VARIATION OF CHROMOSOME NUMBERS AMONG F_2 - AND F_3 - PROGENIES IN THE CROSSES BETWEEN TWO DWARF WHEAT PLANTS¹⁾

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(With One Plate and Two Text-figures)

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INTRODUCTION

The pentaploid hybrid, *Triticum polonicum* $\times$ *T. spelta*, has 35 somatic chromosomes, and both in pollen mother cells and embryosac mother cells there are 14 bivalents and 7 univalents. The bivalents consist of 14 *polonicum*- and 14 *spelta*-chromosomes, the remaining 7 *spelta*-chromosomes being left as univalents (KIHARA, 1924). The chromosomal formula of F_1 -hybrid is therefore $35 = 14_{II} + 7_I$. The 14 bivalent chromosomes divide in the usual manner and behave quite normally throughout maturation divisions. The remaining 7 univalent chromosomes split 'longitudinally in the heterotypic division, and pass at random to either pole in the homeotypic division. Therefore both the pollen-grains and egg-cells have the chromosome number represented by the formula $14 + i$, where "i" is equal to 0, 1, 2, 3, 4, 5, 6 or 7. KIHARA named the 7 chromosomes a, b, c, d, e, f and g respectively. In the following generations the somatic number of chromosomes was found to vary from 28 to 42. Among them 28- and 42- chromosome plants were constant for the chromosome number, but the others were in general not constant. To explain these phenomena KIHARA (1924) assumed that 28- to 42- chromosome plants are divided into two groups, viz. one with fertile chromosome combinations and the other with sterile chromosome combinations. The fertile combinations are also grouped in two classes, one ranging in chromosome numbers from 28 to 34, called the diminishing group, because in subsequent generations the chromosome number gradually (in this case rather more rapid than in the latter class) reverted to 28; the other, ranging from 36 to 42, called the increasing group, because their progeny eventually returned to the 42- chromosome condition of *T. spelta*. In the increasing group the number of bivalents plus the number of univalents uniformly equals 21. Among the progeny any combination of bivalent and univalent elements, the sum of which

¹⁾ Contribution from the Laboratory of Genetics, Biological Institute, Kyoto Imperial University. No. 4

is less than 21, represents a sterile chromosome combination ; consequently, this group automatically and gradually reverts to the 21_{II} condition.

In 40-chromosome plants $20_{II} + 0_I$ represents the sterile combination, and $19_{II} + 2_I$ the fertile combination ; that is the former lacks completely a pair of chromosomes from 21_{II} condition (= *spelta*), but the latter has lost 2 chromosomes from 2 different pairs.

In the F_4 - and F_5 - progeny of the pentaploid hybrids KIHARA obtained two dwarf plants having 40 diploid chromosomes. The combination of chromosomes was $20_{II} + 0_I$ for both plants. They were constant in chromosome number and also in their dwarf habit. He crossed these two dwarf plants and got tall F_1 -plants with fertile chromosome combination ($19_{II} + 2_I$). He assumed from these results, that the two dwarf plants lost 2 different chromosomes (f or g) of their hexaploid ancestor (*spelta*= $19_{II} + ff + gg$). They are called D-2f and D-2g.¹⁾ However he could not go further owing to a journey abroad (1925—27).

Accordingly the present investigation was carried on from 1926 to 1928 under the guidance of Prof. KIHARA.

MATERIAL AND METHOD

The material used in the present investigation was derived from Prof. KIHARA's cultures as already given above. They are F_2 of the original pentaploid hybrid (*T. polonicum* × *spelta*).

One of the parents D-2g (Pl. III, Fig. 1a) shows some characteristics of ateliotic dwarf. Ear, neck and stem are short. Especially the ear (Pl. III, Fig. 3c) is very short and compact. As the neck is very short there are some difficulties for the ears in getting out from the leaf-sheath (Pl. III, Fig. 3a & b). This dwarf plant grows from 40 to 105 cm. high. The dwarf plant has awns and hairs on the ears.²⁾ This dwarf plant reproduced few seeds as compared with D-2f.

D-2f (Pl. III, Fig. 1b) shows also the characteristics of dwarf habit. However, the vegetative development is better than that of D-2g and the plant is much taller. This dwarf plant reaches a height of 75—120 cm. The length of the neck and the ear (Pl. III, Fig. 4) however is almost normal. These plants have awns but no hair on the head. This dwarf plant produced seeds in greater abundance than D-2g. This dwarf plant reminds us of the achondroplasia.

The original species, from which these dwarf plants were produced, were ca.

¹⁾ D-2f and D-2g were described as 3-3-3-6 and 2-8-31 respectively by KIHARA (1924, 1925)

²⁾ D-2g without awns is obtained from the cross *Spelta* × awned D-2g. These plants can push out their awns with no difficulty. Full description of the dwarf plants will be given in the future paper.

155 cm. (*polonicum*) and ca. 150 cm. (*spelta*) in height.

For the karyological observation the usual paraffin method was adopted. For fixing BONN's modification of FLEMMING's fluid combined with CARNOY's fluid and for staining iron-alum-haematoxylin were used.¹⁵

CHROMOSOME BEHAVIOR IN THE MEIOSIS OF F_1 -HYBRIDS

In the crosses between two dwarf plants, $D-2g \times D-2f$ and $D-2f \times D-2g$ (Pl. III, Fig. 1c), the diploid number of chromosomes was 40. At the heterotypic metaphase of the pollen mother cells there were 19 bivalents and 2 univalents (Text-fig. 1c). From this result it is clear that the 19 chromosomes from $D-2g$ paired with 19 chromosomes of $D-2f$, and 2 unpaired chromosomes are no other than f - and g -chromosome, as already described by KIHARA (1925).

The 19 bivalents divide in the usual way and behave quite normally throughout both divisions. But the 2 univalents usually lag behind the 19 bivalents at the heterotypic anaphase. Then they divide longitudinally and go to the poles, where 19 chromosomes have already gathered. In the second division the 2 monad chromosomes, derived from univalents in the heterotypic division, do not divide longitudinally. They pass at random to either pole. I could not study the behavior of chromosomes in embryosac mother cells. After KIHARA (1924) however the mode of chromosome reduction in the pentaploid F_1 -hybrids and of the following generations is the same both in pollen mother cells and embryosac mother cells. Therefore it is quite natural to assume that the behavior of the chromosomes in E. M. C. is as described above.

The chromosome behavior is also the same in the reciprocal crosses $D-2g \times D-2f$ and $D-2f \times D-2g$.

The number and combination of chromosomes in parents and F_1 -plants are given in the following Table (Table I).

The variation of chromosome combination was found, for instance $18_{II} + 4_I$ which arose from failure of conjugation in a pair of chromosomes (See also Table III). Such variations were found in the offspring of this hybrid, but in each the determined chromosome combination usually occurs (cf. KIHARA, 1924, p. 78).

TABLE I

Parents and F_1	Chromosome numbers and combinations
$D-2f$	$40 = 20_{II} + 0_I$
$D-2g$	$40 = 20_{II} + 0_I$
$D-2f \times D-2g$	$40 = 19_{II} + 2_I$
$D-2g \times D-2f$	$40 = 19_{II} + 2_I$

¹⁵ See KIHARA (1924)

The F_1 -plants grow to a high of ca. 140 cm. and the vegetative development is quite normal (Pl. III, Fig. 1c).

VARIATION OF CHROMOSOME NUMBERS AND THE COMBINATION OF CHROMOSOMES IN F_2

As the result of random distribution of f- and g-chromosome in the homeotypic division of F_1 -plant we can easily imagine that there are three kinds of gametes of concerning chromosome numbers. They have 19, 20 or 21 chromosomes. It is, therefore, expected that the number of chromosomes in F_2 -plants ranges from 38 to 42. 220 plants, raised from self pollination of F_1 -hybrids, were examined karyologically and the chromosome numbers of 154 individuals determined.

The number and combination of chromosomes in F_2 -plants are shown in Table II.

TABLE II

Chromosome numbers and combinations		Number of observed plants
Sterile combinations	$38 = 19_{II} + 0_I$	5
	$39 = 19_{II} + 1_I$	28
	$40 = 20_{II} + 0_I$	6
Fertile combinations	$40 = 19_{II} + 2_I$	50
	$41 = 20_{II} + 1_I$	50
	$42 = 21_{II} + 0_I$	12

The chromosomes in the heterotypic metaphase of parents, F_1 - and F_2 -plants are illustrated in Text-fig. 1.

As mentioned above I succeeded in obtaining the series of all expected chromosome combinations in the F_2 -progeny. Besides this, I found three unexpected combinations (Table III) (Text-fig. 2), which are perhaps derived from the irregular division of chromosomes during the gametogenesis of F_1 -plants.

TABLE III

Chromosome numbers and combinations	Variations
$38 = 1_{IV} + 16_{II} + 2_I$	$18_{II} + 2_I, 1_{III} + 17_{II} + 1_I$
$42 = 1_{III} + 18_{II} + 3_I$	} ?
$44 = 21_{II} + 2_I$

Text-fig. 1

All figures are drawn with an ABBE's Camera Lucida; Zeiss $\frac{1}{12}$ objective, Huyghens ocular 15 $\times$. Magnification is ca. $\times 1750$.

Text-fig. 1. a-i, polar view of heterotypic metaphase in pollen mother cells of parents, F_1 and F_2 , a, D-2g. b, D-2f. c, D-2g $\times$ D-2f. d-f, sterile chromosome combinations in the F_2 ; d, $19_{II} + 0_I$. e, $19_{II} + 1_I$. f, $20_{II} + 0_I$, g-i, fertile chromosome combinations in the F_2 ; g, $19_{II} + 2_I$; h, $20_{II} + 1_I$. i, $21_{II} + 0_I$.

Text-fig. 2

Text-fig. 2. Heterotypic division in the pollen mother cells of unexpected chromosome combinations; a-c, $44=21\text{II} + 2\text{I}$.

a, polar view of metaphase. b and c, polar view of anaphase showing 21 chromosomes in b and 23 in c. d and e, $1\text{IV} + 16\text{II} + 2\text{I}$ polar and side view of metaphase showing 1 tetravalent, 16 bivalents and 2 univalents.

FERTILITY IN PARENTS, F_1 AND F_2

It is already known, that environmental conditions have a strong influence on the fertility of plants. In this respect, it is worth while to report that the fertility of D-2f and D-2g was very low in Hokkaido (see KIHARA, 1924) and high in Kyoto (KIHARA, 1925). The experience of our Laboratory tells us that the plants having sterile combinations are easily obtained in Kyoto, the viability of seeds being in this case low. From these facts we may conclude that locality plays an important rôle in sterility.

Our materials were collected with great care in the years 1927—28. The conditions would not be quit identical, but the average fertility of the parents was almost the same in the two years. The results are given in Table IV.

TABLE IV.

Chro. no.		Grains per spike	Grains per spiklet	Spiklets per spike	Number of observed ears	
P	D-2f 40	40.64±1.17	2.0948	19.40±0.18	50	
	D-2g 40	21.50±0.93	1.3371	16.08±0.20	50	
F ₁	D-2g × D-2f 40	56.80±2.61	2.3667	24.00±0.00	5	
	D-2f × D-2g 40	56.50±1.33	2.4565	23.00±0.00	6	
F ₂	Sterile combinations	38	0.78	0.0052	15.05	37
		39	20.80	1.2297	16.92	342
		40	31.46	1.8845	16.69	69
	Fertile combinations	40	42.93	2.0775	20.67	238
		41	44.64	2.1315	20.94	246
		42	48.07	2.2641	21.23	61

A plant having the chromosome combination $38 = 19_{II} + 0_I$ (Pl. III, Fig. 2a) shows nearly complete sterility. On the other hand individuals with $39 = 19_{II} + 1_I$ were not completely sterile, but among these plants two classes in regard to sterility were apparently recognized. While one group of plants had a comparatively rich fertility (Pl. III, Fig. 2c), the other group showed very poor fertility (Pl. III, Fig. 2b). It may be supposed that the difference of fertility is due to the difference of chromosomes which are lost in these chromosome plant classes, because, it is a fact that D-2g is usually lower in fertility than D-2f. Therefore, a difference of fertility grades between the plants which lost f+gg and the plants which lost ff+g may be expected.

In F₂ two types of 40-chromosome plants having sterile combination among dwarf plants were produced: viz. D-2f type (Pl. III, Fig. 2e) and D-2g type (Pl. III, Fig. 2d). However it is very difficult to distinguish them definitely without further investigation. Accordingly, the average number of the fertility of parents compared with F₂-plants having 40 sterile combination are given below (Table V).

TABLE V

Grains per spike	Grains per spiklet	Spiklets per spike	Number of observed ears
40=20 _{II} +0 _I in F ₂31.46	1.8845	16.69	69
40=20 _{II} +0 _I (D-2f31.07 and D-2 g mixed in equal numbers)	1.7514	17.74	100

As shown by Table V the fertility of the 40-chromosome plants and that of the parents is nearly the same. The 40-, 41- and 42-chromosome plants (Pl. III, Fig.

2f) show a high fertility, and among these the 42-chromosome plant has the highest fertility.

VARIATION OF CHROMOSOME NUMBERS IN THE F_3 GENERATION

The seeds (5 in all) of the 38-chromosome plants (F_2) failed to germinate and proved to be completely nonviable. If they were viable, individuals of the chromosome number (38) would be constant.

In 39-chromosome plants there are two kinds of gametes; one having 19 and the other 20 chromosomes. Accordingly, we may expect 38-, 39- and 40-chromosome plants in the next generation. They are found in reality, as given in the following Table.

TABLE VI
Segregates from $19_{II} + 1_I$ - F_2 plants

Chromosome numbers and combinations	Number of observed plants
$F_3 \begin{cases} 38 = 19_{II} + 0_I \\ 39 = 19_{II} + 1_I \\ 40 = 20_{II} + 0_I \end{cases}$	7 50 9

As is clear, in the F_3 -progeny which were produced from 39-chromosome plants of the F_2 , I succeeded in obtaining the series of all expected chromosome combinations. Besides, I found two unexpected combinations (Table VII), which were perhaps derived from irregular division of chromosomes during the gametogenesis of F_2 -plants.

TABLE VII

Chromosome numbers and combinations	Number of observed plants
$F_3 \begin{cases} 37 = 18_{II} + 1_I \\ 38 = 18_{II} + 2_I \end{cases}$	1 3

The chromosome combination of the 40-chromosome plants with $20_{II} + 0_I$ in the F_2 and that of the parents is precisely the same. The number of chromosomes and their combination in the next generation were, as can be expected, constant.

I have not studied especially the F_3 -progeny of the $19_{II} + 2_I$ -plants. However, there is no doubt that these plants produce the 6 types of the chromosome combinations in the F_3 generation as from the F_1 -hybrids.

From the results of NISHIYAMA's investigations (1928), we can say that the 41-chromosome plant produces the 40-chromosome plants with sterile combination and 41- and 42-chromosome plants in the F_3 generation. It is useless to say that the 42-chromosome plant is constant in chromosome number.

From theoretical expectation and from my own results, the variation of chromosome numbers in F_3 is summed up in Table VIII.

TABLE VIII

Number of chromosomes in the F ₂	Number of chromosomes in the F ₃					
	Sterile combinations			Fertile combinations		
Sterile combinations $\begin{cases} 38 \\ 39 \\ 40 \end{cases}$	(38) 38 39 40 40					
Fertile combinations $\begin{cases} 40 \\ 41 \\ 42 \end{cases}$	38	39	40	40	41	42
			40		41	42
						42

Some plants with unexpected chromosome combination, which is found among F₂-plants have shown segregation as shown in Table IX. The trivalent (or tetravalent) chromosome is recognized almost in every nuclear plate. Therefore we might say that such (trivalent or tetravalent) is a constant feature of these individuals. However, further investigation is necessary to determine thier bearing on the heredity.

TABLE IX

Chromosome numbers and combinations in the F ₂	Chromosome numbers and combinations in the F ₃
$38 = 1_{IV} + 16_{II} + 2_I$	$38 = 1_{IV} + 17_{II}$
	$39 = 19_{II} + 1_I$
	$40 = 20_{II} + 0_I$
$42 = 1_{III} + 18_{II} + 3_I$	$39 = 1_{III} + 17_{II} + 2_I$
	$40 = 1_{III} + 18_{II} + 1_I$
	$41 = 20_{II} + 1_I$

STERILITY

It has been already mentioned that D-2g is usually more sterile than D-2f. From this fact I attempted to make clear the correlation of the difference of lost chromosomes and the degree of sterility. In order to study this problem I used 39-chromosome plants ($19_{II} + 1_I$) which are found among F₂-progeny. In this case one univalent chromosome, which appeared in the reduction divisions of pollen mother cells was either f- or g-chromosome. Accordingly, if the univalent be f-chromosome, this plant may be classified as D-2g type (Pl. III, Fig. 2b), all progeny from these plants having no g-chromosome. On the other hand if the univalent chromosome is g-chromosome, these plants and all progeny lack f-chromosome (D-2f type) (Pl. III, Fig. 2c). The classification of the two types is not easy, as the morphological difference between $19_{II} + f$ and $19_{II} + g$ is not quite identical with that of D-2f and D-2g. The combination of 19 chromosomes is different in this case. From this reason we have examined only clear cases. 38-chromosome plants were

very short and showed miserable vegetative development. We could hardly distinguish 38-chromosome plants derived from $19_{II} + f$ and $19_{II} + g$. The seeds were counted in these plants. A comparison between chromosome combination and sterility in F_3 are shown in Table X.

TABLE X

Supposed chromosome combinations	Grains per spike	Grains per spikelet	Spiklets per spike	Number of observed ears
$F_2 \dots\dots 19_{II} + g$	26.03	1.3699	19.00	34
$F_3 \dots\dots \left\{ \begin{array}{l} 19_{II} + 0_I \\ 19_{II} + g \\ 19_{II} + gg \end{array} \right.$	0	0	10.67 ± 1.21	3
	22.25 ± 1.01	1.3485	16.50 ± 0.21	133
	24.69 ± 2.41	1.6134	15.31 ± 0.59	36
$F_2 \dots\dots 19_{II} + f$	24.47	1.3394	16.27	30
$F_3 \dots\dots \left\{ \begin{array}{l} 19_{II} + 0_I \\ 19_{II} + f \\ 19_{II} + ff \end{array} \right.$	1.71 ± 0.61	0.1250	13.71 ± 0.68	14
	17.05 ± 1.13	1.0471	16.29 ± 0.29	86
	13.83 ± 3.47	1.1857	11.67 ± 0.69	6

As shown in Table X the 38-chromosome plant shows nearly complete sterility. The classification of 39-chromosome plants into two types, $19_{II} + f$ and $19_{II} + g$, may be justified, the former being high in sterility, and the latter low. Also in the 40-chromosome plants there are two classes with different combinations namely $19_{II} + ff$ ($20_{II} + 0_I = D-2g$ type) and $19_{II} + gg$ ($20_{II} + 0_I = D-2f$ type). The former shows a higher degree of sterility than the latter.

As mentioned above, it is evident that the plant which lost f-chromosome (from *spelta* = $19_{II} + ff + gg$) showed a higher degree of fertility than the plant which lost g-chromosome. From this assumption we can say that the sterility in the offspring of pentaploid wheat hybrids is not only due to the chromosome combination but also to the difference of the lacking chromosomes.

Although the relative germination faculty may be different, pollen grains having 19 and 20 chromosomes were all effective. The almost complete sterility of 38-chromosome plants is therefore at least partly due to the sterile chromosome combination. Therefore we can decisively conclude, as already mentioned by KIHARA (1924) in his thesis, that "die Sterilität der Gameten nur eine teilweise ist" (p. 131) and "die Art und Weise der Chromosomenkombination scheint aber wenigstens bei den pentaploiden Bastarden des Weizens bei der Verschiebung des Sterilitätsgrades ein wichtiges Moment auszumachen" (P. 132.)¹⁾

THOMPSON and HOLLINGSHEAD (1927) have come to the same conclusion:

¹⁾ Abortion of pollen grains is the cause of non fertilized ovules, while the death of embryos, seeds and young plants are due to the combination of chromosomes. The study on this subject is now in progress by Prof. KIHARA.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

SUMMARY

1. In the F_1 -hybrids between two dwarf plants D-2f ($20_{II} = 19_{II} + g_g$) and D-2g ($19_{II} + ff$) there are 19 bivalents and 2 univalents ($f + g$) in the heterotypic division. The 19 bivalents divide in the usual manner and behave quite normally throughout both divisions. At the time of heterotypic division the 2 univalents divide equationally and pass to each pole, but in the homeotypic division the 2 halves of univalents pass at random to either pole without division. As the result the gametic chromosome number varies from 19 to 21.

2. It is therefore theoretically expected, and experimentally proved that the chromosome numbers and the combination in F_2 are $19_{II} + 0_I$, $19_{II} + 1_I$, $20_{II} + 0_I$, $19_{II} + 2_I$, $20_{II} + 1_I$ and $21_{II} + 0_I$ (Table II).

3. In the offspring of 39-chromosome plant ($19_{II} + 1_I$), individuals with the chromosome combination $19_{II} + 0_I$, $19_{II} + 1_I$ or $20_{II} + 0_I$ are found. Therefore, the pollen grains having 19- and 20-chromosomes must be effective.

4. All offspring from the plant with $19_{II} + f$ have no g -chromosome and the offspring from the plant with $19_{II} + g$ lack f -chromosome. The former shows a higher degree of sterility than the latter. Therefore, we may conclude that the degree of sterility is not only due to the chromosome combination but also to the difference of the lacking chromosomes.

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EXPLANATION OF PLATE III

- Fig. 1. Parents and F_1 -plant; a, b, D-2g. D-2f. c, F_1 (D-2g $\times$ D-2f). $\frac{1}{8}$ normal size.
 Fig. 2. F_2 plants from the F_1 ; a-e, plants with sterile combinations: a, $33 = 19_{II} + 0_I$. b, $19_{II} + f$. c, $19_{II} + g$. d, $19_{II} + ff$ (=D-2g type). e, $19_{II} + gg$ (=D-2f type). f, a plant with fertile combination. $\frac{1}{8}$ normal size.
 Fig. 3. Ears of D 2g $\times \frac{1}{2}$
 Fig. 4. Ear of D-2f $\times \frac{1}{2}$
 Fig. 5. Ear of F_1 -plant (D-2g $\times$ D-2f) $\times \frac{1}{2}$