Genetic effect of the *Aegilops caudata* plasmon on the manifestation of the *Ae. cylindrica* genome

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In the course of reconstructing *Aegilops caudata* from its own genome (CC) and its plasmon, which had passed half a century in common wheat (genome AABBDD), we produced alloplasmic *Ae. cylindrica* (genome CCDD) with the plasmon of *Ae. caudata*. This line, designated (*caudata*)-CCDD, was found to express male sterility in its second substitution backcross generation (SB₂) of (*caudata*)-AABBCCDD pollinated three times with the *Ae. cylindrica* pollen. We repeatedly backcrossed these SB₂ plants with the *Ae. cylindrica* pollen until the SB₅ generation, and SB₅F₂ progeny were produced by self-pollination of the SB₅ plants. Thirteen morphological and physiological characters, including pollen and seed fertilities, of the (*caudata*)-CCDD SB₅F₂ were compared with those of the euplasmic *Ae. cylindrica*. The results indicated that the male sterility expressed by (*caudata*)-CCDD was due to genetic incompatibility between the *Ae. cylindrica* genome and *Ae. caudata* plasmon that did not affect any other characters of *Ae. cylindrica*. Also, we report that the genome integrity functions in keeping the univalent transmission rate high.

Key words: *Aegilops cylindrica*, cytoplasmic male sterility, genome manifestation, plasmon transfer, univalent transmission rate

INTRODUCTION

Genetic autonomy of the cytoplasmic genetic system, or plasmon, from the nuclear genome is one of the central themes in eukaryote genetics. Evidently, the plasmon has diversified along with nuclear genome differentiation during the evolution of the *Triticum* (wheat)-*Aegilops* complex (Tsunewaki, 1996, 2009). However, this does not necessarily mean that plasmon diversification took place after the genome differentiation. *Ae. cylindrica* is a tetraploid species (2n = 28) having the genome constitution CCDD, and is assumed to have originated from spontaneous chromosome doubling of an F₁ hybrid between *Ae. squarrosa* (synonymous to *Ae. tauschii*, 2n = 14, genome DD) and *Ae. caudata* (synonymous to *Ae. markgrafii*, 2n = 14, CC) (Kihara, 1944), the former being the plasmon donor to *Ae. cylindrica* (Tsunewaki, 1996).

To obtain evidence of genetic autonomy of the plasmon from a co-existing genome, we attempted to reconstruct an *Ae. caudata* plant from its own genome (CC) and the plasmon that had passed half a century in common wheat (2n = 42, genome AABBDD). At an early stage of this

work, it became necessary to transfer the *Ae. caudata* plasmon from the alloplasmic common wheat to *Ae. cylindrica*, because its direct transfer to *Ae. caudata* was not possible. Therefore, we bred an alloplasmic line of *Ae. cylindrica*, (*caudata*)-CCDD, by transferring the *Ae. caudata* plasmon of the alloplasmic wheat, (*caudata*)-AABBDD, at its 50th substitution backcross generation (SB₅₀), to *Ae. cylindrica*.

In the SB₂ generation of (*caudata*)-CCDD, we found that some plants exhibited partial male sterility. We carried out an investigation to clarify whether this male sterility was caused by the *Ae. caudata* plasmon alien to *Ae. cylindrica* or by the nuclear gene interaction between the C and/or D genome derived from *Ae. caudata*, *T. aestivum* and *Ae. cylindrica*. To clarify this point, we successively backcrossed the (*caudata*)-CCDD SB₂ to SB₄ plants with the *Ae. cylindrica* pollen, and the SB₅F₂ progeny produced by self-pollination of the SB₅ plants were compared to the self-pollinated euplasmic *Ae. cylindrica* plants. In those successive generations, 11 to 13 morphological and physiological characters of (*caudata*)-CCDD were observed and compared with those of the euplasmic *Ae. cylindrica*.

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MATERIALS AND METHODS

Plant materials The following designation is used for all alloplasmic lines produced in this investigation: a plasmon donor in parentheses, hyphenated to a genome donor or its genome constitution. For example, (*caudata*)-Tve SB₅₀ indicates the 50th substitution backcross generation of the common wheat strain Tve with the *Ae. caudata* plasmon. The *Ae. caudata* strain used as the plasmon donor to this alloplasmic common wheat was *Ae. caudata* var. *polyathera* KU6-1. This alloplasmic common wheat line was produced by Kihara (1959) and its backcrossing has been continued until now by the present senior author (Tsunewaki, 2009, unpublished data). The two *Aegilops* species *Ae. caudata* var. *typica* KU6-2 (2n = 14, genome CC) and *Ae. cylindrica* var. *typica* KU7-2 (2n = 28, CCDD) were also employed in the present investigation. All three *Aegilops* accessions originated from the

Aegilops-Triticum germplasm collection of Kyoto University, Japan (Tanaka, 1983).

Methods Procedures for producing the alloplasmic *Ae. cylindrica* lines (*caudata*)-CCDD SB₂ - SB₅F₂ are summarized in Table 1.

To begin with, (*caudata*)-AABBDD SB₅₀ was crossed as female to *Ae. caudata*, giving rise to the (*caudata*)-ABCD F₁ hybrid. This hybrid was treated with 0.5% aqueous colchicine solution for five days (Sears, 1941), successfully producing 10 seeds of the amphidiploid (*caudata*)-AABBCCDD. Next, this amphidiploid was crossed as female to *Ae. cylindrica*, producing an F₁ hybrid, (*caudata*)-ABCCDD. The F₁ was backcrossed five times with the pollen of *Ae. cylindrica* up to the SB₅ generation, which was self-pollinated, giving rise to a fixed progeny, (*caudata*)-CCDD SB₅F₂ line. In each generation, 2n chromosome numbers in root-tip cells and chromosome pairing configu-

Table 1. Outline of producing an alloplasmic line of *Ae. cylindrica* var. *typica* (2n = 28, genome CCDD) having the plasmon of *Ae. caudata* var. *polyathera* (2n = 14, genome CC), by repeated backcrosses of the (*caudata*)-AABBCCDD by *Ae. cylindrica* as the recurrent pollen parent

Step/Year	Cross (♀ × ♂) ¹⁾	Product	Chr. no. (2n)
1) 2000–'01	(c)-AABBDD SB ₅₀ × <i>Ae. caudata</i>	(c)-ABCD F ₁	2n = 28, 28'
2) 2002–'03	Colchicine treatment of (c)-ABCD	(c)-AABBCCDD	2n = 56, 28"
3) 2005–'06	(c)-AABBCCDD × <i>Ae. cylindrica</i>	(c)-ABCCDD F ₁	2n = 42, 14" + 14'
4) 2006–'07	(c)-ABCCDD F ₁ × <i>Ae. cylindrica</i>	(c)-CCDD + i' SB ₁ ²⁾	2n = 28 + 2 – 12
5) 2007–'08	(c)-CCDD + ii' SB ₁ ³⁾ × <i>Ae. cylindrica</i>	(c)-CCDD + iii' SB ₂ ⁴⁾	2n = 28 + 0 – 5
6) 2008–'09	(c)-CCDD SB ₂ ⁵⁾ × <i>Ae. cylindrica</i>	(c)-CCDD SB ₃	2n = 28, 14"
7) 2009–'10	(c)-CCDD SB ₃ × <i>Ae. cylindrica</i>	(c)-CCDD SB ₄	2n = 28, 14"
8) 2010–'11	(c)-CCDD SB ₄ × <i>Ae. cylindrica</i>	(c)-CCDD SB ₅	2n = 28, 14"
9) 2011–'12	(c)-CCDD SB ₅ selfing	(c)-CCDD SB ₅ F ₂	2n = 28, 14"

¹⁾ SB: substitution backcross; the attached subscript indicates the SB generation.

²⁾ 2n = 30–40 SB₁ plants having 2–12 A and/or B genome chromosomes were obtained.

³⁾ Of these SB₁ plants, 2n = 30–33 plants having 2–5 A and/or B genome chromosomes were used for further backcrossing.

⁴⁾ The 119 SB₂ plants produced had 2n = 28–33 chromosomes, including 0–5 A and/or B genome chromosomes.

⁵⁾ Of these SB₂s, plants with 2n = 28, forming 14" in the first meiotic metaphase (MI) (Fig. 1B), were selected as the female to produce SB₃ offspring by further backcrossing with *Ae. cylindrica*.

Note) (c) in this and other tables means the *Ae. caudata* plasmon.

Table 2. Results of repeated backcrossing of (*caudata*)-AABBCCDD and its offspring with *Ae. cylindrica* pollen

Cross combination (♀ parent) ¹⁾	No. ears poll.	No. florets poll.	No. seeds set	% Seed set	No. seeds sown	No. seeds germ.	% Seed germ.	% Cross success ²⁾
(c)-AABBCCDD (2n = 56)	45	1,002	96	9.6	16	12	75.0	7.2
(c)-ABCCDD F ₁ (2n = 42)	90	2,066	71	3.4	86 ³⁾	62	72.1	2.5
(c)-CCDD SB ₁ (2n = 30–33)	147	2,796	700	25.0	240	194	80.0	20.0
(c)-CCDD SB ₂ -SB ₄ (2n = 28) ⁴⁾	30	328	297	90.5	18	16	88.9	80.5

¹⁾ Pollen parent of all crosses is *Ae. cylindrica* var. *typica*.

²⁾ [Seed setting rate] × [seed germination rate] in percentage.

³⁾ Seeds set in the third florets are included.

⁴⁾ Summary of the SB₂-SB₄ generations.

ration in pollen mother cells (PMCs) were observed by the squash and smear method, respectively. The results are summarized in the rightmost column of Table 1.

All the crossing was made by manual emasculation and hand pollination. Self-pollinated ('selfed' hereafter) seed fertility was estimated by the seed setting in the 1st and 2nd florets in all spikelets except one or two underdeveloped basal and apical ones. The percent cross success was calculated as the product of the percent seed setting of the cross and the percent seed germination rate.

RESULTS

Production of alloplasmic *Ae. cylindrica* with the *Ae. caudata* plasmon The results of crosses in Steps 3 to 8 (Table 1) are collectively shown in Table 2; the results of the last three steps from (*caudata*)-CCDD SB₂ to SB₄ backcrossed with *Ae. cylindrica* pollen were pooled.

The first cross, (*caudata*)-AABCCDD × *Ae. cylindrica*, was difficult as is clear from its low cross success rate of 7.2%. Sixteen seeds selected from 96 seeds produced by hand pollination were sown, and 12 seedlings were grown. An F₁ plant that had 2n = 42 with a probable genome constitution ABCCDD revealed an average meiotic chromosome configuration of 0.98^{'''} + 1.48^{'''} + 10.46^{''} +

12.35['] with 0.09 penta- and 0.02 hexavalents. This indicated that the C- and D-genome chromosomes mostly formed bivalents or multivalents with their homologues, whereas the A- and B-genome chromosomes stayed in univalents.

The second cross, (*caudata*)-ABCCDD F₁ × *Ae. cylindrica*, was the most difficult of those made in the present investigation, being evident from its lowest cross success rate of 2.5%. All 86 seeds produced, including 15 seeds set in the third florets, were sown, and chromosome checking was successfully conducted with 60 of the 62 viable seedlings (Table 3).

We pollinated more than two thousand florets of the F₁ hybrid, expecting to get 2n = 28 plants with the complete CCDD genome constitution devoid of the A- and B-genome chromosomes in the SB₁ progeny. However, we did not get any 2n = 28 plants, or even any 2n = 29 plants. This indicated that the transmission rate of the A- and B-genome univalents was much higher in the ABCCDD hybrid than in the 21 monosomics of common wheat, as discussed later.

As shown in Table 4, we selected 2n = 30 to 33 plants having two to five A- and/or B-genome univalents to increase the chance to obtain 2n = 28 plants with the complete CCDD genome constitution in the SB₂ progeny.

Two SB₁ plants with 2n = 30 and 31 produced no SB₂ progeny. Five SB₁ plants with 2n = 32 and 14 SB₁ plants with 2n = 33 produced 200 and 488 SB₂ seeds, respectively. From each of the SB₂ progeny, 120 seeds were sown and the somatic chromosome numbers of 119 out of the 194 germinated SB₂ seedlings were determined (Table 5).

The somatic chromosome numbers of the 119 SB₂ seedlings ranged from 2n = 28 to 33, of which 89 plants still retained one to five A- and/or B-genome chromosomes. We obtained 30 SB₂ plants having 2n = 28. The meiotic configuration of six of the 30 2n = 28 SB₂ plants was determined (Table 6).

Three of the six plants examined had a modal chromosome configuration of 14^{''}, like the euplasmic *Ae. cylindrica* plant (Fig. 1, A and B). This indicated that the *Ae. cylindrica* CCDD genomes were restored in those plants.

Table 3. Somatic chromosome numbers in root-tip cells of SB₁ plants produced by backcrossing (*caudata*)-ABCCDD F₁s (2n = 42, modal meiotic configuration 14^{''} + 14[']) with *Ae. cylindrica* pollen

Chromosome number (2n)	No. plants obtained	Chromosome number (2n)	No. plants obtained
28	0	36	8
29	0	37	5
30	1	38	1
31	1	39	2
32	5	40	1
33	15	41	0
34	10	42	0
35	11	Total	60

Table 4. Results of backcrossing (*caudata*)-CCDD SB₁ plants with 2n = 30–33 by *Ae. cylindrica* pollen

Cross combination (♀ parent) ¹⁾	No. SB ₁ plants	No. ears poll.	No. florets poll.	No. seeds set	% Seed set	No. seeds sown	No. seeds germ.	% Seed germ.	% Cross success ²⁾
2n = 30 SB ₁ parent	1	3	62	12	19.4	0	—	—	—
2n = 31 SB ₁ parent	1	3	58	0	0.0	—	—	—	—
2n = 32 SB ₁ parents	5	40	772	200	25.9	120	102	85.0	22.0
2n = 33 SB ₁ parents	14	101	1,904	488	25.6	120	92	76.7	19.6
Total	21	147	2,796	690	24.7	240	194	80.8	20.0

¹⁾ Pollen parent of all crosses is *Ae. cylindrica* var. *typica*.

²⁾ [Seed setting rate] × [seed germination rate] in percentage.

Table 5. Somatic chromosome numbers in root-tip cells of (*caudata*)-CCDD + i¹⁾ SB₂ seedlings

Female parent		Chromosome no. (2n) of SB ₂ progenies								%
2n	♀ parent	28	29	29 + t ²⁾	30	31	32	33	Total	
2n = 32	21-4	4	12	1	7	1	2	0	27	—
“	31-2	9	9	1	11	2	0	1	33	—
Subtotal	—	13	21	2	18	3	2	1	60	21.7
2n = 33	24-2	13	9	0	4	3	0	0	29	—
“	31-7	2	4	0	9	1	1	0	17	—
“	31-8	2	8	0	1	1	0	1	13	—
Subtotal	—	17	21	0	14	5	1	1	59	28.8
Total	—	30	42	2	32	8	3	2	119	25.2

¹⁾ i: 4 to 5 univalents of A- and/or B-genome chromosomes.

²⁾ t: telocentric chromosome derived from an A- or B-genome chromosome.

Table 6. Meiotic chromosome configuration analysis of six 2n = 28 SB₂ plants

Plant	Chromo. no. (2n) ¹⁾	No. of PMCs						Modal chromosome configuration ²⁾	
		14"	12" + 1""	13" + 2'	12" + 1"" + 1'	Others	Total		
27-15	28	98	18	15	13	8	152	14"	(64.5%)
27-24	28	90	7	14	10	2	123	14"	(73.2%)
31-22	28	3	2	12	9	11	37	13" + 2'	(32.4%)
31-24	28	37	96	2	1	2	138	12" + 1""	(69.6%)
33-10	28	39	35	59	37	20	190	13" + 2'	(31.1%)
33-14	28	96	51	3	5	1	156	14"	(61.5%)

¹⁾ Determined by root-tip cell mitosis.

²⁾ Percentage of the PMCs showing the modal chromosome configuration is given in parentheses.

Two of the remaining three plants had a modal chromosome configuration of 13" + 2' and one had 12" + 1"". This indicated that one A- or B-genome chromosome remained in the former two plants and one reciprocal translocation was present in the latter.

We backcrossed two of the three SB₂ plants with 14" with *Ae. cylindrica* pollen. Their SB₃ progeny were further backcrossed twice with the same pollen to produce the SB₅ seeds. The results of all these backcrosses are compiled in Table 7.

In every backcross of the SB₂ to SB₄ parents, the seed set rate was about 90% with a seed germination rate of higher than 65%. Thus, the progeny of (*caudata*)-CCDD were stably maintained after the SB₃ generation.

Effects of the *Ae. caudata* plasmon on the genome manifestation of *Ae. cylindrica* In the (*caudata*)-CCDD SB₂ generation, some plants had non-dehiscing anthers in spite of their having 2n = 28 chromosomes. They showed a wide variation in selfed seed fertility, from 0 to 97%, with the average of $57.3 \pm 30.9\%$, in contrast to a narrow variation in the selfed seed fertility ($92.1 \pm 4.2\%$) of the euplasmic *Ae. cylindrica* plants (Table 8).

The genetic effect of the *Ae. caudata* plasmon on various morphological and physiological characters of *Ae.*

cylindrica was investigated with (*caudata*)-CCDD plants in the SB₂₋₅ generations and with euplasmic *Ae. cylindrica* plants grown in each of the corresponding years (Table 9). No consistent effect of the *Ae. caudata* plasmon was observed on any of the examined characters, including spike morphology (Fig. 1G), throughout the four backcross generations, except for the selfed seed fertility.

Seeds from hand-pollination of the alloplasmic lines were smaller than those from self-pollination, which might cause some growth retardation. A critical comparison of the alloplasmic and euplasmic lines was made using the self-pollinated seeds of the (*caudata*)-CCDD SB₅ line (SB₅F₂) and those of the euplasmic line; both lines were grown in the same year. Ten pots containing single plants of each of the two lines were placed in pairs at random on a bench.

The (*caudata*)-CCDD SB₅F₂ line showed significant difference from the euplasmic line in five characters: 3rd internode length, number of spikelets per spike, dry matter weight, pollen fertility and selfed seed fertility. The effects of the *Ae. caudata* plasmon on three characters (3rd internode length, number of spikelets per spike and dry matter weight) were inconsistent between the five generations, SB₂₋₅ and SB₅F₂. On the contrary, semi-sterility induction by the *Ae. caudata* plasmon was consistent in

Fig. 1. Meiotic chromosome pairing in PMCs, pollen grains and ears of *Ae. cylindrica* ($2n = 28$, genome CCDD) and its alloplasmic line (*caudata*)-CCDD. A–C: Meiotic chromosome pairing in a PMC of (A) euplasmic *Ae. cylindrica*, (B) alloplasmic *Ae. cylindrica*, (*caudata*)-CCDD, in the second backcross generation, SB_2 , and (C) (*caudata*)-CCDD in the SB_5F_2 generation. D–F: Pollen grains of (D) euplasmic *Ae. cylindrica* no. N-6 plant (Table 10; 96% pollen and 97% selfed seed fertility), (E) and (F) alloplasmic *Ae. cylindrica*, (*caudata*)-CCDD, no. C-6 and no. C-1 plants, respectively, in the SB_5F_2 generation (Table 10; 95% pollen and 63% selfed seed fertility for the former and 29% pollen and 63% selfed seed fertility for the latter). A high inter-plant variability of the pollen fertility in the alloplasmic *Ae. cylindrica* is evident. G: Ears of euplasmic *Ae. cylindrica* (two on the left) and of an alloplasmic (*caudata*)-CCDD in the SB_5F_2 generation (two on the right). No remarkable morphological difference was noticed between the ears of eu- and alloplasmic *Ae. cylindrica* lines.

Table 7. Results of repeated backcrossing of (*caudata*)-CCDD SB_2 plants with $2n = 28$, $14''$ by the *Ae. cylindrica* pollen one to three times

Cross combination (♀ parent) ¹⁾	No. SB_{2-4} plants	No. ears poll.	No. florets poll.	No. seeds set	% Seed set	No. seeds sown	No. seeds germ.	% Seed germ.	% Cross success
(c)-CCDD SB_2 , $2n = 14''$	2	6	44	42	95.5	6	6	100.0	95.5
(c)-CCDD SB_3 , $2n = 14''$	3	6	94	83	88.3	6	5	83.3	73.6
(c)-CCDD SB_4 , $2n = 14''$	3	6	104	97	93.3	6	4	66.7	62.2
(c)-CCDD SB_2 - SB_4 pooled	8	18	242	222	91.7	18	15	83.3	76.4

¹⁾ Pollen parent of all crosses is *Ae. cylindrica* var. *typica*.

Table 8. Distribution of selfed seed fertility in the (*caudata*)-CCDD SB_2 generation, compared to that of the euplasmic *Ae. cylindrica*

Material	Fertility class (%)					Total no. plants	Average fertility (%)
	0–20	21–40	41–60	61–80	81–100		
(c)-CCDD SB_2	4	3	3 ¹⁾	7	8 ²⁾	25	57.3 ± 30.9
<i>Ae. cylindrica</i>	0	0	0	0	12	12	92.1 ± 4.2

¹⁾ Two of the three plants were confirmed to have $2n = 28$, forming $13'' + 2'$ in MI.

²⁾ Three of the eight plants were confirmed to have $2n = 28$, forming $14''$ in MI.

Table 9. Morphological and fertility characteristics of alloplasmic *Ae. cylindrica* var. *typica* lines having the plasmon of *Ae. caudata* in the SB₂ to SB₅F₂ generations, as compared to those of its euplasmic lines grown in the same years

Character	Difference: (alloplasmics) – (euplasmics)				
	SB ₂	SB ₃	SB ₄	SB ₅	SB ₅ F ₂
No. plants examined ¹⁾	12/12	6/6	5/5	4/6	10/10
Heading date (No. days)	0.00	–0.17	+1.6*	+0.92	0.00
Ear number/plant	–5.3*	–3.2	+5.4	–10.3	–0.3
Plant height (cm)	+2.02	+1.55	+1.46	+4.71*	–0.22
3 rd internode length (cm) ²⁾	–	+0.23	+0.96*	+1.31	–1.00*
2 nd internode length (cm) ²⁾	+1.18*	–0.62	+0.76	+1.98	+0.06
1 st internode length (cm) ²⁾	–0.23	–0.60	–1.56	–0.27	+0.5
Flag leaf length (cm)	+0.55	–	+0.66	–1.10	+0.75
Spike length (cm)	+0.28	–0.22	–0.48	–0.85*	–0.30
No. spikelets/spike	–0.13	–0.27	+0.06	–0.52	+0.73**
Dry matter weight (mg) ³⁾	+0.04	+0.05	–	–0.32	+0.23*
Pollen fertility (%)	–	–	–	–	–34.0**
Selfed seed fertility (%)	–43.5**	–18.0	–36.1**	–19.7**	–25.5**

¹⁾ Number of alloplasmics/number of euplasmics.²⁾ Position from the top.³⁾ Weight of three air-dried culms, including seeds borne.

* and **: significant at the 5 and 1% probability level, respectively.

Table 10. Pollen and selfed seed fertilities of SB₅F₂ plants of (*caudata*)-CCDD compared with those of the euplasmic *Ae. cylindrica* var. *typica* KU7-2

(caudata)-CCDD			Euplasmic CCDD		
Plant	% Pollen fertility	% Selfed seed fertility	Plant	% Pollen fertility	% Selfed seed fertility
C-1	29.4	62.9	N-1	94.7	88.3
C-2	10.0	64.1	N-2	92.0	86.7
C-3	58.7	61.3	N-3	94.0	86.2
C-4	62.0	53.2	N-4	93.5	91.7
C-5	51.1	66.1	N-5	92.5	94.6
C-6	94.7	62.9	N-6	96.0	96.6
C-7	92.1	74.1	N-7	83.7	94.8
C-8	85.4	83.3	N-8	84.2	89.1
C-9	28.2	63.8	N-9	95.7	88.7
C-10	69.9	62.9	N-10	95.3	92.9
Aver. ¹⁾	58.2 ± 28.8	65.5 ± 8.1	Aver. ¹⁾	92.2 ± 4.5	91.0 ± 3.7

¹⁾ Average ± standard deviation.Note) Correlation coefficient between pollen and selfed seed fertility was $r = 0.375$ among the (*caudata*)-CCDD plants and $r = -0.071$ among the euplasmic CCDD plants, both being non-significant at the 5% level of probability for df = 8.

all generations, although the plasmon effect was not statistically significant in the SB₃ generation. The pollen fertility of the SB₅F₂ line was 34.0% lower than that of the euplasmic *Ae. cylindrica*. The normal seed setting rate

(90.5%) of backcrossed plants in the SB₂₋₄ generations (bottom row of Table 2) implied their normal female fertility.

As shown in Table 10, the pollen (Fig. 1D) and selfed seed fertilities of the euplasmic *Ae. cylindrica* were both higher than 90% on average with low intra-line variation. On the contrary, the (*caudata*)-CCDD SB₅F₂ line forming 14" in its meiotic metaphase I (Fig. 1C) showed high within-line variability in pollen fertility (Fig. 1, E and F) that was not correlated with the seed fertility ($r = 0.375$, non-significant). Altogether, we conclude that the *caudata* plasmon caused male sterility accompanied by its high within-line variation in (*caudata*)-CCDD.

DISCUSSION

Possible cause of the male sterility expressed in (*caudata*)-CCDD The *Ae. caudata* plasmon is known to cause cytoplasmic male sterility in many cultivars of 4x and 6x wheats (Kihara, 1959; Maan and Lucken, 1971; Tsunewaki, 1974). Kihara and Ohta (1970) reported hybrid sterility between two varieties of *Ae. caudata* var. *polyathera* and var. *typica*, and Endo and Katayama (1978) and Endo (1979) found some gametocidal chromosomes in *Ae. cylindrica* and *Ae. caudata*, respectively, which caused semi-sterility in the offspring of their hybrids with non-carriers of the chromosomes. All these findings suggest the involvement of different mechanisms in the expression of the male sterility observed in the pedigree of (*caudata*)-CCDD.

Table 11. Female transmission of univalent(s) from hybrids with various genome constitutions to backcrossed progeny

Cross combination (♀ × ♂) ¹⁾	No. univalent ²⁾	No. progeny	Transm. rate (%) ³⁾	Reference
ABCCDD × CCDD	14	60	93.0	Present work, Table 3
[CCDD + i'] ⁴⁾ × CCDD				
2a) i = 4	4	60	69.2	Present work, Table 5
2b) i = 5	5	59	49.5	Present work, Table 5
[AABBDD - 1'] ⁵⁾ × AABBDD	1	2,511	54.0	Tsunewaki (1963)
AABBD × AABB	7	250	88.3	Kihara & Wakakuwa (1935)
AABBD × AABBDD	7	232	80.8	Kihara & Wakakuwa (1935)

¹⁾ Italics: Genome(s) whose chromosomes formed univalents in MI.

²⁾ Univalents formed in the MI of the female parent.

³⁾ [Univalent transmission rate (%)] = 100% - [Univalent elimination rate (%)].

⁴⁾ 'i' shows univalents of A and/or B genome.

⁵⁾ Monosomics of the common wheat cultivar 'Chinese Spring'.

The present investigation indicated that the male sterility observed in the (*caudata*)-CCDD line was expressed under the fixed genotypic condition with normal bivalent formation, as is evident from the consistent expression of the sterility after many generations of backcross and self-pollination. Therefore, the heterozygosity of nuclear gene(s) and the presence of either alien chromosome(s) or a gametocidal chromosome are excluded as causal agents of the male sterility discovered here, and it can be concluded that its real cause is the cytoplasmic effect of the *Ae. caudata* plasmon.

Gandhi et al. (2005) compared chloroplast and nuclear microsatellite loci of 36 *Ae. cylindrica* accessions with those of seven accessions of *Ae. caudata* and 17 accessions of *Ae. squarrosa*. They found that 35 *Ae. cylindrica* accessions had chloroplast short sequence repeat (SSR) alleles similar to those of *Ae. squarrosa*, whereas one accession, CL-TK116, collected in Turkey, had chloroplast SSR alleles similar to those of *Ae. caudata*. Their result mostly confirmed that *Ae. cylindrica* originated from a cross between *Ae. squarrosa* (♀ parent) and *Ae. caudata* (♂ parent), as proposed by Kihara (1944) and Tsunewaki (1996). Murai and Tsunewaki (1986) reported the diphyletic origin of an allotetraploid species, *Ae. triuncialis* (2n = 28, genome CCUU), from the reciprocal crosses between *Ae. caudata* and *Ae. umbellulata* (2n = 14, UU). The presence of one *Ae. cylindrica* accession with the C-type plasmon may indicate a diphyletic origin of this species from the reciprocal crosses between the two parental species or a misclassification of this accession, similar to a case that happened in a group of *Ae. triuncialis* accessions (Tsunewaki et al., 2002a).

Effects of the *Ae. caudata* plasmon on the manifestation of the *Ae. cylindrica* genome The present investigation revealed that the genetic effect of the *Ae. caudata* plasmon on the manifestation of *Ae. cylindrica* genomes was limited to male fertility: the alien plasmon caused pollen and self-pollinated seed sterility, without exerting

any detectable effects on the other morphological and physiological characters investigated. Previously, Tsunewaki et al. (2002b) investigated the genetic effects of the *Ae. caudata* and many other plasmons of the *Triticum-Aegilops* complex on the genome manifestation of 12 genotypes of common wheat. They found that the *Ae. caudata* plasmon caused pollen and seed sterility in nine of the 12 genotypes and induced haploid and twin seedling formation in a single genotype, 'Salmon', which carries a 1BL-1RS wheat-rye translocation. They also found that the *Ae. caudata* plasmon did not affect 14 vegetative characters, including the ten characters studied in this investigation. It was not expected that the (*caudata*)-CCDD line would produce haploid and twin seedlings, because *Ae. cylindrica* has no 1RS chromosome arm, and actually no such seedlings were observed during the production of the (*caudata*)-CCDD line. Thus, the *Ae. caudata* plasmon exerted a similar effect on the genome manifestation of *Ae. cylindrica* and common wheat.

Effect of genome integrity on univalent transmission in *Triticum-Aegilops* hybrids Univalents formed in the first meiotic metaphase (MI) move in the first meiotic anaphase (AI) toward either opposite pole independently. Although a gamete is expected to receive a specific univalent at the probability of 1/2, the univalent is eliminated at a certain rate in the MI-AI and/or MII-AII (second meiotic metaphase and anaphase) stages, forming micronuclei in the cytoplasm. Univalent transmission rate is given by t, and its elimination rate is by 1-t, where t is estimated by the following formula:

$$t = T / (m / 2 \times N) = 2 \times T / (m \times N),$$

where m is the number of univalents in the female parent, N is the number of its next-generation offspring and T is the total number of univalents transmitted to the next-generation offspring. T is obtained by the formula $\sum_{ij} i$, where i is the univalent number class from 0 to m,

and j is the number of plants in each i class.

In the present investigation, the univalent transmission rate was estimated for the two cross combinations ABCCDD $\times$ CCDD and [CCDD + i'] $\times$ CCDD. In the latter cross combination, two types of female parent were used, namely, CCDD + 4' and CCDD + 5' (Table 11).

Eighty years ago, Kihara and Wakakuwa (1935) reported the transmission of the seven D-genome univalents in pentaploid wheat hybrids that were backcrossed with the tetra- and hexaploid parents. From their results, the average transmission rate of the D-genome univalents was estimated. Tsunewaki (1963) reported the average transmission rate of the 21 monosomes in the Chinese Spring monosomic series. All those results are compiled in Table 11.

The results shown in Table 11 indicate that when all seven chromosomes of a genome or 14 chromosomes of two genomes formed univalents, their average transmission rate became very high (93% for ABCCDD, 88% and 81% for AABBD). In other words, the rate of univalent elimination was very low (7, 12 and 19%, respectively). On the other hand, the average univalent transmission rate was 68% in CCDD + 4', 50% in CCDD + 5' and 54% in AABBD-1', where part of the chromosome complement of a genome(s) formed univalents. Their elimination rate was close to 50%.

A few investigations have been carried out to clarify the genetic factors that might be involved in controlling meiotic univalent transmission. Kaltsikes et al. (1970) extracted the AABB genomes from three common wheat cultivars and studied female and male univalent transmission in three 5x hybrids between the extracted AABB lines and their original 6x parents. They intended to determine whether univalent elimination was controlled by the D-genome univalents themselves or by the background AB genomes, because the AABB genomes of the three common wheat cultivars and those of their respective extracted AABBs should have been nearly the same. They found a distinct difference in the univalent transmission rate between the three cultivars, but did not find any clear association between meiotic behavior and the number of univalents transmitted. Recently, Silkova et al. (2014) analyzed the effect of six cases of the substitution of one or two wheat chromosomes by their rye homoeologues on the meiotic behavior of univalents, and found that the 2R/2D substitution suppressed the equational univalent division, whereas 6R/6A substitution resulted in a high frequency of univalent elimination.

In conclusion, the complete genome appears to have a function to suppress univalent elimination in meiosis to retain its own integrity.

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