Cytoplasmic Male Sterility in Relation to Hybrid Wheat Breeding*[1]

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Summary. The method of substitution and restoration of nucleus is briefly described.

Three species, *Aegilops caudata*, *Ae. ovata* and *Triticum timopheevi*, were used as donors of male sterility cytoplasms.

The characteristics of these three cytoplasms are summarized as follows:

Caudata-cytoplasm: This cytoplasm has in many respects deleterious effects on the manifestation of alien genomes. Substitution lines having hexaploid wheat genome constitution are mostly male sterile while the female organ is normal. Some lines set frequently germless seeds. Haploid and twin seedlings are of common occurrence in other lines. Pistillody is common in the substitution lines with tetraploid wheat genomes.

Ovata-cytoplasm: No pistillody was found in the substitution lines, both with hexaploid and tetraploid wheats. Male sterility is always complete in the substitution lines of hexaploid wheats with the exception of P 168, a variety of common wheat having a pair of satchromosomes of *Ae. caudata*. This variety restores male fertility completely. No effective restorers were found for the substitution lines of emmer wheat. Delayed heading is common in the 4x substitution lines.

Timopheevi-cytoplasm: Substitution lines of 6x wheats are mostly male sterile, while those of 4x wheats are more or less male fertile. Only the genome of *T. spelta duhamelianum* restores completely pollen fertility.

Among the indispensable factors for the success of hybrid wheat, five were discussed. They were (1) heterosis, (2) selection of male sterile cytoplasms, (3) discovery of restoring genes, (4) production of hybrid seeds and (5) quality.

This paper deals with our investigations on cytoplasmic male sterility and the fertility restoring genes hitherto obtained in wheat and its allies. This line of work was started in Kyoto since 1935. The first report on this problem was written in Japanese (1949) and a full paper was published in Cytologia in 1951.

The finding of a male sterile cytoplasm in crosses with *Aegilops caudata* attracted the eyes of wheat geneticists and wheat breeders. Soon the cytoplasms of *Ae. ovata* and *Triticum timopheevi* became known to cause male sterility and later many new findings were added. All are briefly summarized in the following.

Method

First of all I should like to describe the method of our investigations.

Substitutions of nucleus can be accomplished by successive backcrosses. Let us assume that two diploid species, AA and BB, are used in an experiment in which the nucleus of BB has to be transferred to the cytoplasm of AA. The first step will be to produce the F_1 hybrid AB, with AA as the female parent. The further step will consist of successive backcrosses of BB (♂) to F_1 and to the subsequent backcrossing products.

For the sake of simplicity we will assume that the chromosome pairing between the genomes A and B is complete. The first backcross, BB to F_1, namely AB ♀ × BB ♂, will produce the genome complement B^1B, in which B^1 represents the first combination genome, resulting from the meiotic divisions in F_1 (AB). Accordingly, the second backcross, $B^1B \times BB$, will result in B^2B and so forth until the n^{th} backcross, B^nB. If n is large enough, the offspring of the n^{th} backcross will have the genome BB in the AA plasma. This series of backcrosses may be called substitution backcrosses (SB). With the n^{th} backcross the substitution of AA genomes with BB genomes would be completed.

On the other hand, if AA is backcrossed to the F_1 hybrid, AB ♀ × AA ♂, we can expect to obtain after a sufficient number of backcrosses plants which will not be different, either in plasma or genome constitution, from AA. Here the restoration of the AA genomes to the AA plasma would have taken place. Hence the term: restoration backcrosses (RB).

Both above described procedures are given in the following schema:

			Number of backcrosses
Successive backcrosses	Substitution backcrosses	$\alpha AB \times BB = \alpha B^1B$	SB_1
		$\alpha B^1B \times BB = \alpha B^2B$	SB_2
		$\alpha B^2B \times BB = \alpha B^3B$	SB_3
		⋮	
		$\alpha B^{n-1}B \times BB = \alpha B^nB$	SB_n
	Restoration backcrosses	$\alpha AB \times AA = \alpha A^1A$	RB_1
		$\alpha A^1A \times AA = \alpha A^2A$	RB_2
		$\alpha A^2A \times AA = \alpha A^3A$	RB_3
		⋮	
		$\alpha A^{n-1}A \times AA = \alpha A^nA$	RB_n

α denotes the plasma of species AA. The genome complements A^nA and B^nB will correspond to AA and BB, respectively, from which the experiments are supposed to have started, provided that n is large enough.

The completion of substitution or restoration will be assumed on grounds of morphology and fertility as well as conjugation of chromosomes in the backcross offspring. Both procedures, substitution and restoration, are clearly demonstrated in Fig. 1.

Should the genomes A and B be non-homologous, with only univalents and unreduced gametes in F_1, the backcross would give rise to ABB (AB ♀ × BB ♂ = αA^1B^1B; A^1 and B^1 are the first recombination genomes). The continuous backcrossing would result in placing the BB genomes in α plasma (Fig. 1). This process may be greatly simplified by doubling the chromosome number of F_1 plants through colchicine treatment (Fig. 2). In such case the chance

* Dedicated to Professor HANS STUBBE on the occasion of his 65th birthday.

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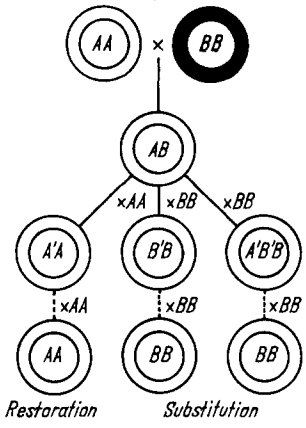


Fig. 1.

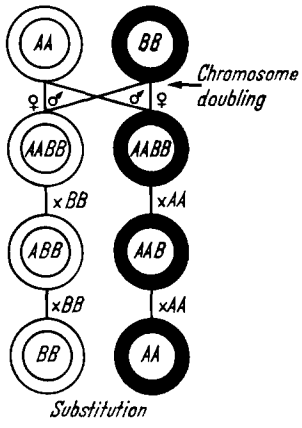
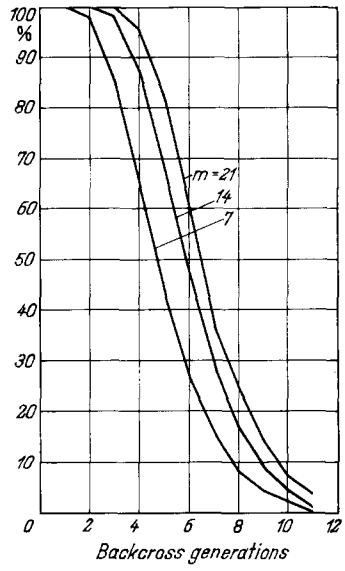


Fig. 2.

Fig. 1. Diagram showing substitution and restoration processes of genome complements through successive backcrosses to F_1 in two diploid species, AA and BB, without chromosome doubling. A^1 and B^1 are the first recombination genomes. End-result: AA restored to α plasma, BB transferred to α plasma (KIHARA 1949).

Fig. 2. Diagram showing substitution of genome complements after doubling of chromosomes through colchicine treatment. End-result: AA in β plasma and BB in α plasma (KIHARA 1949).

Fig. 3. Frequency percentage of individuals (ordinate) with nonsubstituted chromosome segments in successive generations. In each chromosome pair 30 per cent crossing-over assumed. Abscissa: number of backcrossings; m: number of chromosome pairs (after KIHARA 1959).



of crossing over between the paternal and maternal chromosomes during the F_1 meiosis would be practically eliminated.

It is assumed that crossing over occurs between the chromosomes of the non-homologous genomes A and B. The theoretical decrease in the number of heterozygotes in the subsequent generations was calculated by KIMURA (1950). KIMURA showed that it is slow in several of the first backcross generations, depending on the haploid chromosome number and the amount of crossing over. But later the decrease of heterozygotes is quick like in calculations made on the basis of non-crossing over (see Fig. 3).

Our objectives of nucleus substitution are:

1) to know the effects of foreign cytoplasms on genome manifestations,
2) to study (if any) the mutagenic effects of foreign genomes on the cytoplasmic elements (and *vice versa*), and
3) to examine the possibility of cytoplasmic transmission from pollen to egg cells.

Restoration backcrosses are indispensable for our understanding of the relationship between genomes and cytoplasms. As the progress of nuclear substitution and restoration in the course of backcrosses proceeds in similar manner, the difference in genome manifestation of SB and RB strains may be attributed to cytoplasm. If n is large enough, both lines will have quite identical genomes (AA).

Results

1. Experiments with *Ae. caudata* and *T. vulgare erythrospermum*. First experiment: In my first report (KIHARA 1951), a case of substitution was described, where the hexaploid genome complement (VV) of *Triticum vulgare erythrospermum* (abbreviated *T. v. e.*)[1] was introduced to the cytoplasm of *Aegilops caudata*, a diploid species having the genome formula CC. By two successive backcrosses of the hybrid, *T. v. e.* (♀) × *Ae. caudata* (♂), with *T. v. e.*, as the male parent we obtained an SB_2 plant having $21_{II} + 2_I$. Its offspring were obtained from open pollination, as the third backcross was not available.

The offspring of this SB_2 plant were found to have 21_{II} in later generations and the line was maintained by self-pollination in our collection as a pure strain (P 174).

After karyological examination, this strain was proved to possess one *caudata* chromosome, called C-Sat-2, which carries a gene (or genes) for male fertility restoration in the *caudata* cytoplasm and a gene for black awns. The modified genome of P 174 was designated V^b. C-Sat-2 is homoeologous to chromosome XVII or 1 D (KIHARA and MURAMATSU 1955, MURAMATSU 1959).

P 168 was obtained from the cross *T. v. e.* (♀) and P 174 (♂). This strain has *vulgare* cytoplasm and the genomes of P 174. Accordingly the plasmatic genome formulas for *T. v. e.*, P 174 and P 168 are:

	Old		New
	T. v. e. - - -	$\beta V\ V$	(*aestivum*) $V\ V$
	P 168 - - - -	βV^bV^b	(*aestivum*) V^bV^b
and	P 174 - - - -	αV^bV^b	(*caudata*) V^bV^b

respectively, where α = plasma of *Ae. caudata* and β = plasma of *T. v. e.*

As the number of male sterile cytoplasms available for our studies is increasing, we can no longer use Greek letters for distinguishing each of them. This was the reason why I am using now a new system for plasma-genome combinations. The new formulas are given above at the right side of the old ones. For a variety with an alien cytoplasm, for instance, Norin 26 with *ovata* plasma, the symbol should be (*ovata*) Norin 26.

Second experiment: Our backcrosses were stopped at the 2nd generation (SB_2) and we had no restoration lines. This means that our first investigations were not adequately planned. Therefore a new series of substitution as well as restoration backcrosses was started in 1949. Reciprocal hybrids between *T. v. e.* and *Ae. caudata* were used.

As far as the chromosome behavior in F_1 and the procedure of restoration or substitution are concerned, the reciprocal hybrids behaved quite simi-

[1] For convenience' sake, the genome symbol (*AABBDD*) for *T. v. e.* is abbreviated to VV.

larly. The F_1 had 0—6 bivalents. Often restitution nuclei were observed. Accordingly functional unreduced gametes were expected. The first backcross offspring (both in SB_1 and RB_1) had very often 49 somatic chromosomes. They showed most frequently the chromosome configuration, $21_{II} + 7_I$, representing the genome combination *VVC*. These B_1 plants were used for further backcrosses.

After RB_2 in the restoration series and SB_3 in the substitution series the chromosome number became constant at 42. The successive backcrosses have reached at present (1966) to the 17th generation (RB_{17} and SB_{17}). After SB_4 pollen fertility was always nearly zero, but seed fertility by backcrossing was normal. On the contrary, after RB_2, pollen fertility as well as seed fertility was quite normal. There was no indication that the cytoplasm of *T. v. e.* was affected by the nuclear genes of *caudata*. Also there was no indication of cytoplasmic transmission from the male parent (*T. v. e.*) to *caudata* cytoplasm in the advanced backcross generations.

Thus a male sterile *T. v. e.* strain with *caudata* cytoplasm was established. Using this strain for the female parents, further substitution works were started. In the course of our studies, it was found that not only *caudata* cytoplasm, but also cytoplasms of *Ae. ovata* and *T. timopheevi* cause male sterility in many wheat species and varieties. These two cytoplasms were also included into our substitution program.

The results of our substitution work with three cytoplasms are given below.

Caudata cytoplasm: The male sterile line of *T. v. e.* = (*caudata*) *T. v. e.* has been used as donor of *caudata* cytoplasm to various wheat varieties. Up to date (1966), it was crossed to 12 varieties of common wheat, five emmer strains, a variety of *T. timopheevi* and two strains of synthesized hexaploids.

Ovata cytoplasm: The cytoplasm of *Ae. ovata* causes also male sterility. We used two male-sterile strains, whose genomes were obtained from *Triticum dicoccum* var. Khapli or *T. aestivum* var. Norin 26. They were produced by FUKASAWA (1953, 1959). Using these two strains as donors of *ovata*-plasma, we have developed many new male sterile lines involving 49 varieties of common wheat, three of emmer, a variety of *T. timopheevi* and a synthesized hexaploid.

Timopheevi cytoplasm: In the meantime a third male-sterile plasma has become available for our study. This time it was the cytoplasm of a *Triticum* species, namely *T. timopheevi* Zhuk. From genome analytical investigations, this tetraploid species was ascertained to have a new genome type *AAGG* differing from all other tetraploid species (*AABB*) (LILIENFELD and KIHARA 1934).

Substitution lines with *timopheevi* cytoplasm were obtained, namely 11 with common wheat, 6 with emmer wheat and two synthesized hexaploids. The most advanced materials have reached to SB_7, while several are still in SB_2 or SB_3 generation.

Comparison of three cytoplasms: The hereditary characteristics of these three cytoplasms are revealed when we observe the manifestation of the genomes introduced by backcrosses. For instance let us compare normal *T. v. e.* with *T. v. e.* having *caudata* cytoplasm i. e., (*vulgare*) *T. v. e.* and (*caudata*) *T. v. e.*, morphologically and physiologically. Fig. 4 shows the anthers and pistills of normal (a) and male sterile strains (b). By such observations, the genetic differences between the above three cytoplasms were disclosed as shown in Table 1.

The cytoplasm of *Ae. caudata* is characterized by frequent formation of germless grains, production of haploid or twin seedlings when combined with hexaploid wheat, and induction of pistillody with emmer wheat genomes (Fig. 4c). In some cases, female fertility is reduced. P 168 and *compactum* 44 act as partial fertility restorers. It is characteristic of *ovata* cytoplasm to induce male sterility in most wheat varieties and to prolong extremely the vegetative growth in emmer wheat. No pistillody is caused. P 168 restores male fertility completely. *Timopheevi* cytoplasm is different from the preceding two, as *T. spelta* and all emmer varieties having it restore male fertility to various levels. This cytoplasm does not exhibit any other remarkable effect.

2. Genome manifestation in alien cytoplasms.

Pollen sterility and pollen fertility: From Fig. 5, we see that many substitution lines show complete male sterility. However some strains restore pollen fertility in the male sterile cytoplasms. The degree of fertility restoration is different in different genome-cytoplasm

Table 1. *Hereditary characteristics of three cytoplasms revealed by substitution of a set of wheat nuclei.*

Nucleus	Cytoplasm		
	Ae. caudata	*Ae. ovata*	*T. timopheevi*
Hexaploid wheat			
T. v. e.	male-sterile, germless grains	male-sterile	male-sterile
P 168	partially male-fertile	completely male -fertile	male-sterile
Salmon	male-sterile, haploid and twin seedlings	male-sterile	male-sterile
Compactum 44	partially male-fertile	male-sterile	male-sterile
T. spelta duhamelianum	male-sterile, reduced female fertility	male-sterile	completely male-fertile
Tetraploid wheat			
T. durum reich.	pistillody, abortive ovules	male-sterile, delayed heading	partially male-fertile
T. polonicum vest.	pistillody	male-sterile, delayed heading	completely male-fertile
T. dicoccoides spont.	pistillody	male-sterile, delayed heading	slightly male-fertile

(KIHARA and TSUNEWAKI 1966)

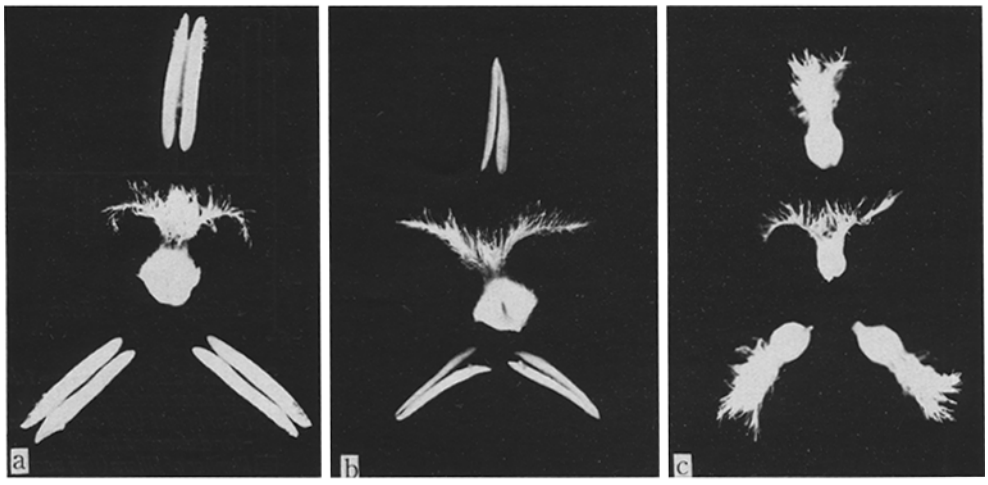


Fig. 4. Stamens and pistils of normal and substitution lines. a) *T. v. e.* (normal), b) *T. v. e.* with *caudata* cytoplasm, c) *T. durum reichenbachii* with *caudata* cytoplasm. Three pistils transformed from anthers are completely sterile, while the pistil in the center is only 10% fertile.

combinations. One genome, which is effective in one cytoplasm, is not necessarily effective in another. It is also very interesting to note that the pollen fertility in F_1 of some combinations is low and increases with the advance of backcross generations, while fertility of other combinations is high in F_1 and decreases in later generations. The combinations of the former type (group 1) are marked with (O) and of the latter (group 2) with (×).

This second group (×) is represented in Fig. 6 by *T. durum* var. *reichenbachii* in *timopheevi* plasma. The self-fertilized F_1 shows complete male fertility, gradually diminishing in the course of continued backcrosses. In contrast, the other group (O), represented by *compactum* 44 in *caudata* plasma, shows in F_1 very low male fertility, which is recovered however in the course of backcrosses. For the (O) group it may be assumed that homozygosity of the restoring gene or genes is required for recovery, while for the (×) group the presence of complementary genes or restoring genes contributed from the parents which produce a heterotic effect in F_1 is indicated. The determination of pollen fertility is difficult as it varies according to environmental conditions. It is also difficult to investigate the pollen fertility for all individuals. Therefore it was estimated by the seed set obtained from selfing, as shown in Figs. 5 and 6.

So far complete seed set could be obtained only in F_1, namely in (*ovata*) Norin 26 × P 168 (Fig. 7), (*timopheevi*) *T. durum reichenbachii* × *T. polonicum vestitum* and (*timopheevi*) *T. aestivum* Bison × *T. spelta duhamelianum*.

Fertility of egg cells: Fertility of egg cells of substitution lines is estimated by seed production in the backcrosses. Hence it is called seed fertility.

Seed fertility is normal in almost all combinations. However we found two cases, where it was reduced to a certain degree. One is *T. spelta duhamelianum* in *caudata* plasma, whose seed fertility was slightly reduced. The other is *T. durum reichenbachii* in *caudata* plasma, where only ca. 10% egg cells were functional.

Deleterious effects of male-sterile cytoplasms: Some examples of deleterious effects of male sterile cytoplasms are given below. They were mostly found in the substitution lines with *caudata* cytoplasm.

1) Effect of *caudata* cytoplasm on plant vigor: Nucleus substitution lines of *T. v. e.*, Salmon, Comp. 44 and *T. spelta duhamelianum* were used together with their normal strains for the examination of heading date, plant height, number of tillers and dry matter weight, and the difference between the normal lines and the substitution lines was statistically tested. The result was reported by TSUNEWAKI (1964), and is here diagrammatically given in Fig. 8.

As Fig. 8 shows, Salmon's nucleus performed better in *caudata* cytoplasm than in its own. This type of

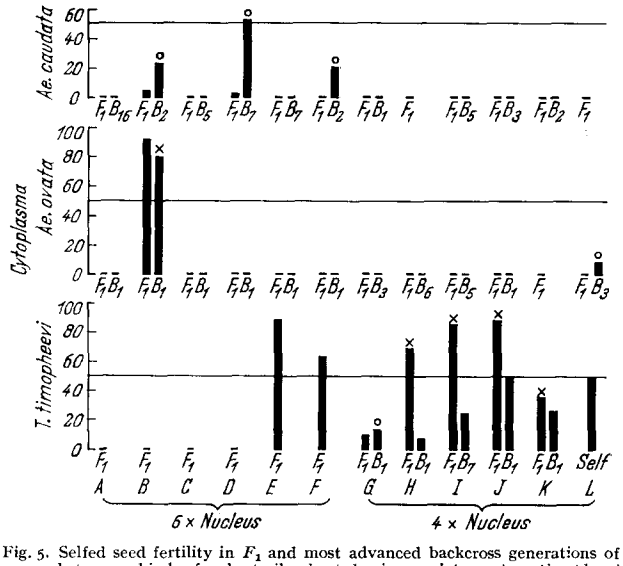


Fig. 5. Selfed seed fertility in F_1 and most advanced backcross generations of crosses between 3 kinds of male-sterile wheats having *caudata*, *ovata* or *timopheevi* cytoplasm and normal, fertile wheats as recurrent male parents.
A. *T. v. e.*, B. P 168, C. Salmon, D. *Comp.* 44, E. *Spelta*, F. ABD-13, G. *dicoccoides*, H. Khapli, I. *durum*, J. *polonicum*, K. *turgidum*, *L. timopheevi* (KIHARA and TSUNEWAKI 1966).

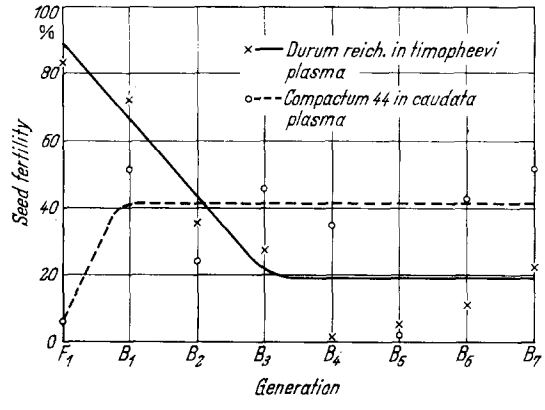


Fig. 6. Behavior of 2 representative restorers in F_1 and backcross generations (KIHARA and TSUNEWAKI 1965).

Fig. 7. Spikes and pollen grains of normal Norin 26 (a), male sterile Norin 26 (b), F_1 from the cross, male sterile Norin 26 × P 168 (c) and P 168 (d) (KIHARA and TSUNEWAKI 1966).

heterosis, caused by nucleus-cytoplasm hybridity, was called by KIHARA (1963) plasmatic heterosis. Substitution lines of all other wheats were more or less inferior to their respective normal lines. According to TSUNEWAKI (1964), the gene complement of Salmon[1] is modified from that of ordinary *vulgare* by integration of some genes of rye and eventual loss of some of wheat. This change in gene content was assumed to be the cause of plasmatic heterosis in this combination. Therefore, it can be said that, in general, alien male-sterile cytoplasm reduces plant vigor, causing some delay in heading and reduction in plant height, tiller number and dry matter weight.

The delay of heading was observed in all male sterile lines with *ovata* cytoplasm. However no investigation was undertaken for plant height, number of tillers, etc.

2) Occurrence of germless grains: It was previously noted that male-sterile plants set rather frequently germless grains. To get more information on their occurrence, an investigation was carried out using male-sterile *T. v. e.* with *caudata* cytoplasm. In this experiment, plants of normal and male-sterile lines were interplanted in an isolated field. About 40 spikes in each line were emasculated two days before flowering and then artificially pollinated with pollen of normal *T. v. e.* a few days after emasculation. The grains set on those spikes were husked and examined for the presence of an embryo. At the same time, naturally open pollinated spikes were harvested and the husked grains were examined in the same way. The result is shown in Table 2.

[1] Var. Salmon was derived from a *Triticale* (KIHARA and TSUNEWAKI 1962).

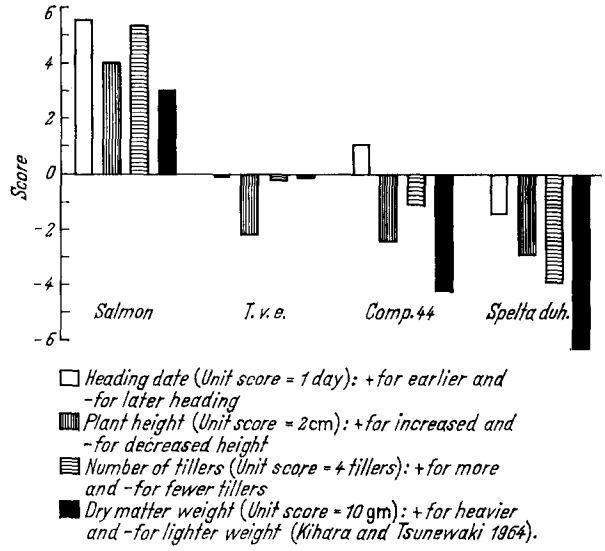


Fig. 8. Effect of *caudata* cytoplasm on the main quantitative characters of four common wheats: Performance of the nucleus-substitution lines is indicated by the difference from that of the respective normal lines.

The frequency of germless grains in normal *T. v. e.* was, on the average, 0.1%, and no difference was found between artificial and natural open pollination. On the contrary, germless grains were found in abundance among seeds set on male-sterile plants. Their frequency was higher when the seeds were produced by natural open pollination than when they were obtained by hand pollination. From those results, it can be said that alien male-sterile cytoplasm increases the frequency of germless grains and that open pollination of male-sterile plants further increases their frequency probably due to inadequate timing of pollen transfer.

In order to test the germination ability of the germless grains, they were seeded on moist filter paper and their germination was compared to that of normal grains. In this test all normal grains have germinated, while none of the germless have elongated the coleoptile. Their contrasting appearance three days after seeding is shown in Fig. 9.

Table 2. *Frequency of germless grains among seeds produced by normal and male-sterile T. v. e.*

Lines	Type of pollination	No. of seeds examined	No. of germless grains	%
Normal line	Artificial pollination	593	1	0.2
Normal line	Natural open-pollination	15,520	17	0.1
Male-sterile line	Artificial pollination	506	48	9.5
Male-sterile line	Natural open-pollination	1,607	276	17.2

(KIHARA and TSUNEWAKI 1964)

Accordingly, seeds raised on male-sterile plants, interplanted with another variety as pollinator, will contain an appreciable amount of germless grains. This would probably require either a larger amount of seeds or mechanical removal of germless grains for assuring a normal stand of the crop.

3) Occurrence of haploids: As already reported by KIHARA and TSUNEWAKI (1962) and KIHARA (1964), male-sterile *caudata* cytoplasm induces a frequent occurrence of haploids in the progeny of substitution lines. Our most recent data in this respect are summarized in Table 3, which includes all data previously reported.

Since the cytological examination of chromosome numbers was made for about one-third of the entire progeny, the frequency of haploids was estimated in two ways, as given in the last two columns of Table 3. The real frequency is expected to fall between the two estimates. In all cases, haploids were much more frequent in the substitution lines than in the normals, especially in Salmon. In all 6x varieties except Salmon the average percentage of haploid individuals in the substitution lines was between 1.6 and 4.3%.

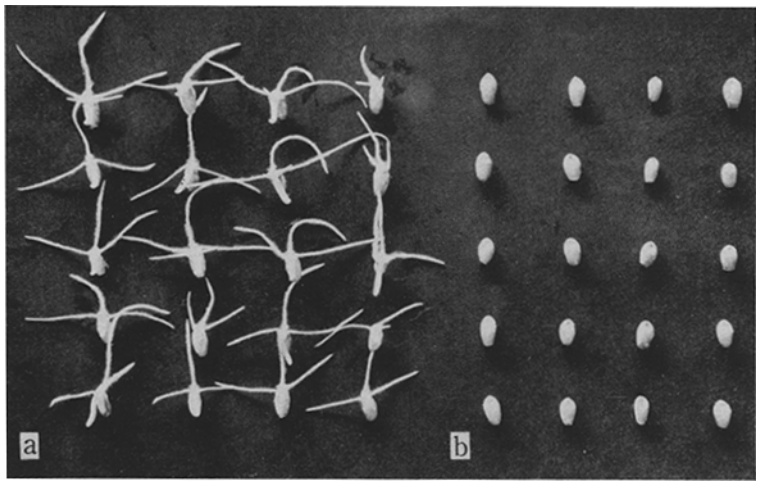


Fig. 9. Germination test of normal and germless grains obtained from male-sterile *T. v. e.* a. normal, b. germless. (KIHARA and TSUNEWAKI 1964).

4) Occurrence of twin seedlings: It was also noted that twin seedlings occur frequently in the progeny of substitution lines (KIHARA and TSUNEWAKI 1963; KIHARA 1964). Our most recent result is presented in Table 4, including all records up to 1964.

Twin seedlings were found in both normal and substitution lines, but their frequency in the latter was ten to thirty times higher than in the former.

Table 3. *Frequency of haploids in substitution lines with caudata cytoplasm.*

Strains	No. of plants grown (N_1)	No. of plants examined (N_2)	No. of haploids (n)	Freq. of haploids (%)	
				n/N_1	n/N_2
T. vulgare Salmon					
Normal line	100	100	0	0.00	0.00
Substitution lines	186	179	54	29.03	30.17
T. vulgare erythrospermum					
Normal line	2,306	357	1	0.04	0.28
Substitution lines	1,914	493	19	0.99	3.85
All other strains					
Normal lines	1,076	836	0	0.00	0.00
Substitution lines	901	325	14	1.55	4.31

(KIHARA and TSUNEWAKI 1964)

The haploids showed very high sterility, even after artificial pollination. Some haploids of Salmon were pollinated by hand with normal pollen grains. Seed fertility, in this case, was 7.5%, while the average seed fertility of male-sterile Salmon (6x) was 74.4% under the same conditions. Nevertheless such a high frequency of haploids in male-sterile lines would reduce the productivity of hybrid seed.

Chromosome numbers of twin pairs were studied. The result is summarized in Table 5.

A great majority of twin pairs (86%), whose chromosome numbers could be checked for both twins, were of diplo-haplo type. Diplo-diplo or haplo-haplo pairs were rare. On the whole, about 50% of all twins were haploid. Consequently, a high frequency of twin seedlings should also reduce, to a certain extent, seed production.

Table 4. *Frequency of twin seedlings in nucleus-substitution lines with caudata cytoplasm.*

Strains	No. of seeds germinated	No. of twin pairs	Freq. of twin pairs (%)
T. vulgare Salmon			
Normal line	292	1	0.34
Substitution lines	196	21	10.71
T. vulgare erythrospermum			
Normal line	323	1	0.31
Substitution lines	301	12	3.99
All other varieties	14,119	9	0.06
Substitution lines	957	5	0.52

(KIHARA and TSUNEWAKI 1964)

Table 5. *Frequency of three types of twin pairs.*

Type	Freq. (No. of twin pairs)
Diplo-diplo	1
Diplo-haplo	19
Haplo-haplo	2
Diplo-?	5
Haplo-?	1

?: One of the twins was not checked for its chromosome number.

(KIHARA and TSUNEWAKI 1964)

Discussion

Our investigations on the substitution of nucleus were carried out primarily from the theoretical standpoint. However in the course of our investigations, we have found several effective systems of male sterility and fertility restoration which might find practical application. Now it seems that hybrid wheat is not a mere dream.

There are many indispensable factors necessary for the success of hybrid wheat. So far as I am aware, I can give at least five that are apparently most important.

1) Heterosis is an essential factor in the breeding of hybrid wheat. It was proved by many investigators that the F_1 hybrids were in many combinations superior to the more productive parent.

Using 31 spring wheat hybrids, SHEBESKI obtained a very interesting result which was reported by JOHNSON (1966b). His description is as follows: "Of particular interest is the demonstration of significant heterosis for yield in hybrids from crosses of intraclass varieties of related parentage. It has not been necessary to resort to crosses of unrelated varieties from diverse origins to demonstrate good hybrid vigor for yield. This is particularly significant from the standpoint of maintenance of acceptable levels of milling and baking quality in wheat."

From our studies, the genome-cytoplasm combination can not be ignored, as genome manifestation may be affected by the cytoplasm resulting in deleterious effects on one hand and cytoplasmic heterosis on the other.

2) Selection of male sterile cytoplasms is very important.

Male sterility and at the same time complete female fertility should be maintained. As given above some cytoplasms have deleterious effects on plant vigor and other characters. In this respect, 4x wheats are usually more sensitive than 6x wheats, therefore no suitable cytoplasms for 4x wheats are known yet.

Though *timopheevi* cytoplasm has no deleterious effect on 4x genomes, its substitution lines are not strictly male sterile (cf. Tab. 1).

3) The restoration of male fertility in F_1 should be perfect. Restoring genes function as incomplete dominants. Our research indicates that complete fertility restoration in F_1 could be obtained by interaction of the parental genes.

The fertility of F_1 hybrids depends very much upon the external conditions, namely late sowing, humidity, low temperature, etc. The fertility differs significantly in different ears of the same plant. Therefore utmost caution must be taken in the selection of sites for cultivation, until we definitely ascertain effective restorer genes or good parental combinations.

4) Production of hybrid seeds is one of the greatest concerns to seed growers. As wheat is a self pollinating crop, spontaneous hybridisation is rather rare in normal fertile plants. But if we leave emasculated florets open for pollination, we get seeds. The seed set varies to a great extent according to environmental conditions. PERCIVAL (1921) mentions that c. Salmon found in S. Dakota seed setting amounted to ca. 76%. Also WILSON and ROSS (1962) reported a similar fertility (69.8—72.6%) for male sterile lines. Those investigators' data correspond with each other very well. But in our conditions in Japan, the highest seed fertility was only 20.4%, when a row of male steriles was placed between two rows of normal plants (KIHARA and TSUNEWAKI 1964).

Air movement, temperature, relative humidity, and precipitation during anthesis may be important for a good set. Also we have to find suitable parental varieties synchronizing in flowering date. We should select pollinators which shed abundant pollen grains. For male steriles it is necessary to find strains with suitable mechanisms for wind pollination.

5) Quality. This problem was not the object of our investigations. Therefore we have nothing to say about it. But so far as we are informed, the breeding for protein quantity and quality seems to be rather simple and might not be very difficult for other agronomic characters (JOHNSON 1966a).

Conclusion

We stand now on the threshold of a new era of wheat breeding. The necessary tools for hybrid wheat now exist. However the economic feasibility has not yet been demonstrated. This awaits an extensive study through joint efforts in all fields of wheat science.

Zusammenfassung

Die Methode der Substitution und Restoration des Nucleus wird kurz beschrieben.

Drei Arten, *Aegilops caudata*, *Ae. ovata* und *Triticum timopheevi*, wurden als Donor cytoplasmatisch bedingter männlicher Sterilität verwendet. Die Charakteristika der jeweiligen Cytoplasmen lassen sich wie folgt zusammenfassen:

Caudata-Cytoplasma: Dieses Cytoplasma hat in vieler Hinsicht einen schädlichen Einfluß auf die Manifestation fremder Genome. Substitutionslinien mit einem hexaploiden Weizengenom sind meist männlich steril, das weibliche Organ ist normal. Einige Linien bringen häufig keimlose Samen; in anderen Linien treten haploide und Zwillingssamen auf. Bei Substitutionslinien mit tetraploiden Weizengenomen werden häufig andere Blütenorgane in Karpelle umgewandelt.

Ovata-Cytoplasma: In den Substitutionslinien sowohl der hexaploiden wie tetraploiden Weizen wurden keine anderen Blütenorgane in Karpelle umgewandelt. Die Substitutionslinien der hexaploiden Weizen sind stets vollkommen männlich steril mit Ausnahme von P 168, einer Weizenvarietät, die ein Paar Sat-Chromosomen von *Ae. caudata* besitzt. Diese Varietät stellt die männliche Fertilität vollkommen wieder her. In den Emmer-Substitutionslinien wurden keine wirksamen Restorer gefunden. Bei den 4x-Substitutionslinien zeigt sich häufig verzögertes Ährenschieben.

Timopheevi-Cytoplasma: Die Substitutionslinien der 6x-Weizen sind meist männlich steril, die von 4x-Weizen dagegen mehr oder weniger männlich fertil. Nur das Genom von *T. spelta duhamelianum* stellt die Pollenfertilität völlig wieder her.

Von den für den Erfolg der Hybridweizenzüchtung unabdingbaren Faktoren wurden die folgenden 5 besprochen: 1. Heterosis, 2. Selektion männliche Sterilität bedingender Cytoplasmen, 3. Auffinden von Restorergenen, 4. Produktion von Hybridsaatgut und 5. Qualität.

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The Interaction of Selection and Linkage III* Synergistic Effect of Blocks of Genes

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Summary. We have examined the effect of selection in populations when the genes controlling the selected character do not have uniform recombination relations. In particular we have examined the outcome of selection for an intermediate optimum phenotype controlled by two blocks of genes additive within blocks, but multiplicative between blocks. This is analogous to "main effect" genes and "modifiers". The question examined was what effect linkage structures of these groups had on the changes in gene frequency and rate of advance under selection.

The results of replicated Monte Carlo runs of large populations at three intensities of selection were:

1. A tightly linked block of genes maintains genes in intermediate gene frequencies, undergoes rapid selections of balanced gametic types and shows a very rapid rise in fitness followed by a long period of plateau at a fairly high fitness value.

2. A loosely linked block of genes goes to fixation of balanced numbers of loci at $q = 0.0$ and $q = 1.00$. This results in a slower rise in fitness but a higher plateau, nearly at a fitness of unity.

3. When one block of genes is tightly linked and the other loosely linked the effects of each type of block are exaggerated. The loosely linked genes go to fixation more rapidly, the tightly linked genes stay closer to intermediate values, fitness rises more quickly than for loosely linked genes but goes to a higher plateau than for tightly linked genes.

The previous publications of this series (LEWONTIN 1964a, b) as well as other works on the theory of linkage and selection (see BELLMANN and AHRENS, 1966, for a substantial bibliography) have concentrated on fairly simple genetical systems. In particular it has been convenient to investigate simple genomes consisting of n loci formed into 1 or several linkage groups but with the same amount of recombination between adjacent genes. Using such models it has been possible to map out the effect of changing the intensity of linkage on the rate of progress under selection, the kinds of gametic combinations built up in the population, the nature of equilibria if any, and so on. Because of the analytic difficulties of these complex processes most of the work has proceeded by the numerical analysis of various selection models with various parameter values. Such quasi-empirical studies can obviously be extended in any direction. More models of selection, more complex physiological interaction between loci, variations in mating structure, increasing numbers of loci, are all open for study. It seems to us, however, that an important lacuna in our knowledge exists with respect to the structure of the genetical system itself.

That is, one may imagine that the genes controlling some character are not homogeneously distributed through the genome with completely additive effects but rather that the genome is organized into blocks of genes, roughly additive among themselves, but interacting with other such blocks to produce the phenotype upon which selection acts. The introduction of such structure into our models results in a very large field of possible inquiry. The number of blocks, the degree of their interaction with each other, the linkage relations among them, are all variables. The problem must then be attacked piecemeal, but

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