>
<td>1.3</td>
<td>0</td>
</tr>
<tr>
<td><em>T. monococcum</em> (16)</td>
<td>◯</td>
<td>13</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>T. dicoccoides</em> (21)</td>
<td>B</td>
<td>592</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>T. dicoccum</em> (22)</td>
<td>◯</td>
<td>27</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>T. aestivum</em> (52)</td>
<td>◯</td>
<td>510</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. caudata</em> (02)</td>
<td>C</td>
<td>500</td>
<td>113</td>
<td>22.6</td>
<td>69</td>
</tr>
<tr>
<td>Syn-triuncialis (27)</td>
<td>◯</td>
<td>51</td>
<td>24</td>
<td>47.1</td>
<td>4</td>
</tr>
<tr>
<td><em>Ae. triuncialis</em> (38)</td>
<td></td>
<td>37</td>
<td>2</td>
<td>5.4</td>
<td>1</td>
</tr>
<tr>
<td><em>Ae. umbellulata</em> (03)</td>
<td>C&lt;sup&gt;a&lt;/sup&gt;</td>
<td>298</td>
<td>26</td>
<td>8.7</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. triuncialis</em> (26)</td>
<td>◯</td>
<td>308</td>
<td>92</td>
<td>29.9</td>
<td>15</td>
</tr>
<tr>
<td><em>Ae. binucialis</em> (29)</td>
<td>◯</td>
<td>174</td>
<td>70</td>
<td>40.2</td>
<td>8</td>
</tr>
<tr>
<td><em>Ae. triaristata</em> 4x (32, 37)</td>
<td>◯</td>
<td>186</td>
<td>27</td>
<td>14.5</td>
<td>9</td>
</tr>
<tr>
<td><em>Ae. triaristata</em> 6x (54, 57)</td>
<td>◯</td>
<td>187</td>
<td>32</td>
<td>17.1</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. columnaris</em> (30)</td>
<td>C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>401</td>
<td>54</td>
<td>13.5</td>
<td>17</td>
</tr>
<tr>
<td><em>Ae. squarrosoa</em> (04)</td>
<td>D</td>
<td>277</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. cylindrica</em> (28)</td>
<td>◯</td>
<td>217</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. ventricosa</em> (36)</td>
<td>◯</td>
<td>110</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. croassa</em> 4x (35)</td>
<td>D&lt;sup&gt;2&lt;/sup&gt;</td>
<td>168</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. croassa</em> 6x (55)</td>
<td>◯</td>
<td>262</td>
<td>0</td>
<td>0.0</td>
<td>2</td>
</tr>
<tr>
<td><em>Ae. juvenalis</em> (53)</td>
<td>◯</td>
<td>121</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. vavilovii</em> (56)</td>
<td>◯</td>
<td>164</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. speltoides</em> (09)</td>
<td>G</td>
<td>103</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td><em>T. araraticum</em> (23, 24)</td>
<td>◯</td>
<td>215</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>T. timopheevi</em> (25)</td>
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<td>197</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>T. zhukovskyi</em> (51)</td>
<td>◯</td>
<td>135</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. comosa</em> (05)</td>
<td>M</td>
<td>145</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td><em>Ae. heldreichii</em> (06)</td>
<td>M&lt;sup&gt;b&lt;/sup&gt;</td>
<td>202</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. ovata</em> (31)</td>
<td>M&lt;sup&gt;c&lt;/sup&gt;</td>
<td>236</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. uniaristata</em> (07)</td>
<td>M&lt;sup&gt;b&lt;/sup&gt;</td>
<td>215</td>
<td>29</td>
<td>13.5</td>
<td>1</td>
</tr>
<tr>
<td><em>Ae. mutica</em> (13)</td>
<td>Mt</td>
<td>257</td>
<td>46</td>
<td>17.9</td>
<td>10</td>
</tr>
<tr>
<td><em>Ae. mutica</em> (14)</td>
<td>Mt&lt;sup&gt;c&lt;/sup&gt;</td>
<td>143</td>
<td>29</td>
<td>20.3</td>
<td>6</td>
</tr>
<tr>
<td><em>Ae. speltoides</em> (08, 17)</td>
<td>S</td>
<td>593</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td><em>Ae. bicorins</em> (12)</td>
<td>Sb</td>
<td>205</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. sharoneosis</em> (10)</td>
<td>S&lt;sup&gt;1&lt;/sup&gt;</td>
<td>92</td>
<td>23</td>
<td>25.0</td>
<td>6</td>
</tr>
<tr>
<td><em>Ae. longiasma</em> (20)</td>
<td>S&lt;sup&gt;12&lt;/sup&gt;</td>
<td>14</td>
<td>4</td>
<td>28.6</td>
<td>1</td>
</tr>
<tr>
<td><em>Ae. searsii</em> (18)</td>
<td>S&lt;sup&gt;c&lt;/sup&gt;</td>
<td>442</td>
<td>111</td>
<td>25.1</td>
<td>9</td>
</tr>
<tr>
<td><em>Ae. kotzekyi</em> (33)</td>
<td>◯</td>
<td>443</td>
<td>96</td>
<td>21.7</td>
<td>31</td>
</tr>
<tr>
<td><em>Ae. variabilis</em> (34)</td>
<td>◯</td>
<td>257</td>
<td>55</td>
<td>21.4</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.0</td>
<td></td>
</tr>
</tbody>
</table>
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<sup>n</sup>, C<sup>nu2</sup>, M<sup>1</sup>, M<sup>t</sup>, M<sup>t2</sup>, S<sup>1</sup>, S<sup>2</sup> and S<sup>v</sup>, induced haploid parthenogenesis, whereas those of ten plasma types, A, B, D<sup>2</sup>, G, M, M<sup>b</sup>, M<sup>o</sup>, S and S<sup>b</sup>, 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)

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>No. ovules observed</th>
<th>Ovule type (%)</th>
<th>Embryo</th>
<th>Endosperm</th>
<th>+</th>
<th>+</th>
<th>+</th>
<th>Twin embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Unpollinated&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>99</td>
<td>99</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>172</td>
<td>81</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>154</td>
<td>58</td>
<td>42</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>b) Pollinated&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>134</td>
<td>12</td>
<td>11</td>
<td>2</td>
<td>75</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>122</td>
<td>2</td>
<td>28</td>
<td>0</td>
<td>70</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>76</td>
<td>1</td>
<td>26</td>
<td>0</td>
<td>72</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Date of fixation after flowering.
<sup>b</sup> 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 cytoplasm 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)

<table>
<thead>
<tr>
<th>Size</th>
<th>Ploidy</th>
<th>Total no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n-2n</td>
<td>2n-2n</td>
</tr>
<tr>
<td>Large-large</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Large-small</td>
<td>51</td>
<td>65</td>
</tr>
<tr>
<td>Small-large</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Small-small</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Total (%)</td>
<td>75</td>
<td>3</td>
</tr>
</tbody>
</table>

(92.5) (3.7) (3.7)
since all the egg cell and synergidgs are produced through mitotic divisions of a single tetrad. To prove this, a genetic test was made, using the following three marker genes: \textit{Hg} for glume hairiness on chromosome arm 1AS, ca. 50 map units from the centromere; \textit{Hp} for peduncle hairiness on 4BS (following the new designation), ca. 30 map units from the centromere; and \textit{C} for compact ear on 2DL, ca. 2 map units from the centromere. \textit{(Caudata)- or (kotschyi)-S}lm was crossed as female parent to a wheat strain with genotype, \textit{HgHgHpHpCC}, where Slm was triple recessive, \textit{hghghphhpc}. 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.

\textbf{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)

<table>
<thead>
<tr>
<th>Linea</th>
<th>2n</th>
<th>Offspring Category</th>
<th>No. plants</th>
<th>Diploid</th>
<th>Haploid</th>
<th>Twins</th>
</tr>
</thead>
<tbody>
<tr>
<td>(kot)-20&quot; + T&quot;</td>
<td>42</td>
<td>—</td>
<td>125</td>
<td>10</td>
<td>54</td>
<td>37</td>
</tr>
<tr>
<td>(kot)-20&quot; + T&quot;</td>
<td>41</td>
<td>with T</td>
<td>37</td>
<td>57</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>(kot)-20&quot; + 1BL&quot;</td>
<td>42</td>
<td>without T</td>
<td>95</td>
<td>66</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>(kot)-20&quot; + 1BS&quot;</td>
<td>42</td>
<td>—</td>
<td>41</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(kot)-20&quot; + T&quot; + 1BS&quot;</td>
<td>44</td>
<td>with 1BS</td>
<td>78</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(kot)-20&quot; + T&quot; + 1BS&quot;</td>
<td>43</td>
<td>without 1BS</td>
<td>6</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

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

linkage between the translocation chromosome and the rfo1 gene for male sterility induction by S' 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, (kotschyi)-Salmon × Chinese Spring

<table>
<thead>
<tr>
<th>Generation</th>
<th>No. of plants</th>
<th>% Haploids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Haploid</td>
</tr>
<tr>
<td>F₁</td>
<td>1209</td>
<td>304</td>
</tr>
<tr>
<td>B₁</td>
<td>1314</td>
<td>50</td>
</tr>
<tr>
<td>B₄</td>
<td>912</td>
<td>39</td>
</tr>
<tr>
<td>B₅</td>
<td>717</td>
<td>67</td>
</tr>
<tr>
<td>Control</td>
<td>790</td>
<td>0</td>
</tr>
</tbody>
</table>

a) Control is (kotschyi)-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 Slm 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.

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Genome-plasmon interactions in wheat


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