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Studies on Cytodemes in Subtribe *Brassicinae* (Cruciferae)

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Summary

Hybridization and cytogenetical investigations of several wild species in the subtribe *Brassicinae* (Cruciferae) were carried out to learn inter- and intra-cytodeme differentiations of this plant group and to determine the cytodeme of 11 taxa whose chromosome numbers were recently studied by the authors. In all the intra-cytodeme hybrids, regular meiotic division was generally observed, though certain amounts of chromosomal changes have been accumulated in *B. fruticulosa* cytodeme. In contrast, every inter-cytodeme hybrid exhibited meiotic irregularities, while certain amounts of bivalent formation indicated the existence of partial chromosomal homology between different genomes. Of the 11 taxa under observation, 4 were classified into the existing cytodemes; *B. desnottesii* belongs to *Brassicaria* cytodeme, *B. fruticulosa* ssp. *mauritanica* and ssp. *radicata* to *B. cossoneana* cytodeme and one strain LEVGT-4 to *B. fruticulosa* cytodeme. The LEVGT-4 was once classified into *Es. laevigatum* ssp. *glabrum* but its taxonomical position was doubted. The remaining 7 species; *B. deflexa* (n=7), *B. gravinae* (n=20), *D. berthautii* (n=9), *D. siettiana* (n=8), *Es. strigosum* (n=8), *Es. varium* (n=7) and *S. aucheri* (n=7) were speculated to be independent from the known cytodemes because of the difficulty of hybridization. Meiotic behavior was described for 11 new inter-cytodeme hybrids; *B. barrelieri* × *B. campestris*, *B. barrelieri* × *B. nigra*, *B. maurorum* × *B. barrelieri*, *B. fruticulosa* × *Es. cardaminoides*, *B. maurorum* × *B. campestris*, *B. maurorum* × *B. nigra*, *B. maurorum* × *Es. varium*, *D. siettiana* × *B. nigra*, *D. virgata* × *B. campestris*, *E. sativa* × *D. tenuifolia* and *Es. varium* × *B. nigra*.

Of many wild cruciferous plants, the genera in the subtribe *Brassicinae* hold our attention because these members are related to crop Brassicas and are potential gene sources for improving these crops. Cytogenetics has provided important information for understanding the relations of these species that was defined by traditional taxonomy. A differentiation of aneuploidy which was speculated to occur as a result of numerical and constructural changes of chromosomes from an original genome, is the characteristic of this group (7, 8). Incorporating the

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Abbreviations: *B.*: *Brassica*, *D.*: *Diplotaxis*, *E.*: *Eruca*. *Es.*: *Erucastrum*, *S.*: *Sinapis*.

concept of 'deme' (2), Harberd (5) distinguished 36 diploid and 10 tetraploid cytodemes in this plant group.

The present investigation was carried out to learn the traits of inter- and intra-cytodeme differentiation of this plant group and to determine the cytodeme of 11 taxa whose chromosome number has been recently studied by the authors (10).

Materials and methods

The eleven species under investigation are listed with their origins in Table 1. These 11 species and other materials were taken from the genetic stocks in the Laboratory of Plant Breeding, Tohoku University. They are the same as those used in the numerical classification experiment by us (10, 11), in which their morphological characteristics and the chromosome numbers were described.

Plants were grown in a pot 24 cm in diameter in a glass house. Pistils of open flowers and buds were pollinated with fresh pollen. Care was taken to free the crossed inflorescences from the contamination of other pollen by bagging them. The harvested seeds were grown the next season, and true hybrids were determined by morphology and somatic chromosome counting. Root tip chromosomes were observed by Feulgen's squash method. Meiotic anthers were fixed in Carnoy's fluid containing a few drops of ferric acetate, and the meiotic chromosomes were observed by the acetocarmine smear method. Pollen fertility was determined by counting the acetocarmine stainable pollen grains to total under a sampled microscopic area. About 200 pollen grains were counted for one plant and the pollen fertility of a cross combination was presented by averaging the values for three or four hybrid plants.

TABLE 1. *List of Eleven Species Used as the Main Material*

Species	Ref. no* in stock	Gametic chromosome number	Origin
<i>Brassica</i>			
<i>deflexa</i>	Df-1	7	Iran
<i>desnottesii</i>	Ds-1	10	Morocco
<i>fruticulosa</i> ssp. <i>mauritanica</i>	Fr-401	16	Algeria
<i>fruticulosa</i> ssp. <i>radicata</i>	Fr-301	16	Algeria
<i>gravinae</i>	Gr-2	20	Algeria
<i>Diplotaxis</i>			
<i>berthautii</i>	BERTH-1	9	Morocco
<i>siettiana</i>	SIETA-1	8	Alboran
<i>Erucastrum</i>			
<i>laevigatum</i> ssp. <i>glabrum</i>	LEVGT-4	8	Morocco
<i>strigosum</i>	STRIG-1	8	S. Africa
<i>varium</i>	VARIM-2	7	Algeria
<i>Sinapis</i>			
<i>aucheri</i>	AUCHR-1	7	Iran

* Reference number of the genetic stocks in Laboratory of Plant Breeding, Tohoku University, Sendai.

Results

I. Intra-cytodeme hybridization

a) *B. fruticulosa* cytodeme

Hybridization was carried out for every combination of three species; *B. fruticulosa*, *B. maurorum* and *B. spinescens*, which belong to the *B. fruticulosa* cytodeme (5). Hybrid formation between these species was not difficult (TABLE 2-a). Eight bivalents were predominant more than 78 per cent, in the meiotic cells of these hybrids (FIG. 1-a). Cell plates with 7 bivalents and 2 univalents were also observed, though the frequency was low (FIG. 1-b). Pollen fertility varied from 95.3 to 34.3 per cent. Of these hybrids the F_1 s of *B. maurorum* \times *B. fruticulosa* showed high 8 bivalent formation and high pollen fertility as compared with the other hybrids with *B. spinescens*.

Besides these three species, LEVGT-4 was found to be easily crossable with *B. fruticulosa* as well as with *B. maurorum*. Eight bivalent formation of the former hybrid was as frequent as that of the intra-cytodeme hybrids (FIG. 1-c). The LEVGT-4 was once classified as *Es. laevigatum* ssp. *glabrum*, but this must belong to the *B. fruticulosa* cytodeme, being miss-classified.

b) *B. cossoneana* cytodeme

We pointed out that *B. fruticulosa* ssp. *mauritanica* and ssp. *radicata* had 16 pairs of chromosomes (10). *B. cossoneana* (= *B. fruticulosa* ssp. *cossoneana*) also has 16 pairs of chromosomes, being an autotetraploid of *B. fruticulosa* (5). The subspecies *mauritanica* and *radicata* were crossed with *B. cossoneana* and the hybrids were easily obtained. The hybrids showed regular chromosome configuration in the meiotic divisions (TABLE 2-b and FIG. 1-d). These two subspecies could be classified into the *B. cossoneana* cytodeme.

c) *Brassicaria* cytodeme

B. desnottesii was easily hybridized with *B. repanda*, and their hybrid showed regular meiotic chromosome behavior (TABLE 1-c). These two species were classified in *Brassicaria* section by Schulz (9). *Brassicaria* cytodeme involves *B. repanda*, *B. nudicaulis* and *B. saxatilis* according to Harberd (4, 5). *B. desnottesii* could be added to the cytodeme as a member.

A few plants were obtained in the cross between *B. gravinae* and *B. repanda*, but all the hybrids were matromorphic (TABLE 2-c). These two species have a similar plant type, but the seed shape is different. *B. gravinae* has globular seeds while the other species in the *Brassicaria* cytodeme have elliploidal ones. Although *B. gravinae* was provisionally classified into the *Brassicaria* cytodeme without a crossing experiment by Harberd (5), this species is possibly independent from the *Brassicaria* cytodeme.

TABLE 2. Data for Intra-cytodeme Hybrids; Crossability,

Cross combinations	Number of flowers (A) pollinated	Number of seeds (B) obtained	(B)/(A)
a. <i>Brassica fruticulosa</i> cytodeme (n=8)			
<i>B. fruticulosa</i> × <i>B. spinescens</i>			
Fr-103* × Sp-1	123	115	0.93
Fr-104 × Sp-1	161	683	4.24
Fr-202 × Sp-1	154	529	3.43
Fr-503 × Sp-1	66	1252	18.97
<i>B. spinescens</i> × <i>B. fruticulosa</i>			
Sp-1 × Fr-103	7	22	3.14
Sp-1 × Fr-104	24	0	0
<i>B. maurorum</i> × <i>B. fruticulosa</i>			
Ma-1 × Fr-202	32	147	4.59
Ma-2 × Fr-104	11	143	13.00
Ma-5 × Fr-104	14	196	14.00
<i>B. maurorum</i> × <i>B. spinescens</i>			
Ma-2 × Sp-1	78	138	1.77
<i>B. fruticulosa</i> × <i>Erucastrum laevigatum</i> ssp. <i>glabrum</i>			
Fr-103 × LEVGT-4	30	37	1.23
Fr-104 × LEVGT-4	133	107	0.80
<i>Es. laevigatum</i> ssp. <i>glabrum</i> × <i>B. fruticulosa</i>			
LEVGT-4 × Fr-104	45	0	0
<i>B. maurorum</i> × <i>Es. laevigatum</i> ssp. <i>glabrum</i>			
Ma-2 × LEVGT-4	68	127	1.86
Ma-5 × LEVGT-4	41	9	0.22
b. <i>B. cossoneana</i> (<i>B. fruticulosa</i> ssp. <i>cossoneana</i>) cytodeme (n=16)			
<i>B. fruticulosa</i> ssp. <i>cossoneana</i> × ssp. <i>mauritanica</i>			
Fr-201 × Fr-401	28	253	9.03
ssp. <i>radicata</i> × ssp. <i>cossoneana</i>			
Fr-301 × Fr-201	37	207	5.59
ssp. <i>radicata</i> × ssp. <i>mauritanica</i>			
Fr-301 × Fr-401	42	251	5.98
ssp. <i>mauritanica</i> × ssp. <i>cossoneana</i>			
Fr-401 × Fr-201	27	0	0
c. <i>Brassicaria</i> cytodeme (n=10)			
<i>B. gravinae</i> × <i>B. repanda</i>			
Gr-1 × Re-4	103	1	0.01
Gr-1 × Re-5	86	2	0.02
Gr-1 × Re-7	83	33	0.40
<i>B. gravinae</i> × <i>B. repanda</i>			
Re-4 × Gr-1	32	0	0
Re-5 × Gr-1	67	0	0
<i>B. gravinae</i> × <i>B. desnottesii</i>			
Gr-1 × Ds-1	26	8	0.31
<i>B. desnottesii</i> × <i>B. gravinae</i>			
Ds-1 × Gr-1	32	5	0.16
<i>B. repanda</i> × <i>B. desnottesii</i>			
Re-4 × Ds-1	16	89	5.56
Re-5 × Ds-1	28	46	1.64

Chromosome Conjugation at MI and Pollen Fertility

Somatic chromosome number	Number of plants observed	Number of PMCs observed	Chromosome conjugation at MI			pollen fertility (%)
			8II	7II+2I	others	
16	3	75	61 (81)	13	1	
16	4	100	93 (93)	6	1	36.9
16	8	200	176 (88)	19	6	48.6
16	4	100	78 (78)	13	9	
16	4	100	97 (97)	2	1	43.8
16	8	186	184 (99)	2	0	94.5
16	7	175	171 (98)	4	0	95.3
16	4	100	88 (88)	11	1	34.3
16	4	100	76 (76)	22	2	

			16II	15II+2I	others	
32	1	25	23 (92)	1	1	
32	2	50	42 (84)	1	7	

			10II	9II+2I	others	
20	3	75	66 (88)	8	1	
20	1	25	23 (92)	1	1	

TABLE 2.

Cross combinations	Number of flowers (A) pollinated	Number of seeds (B) obtained	(B)/(A)
d. <i>Diplotaxis pitardiana</i> cytodeme (n=11)			
<i>D. pitardiana</i> × <i>D. tenuifolia</i> PITAL-1 × TENFO-3	71	702	9.89
e. <i>Eruca</i> cytodeme (n=11)			
<i>E. sativa</i> × <i>E. vesicaria</i> ESATV-9 × EVESC-3	26	100	3.85
<i>E. vesicaria</i> × <i>E. sativa</i> EVESC-3 × ESATV-9	38	254	6.68
EVESC-5 × ESATV-9	7	85	12.14
f. <i>Erucastrum nasturtifolium</i> cytodeme (n=8)			
<i>Es. nasturtifolium</i> × <i>Es. leucanthum</i> NASTF-1 × LEUCT-1	72	389	5.40
NASTF-2 × LEUCT-1	131	1357	9.60
<i>Es. leucanthum</i> × <i>Es. nasturtifolium</i> LEUCT-1 × NASTF-2	194	993	5.12
g. <i>Sinapis arvensis</i> cytodeme (n=9)			
<i>S. turgida</i> × <i>S. arvensis</i> TURGD-1 × ARVNS-16	168	131	0.78

* Reference number of the genetic stocks in Laboratory

- d) *D. tenuifolia* cytodeme, *Eruca* cytodeme, *Es. nasturtifolium* cytodeme and *S. arvensis* cytodeme

The proposition made by Harberd (5) was confirmed for the respective cytodemes (TABLE 2-d, e, f, g): *D. pitardiana* was included in the *D. tenuifolia* cytodeme, *E. vesicaria* in the *Eruca* cytodeme, *Es. leucanthum* in the *Es. nasturtifolium* cytodeme and *S. turgida* in the *S. arvensis* cytodeme.

II. Hybridization of species whose cytodemes have not been defined

Regarding the following seven species; *B. deflexa*, *B. gravinae*, *D. berthautii*, *D. siettiana*, *Es. strigosum*, *Es. varium* and *S. aucheri*, we have tried to hybridize them with several other species in the genetic stocks to determine their cytodemes. Because species in an identical cytodeme should have the same number of chromosomes, the crossing was tried between species that have the same chromosome numbers. For example, *B. deflexa* (n=7) was used to cross with *D. eruroides*, *Hirschfeldia incana*, *Es. varium* and *S. aucheri*. We could not obtain any hybrids in these species combinations except one cross, *D. siettiana* × *B. nigra*. The hybrid obtained, however, revealed that the parental species belong to different cytodemes (TABLE 3). Because of the failure of hybridization and their morphological traits, these seven species were speculated to be independent from the cytodemes described by Harberd (5).

Continued

Somatic chromosome number	Number of plants observed	Number of PMCs observed	chromosome conjugation at MI			pollen fertility (%)
			11II	10II+2I	others	
22	3	66	59 (89)	6	1	
22	3	100	99 (99)	1	0	76.4
22	1	25	25 (100)	0	0	77.4
22	1	50	48 (96)	1	1	
16	2	50	43 (86)	5	2	82.3
16	1	25	21 (84)	3	1	58.9
18	3	75	70 (93)	5		

of Plant Breeding, Tohoku University. (): Percentage.

III. Inter-cytodeme hybridization

Data about inter-cytodeme crossing experiments and cytological observations on the hybrids are summarized in Table 3. Since examination for all the obtained seeds has not been accomplished, the number of seeds in the table includes false hybrid seeds and the value is larger than the true crossability.

a) *B. barrelieri*

Hybridizations of *B. barrelieri* with *B. campestris*, *B. nigra*, *B. fruticulosa* and *B. maurorum* were successful. In the hybrid, *B. barrelieri* × *B. campestris*, the mean frequency of bivalent formation was 2.60 and the number of bivalents ranged from 6 to 0. (FIG. 2-a). The number of bivalents was less in the hybrids, *B. barrelieri* × *B. nigra* and *B. barrelieri* × *B. fruticulosa* (FIG. 2-b).

While *B. fruticulosa* and *B. maurorum* belong to the same cytodeme, a difference of the bivalent formation was observed between the hybrids *B. barrelieri* × *B. fruticulosa* and *B. maurorum* × *B. barrelieri*. This may be due to intra-cytodeme differentiation of chromosomes or to the cytoplasmic effect of parental species.

b) *B. fruticulosa* and *B. maurorum*

B. fruticulosa was successfully hybridized with *B. campestris*, *B. barrelieri* and

TABLE 3. Data for Inter-cytodeme Hybrids:

Cross combinations	Number of flowers (A) pollinated	Number of seeds (B) obtained
<i>B. barrelieri</i> (n=10) × <i>B. campestris</i> (n=10)** Ba-103* × C-333	64	148
<i>B. barrelieri</i> (n=10) × <i>B. fruticulosa</i> (n=8) Ba-109 × Fr-104	15	48
<i>B. barrelieri</i> (n=10) × <i>B. nigra</i> (n=8)** Ba-109 × Ni-141	35	22
<i>B. maurorum</i> (n=8) × <i>B. barrelieri</i> (n=10)** Ma-1 × Ba-109	28	75
<i>B. fruticulosa</i> (n=8) × <i>B. campestris</i> (n=10) Fr-503 × C-333	29	31
<i>B. fruticulosa</i> (n=8) × <i>Es. cardaminoides</i> (n=9)** Fr-104 × CARDM-2	48	11
<i>B. maurorum</i> (n=8) × <i>B. campestris</i> (n=10)** Ma-1 × C-333	105	20
	123	94
<i>B. maurorum</i> (n=8) × <i>B. nigra</i> (n=8)** Ma-1 × Ni-141	110	66
	107	99
<i>B. maurorum</i> (n=8) × <i>Es. varium</i> (n=7)** Ma-1 × VARIM-8	37	48
<i>B. nigra</i> (n=8) × <i>B. oleracea</i> (n=9) Ni-141 × O-171	19	24
<i>D. siettiana</i> (n=8) × <i>B. nigra</i> (n=8)** SIETA-1 × Ni-116	35	436
<i>D. virgata</i> (n=9) × <i>B. campestris</i> (n=10)** DVIRG-12 × C-333	129	125
<i>E. sativa</i> (n=11) × <i>D. tenuifolia</i> (n=11)** ESATV-12 × TENFO-3	13	3
<i>Es. gallicum</i> (n=15) × <i>Es. nasturtifolium</i> (n=8) GALCM-1 × NASTF-3	18	2
<i>Es. varium</i> (n=7) × <i>B. nigra</i> (n=8)** VARIM-2 × Ni-116	15	46
	40	101

Reference number of the genetic stocks in

** Hybrids newly described by the present

Es. cardaminoides; and *B. maurorum* with *B. campestris*, *B. nigra*, *B. barrelieri* and *Es. varium*. The number of bivalent in the hybrid *B. fruticulosa* × *B. campestris* ranged from 5 to 0 and its mean value was 2.60 (FIG. 2-c). A similar range but a little lower mean value for bivalent formation was observed in the hybrid *B. maurorum* × *B. campestris*. Intra-cytodeme variation was found in the mean values in this case. The bivalent formations of the present materials were lower than those of Mizushima's observation, in which he described 7 bivalents in maximum and mean frequencies of 2.60–3.10 in four hybrid plants between *B. campestris* and *B. fruticulosa* (8).

In the hybrids *B. maurorum* × *B. nigra*, chromosome behavior varied with the different strains of *B. maurorum* used as the maternal parent. Maximum and mean values of bivalent formation were 5 and 1.73, respectively, when Ma-1 was

Crossability and Chromosome Conjugation at MI

(B)/(A)	Somatic chromosome number	Number of plants observed	Number of PMCs observed	Bivalents at MI	
				Range	Mean
2.31	20	3	127	6-0	2.60
3.20	18	1	51	4-0	1.20
0.63	18	2	100	4-0	1.73
2.68	18	1	24	2-0	0.58
1.07	18	1	50	5-0	2.60
0.23	17	2	50	3-0	0.50
0.19	18	1	50	5-0	2.38
0.76	18	1	32	5-0	2.03
0.60	16	7	217	5-0	1.73
0.93	16	2	125	7-0	2.58
1.30	15	1	26	4-0	2.38
1.26	17	1	75	5-0	2.43
8.23	16	1	53	6-0	3.51
0.97	19	2	54	6-0	1.46
0.23	22	3	64	5-0	1.72
0.11	23	2	26	8-7	7.96
3.07	15	3	100	5-0	1.72
2.53	15	3	37	4-0	1.27

Laboratory of Plant Breeding, Tohoku University.
experiment.

used; while they were 7 and 2.58 in the case of Ma-5 (FIG. 2-d).

In the inter-genus hybrid, *B. fruticulosa* × *Es. cardaminoides*, more than half of the observed PMC (pollen mother cells) did not form any bivalents. Its mean value of 0.50 was the lowest value among the hybrids observed in the present study. Another inter-genus hybrid, *B. maurorum* × *Es. varium* showed moderate bivalent formation; its mean value was 2.38.

c) Other crosses

Hybrids *B. nigra* × *B. oleracea*, *Es. varium* × *B. nigra*, *D. siettiana* × *B. nigra*, *D. virgata* × *B. campestris* and *E. sativa* × *D. tenuifolia* were raised. In all the hybrids, a certain amount of bivalent formation was observed, indicating partial homology of chromosomes between parent species (FIG. 2-e).

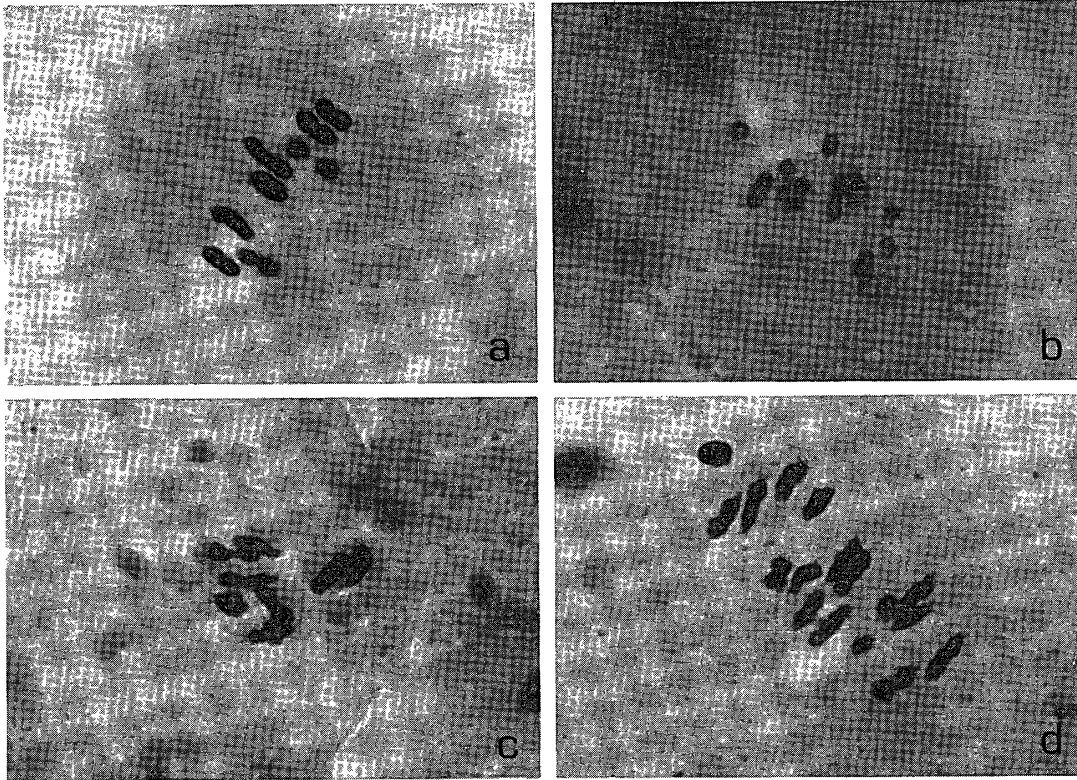


FIG. 1. Chromosomes at Metaphase I in intra-cytodeme hybrids.

- a. *B. maurorum* (Ma-2) × *B. fruticulosa* (Fr-4): 8II.
- b. *B. fruticulosa* (Fr-202) × *B. spinescens* (Sp-1): 7II+2I.
- c. *B. fruticulosa* (Fr-103) × *Es. laevigatum* ssp. *glabrum* (LEVGT-4): 8II.
- d. *B. fruticulosa* ssp. *radicata* (Fr-301) × *B. fruticulosa* ssp. *cossoniana* (Fr-201): 16II.

In the hybrid *Es. gallicum* × *Es. nasturtiifolium*, 8 bivalents and 7 univalents were predominant in the PMC (FIG. 2-f). This supports the view of Harberd and McArthur (6) that *Es. gallicum* is an amphidiploid between the *Es. nasturtiifolium* cytodeme and another one.

Discussion

Species in a cytodeme are considered to be originally identical but have recently differentiated ecologically and morphologically into different species that are classifiable by ordinary taxonomy. It is shown that they are accumulating a certain amount of chromosomal changes in a certain cytodeme. An example is the *B. fruticulosa* cytodeme in which *B. fruticulosa*, *B. maurorum* and *B. spinescens* are involved (4, 5). *B. fruticulosa* grows on dunes or slopes in coastal regions, *B. maurorum* is found on fields or roadsides extending inland from coastal regions and *B. spinescens* inhabits coastal rocky cliffs against the sea. *B. maurorum* (Ca. 200 cm in height) is taller than *B. spinescens* (30–50 cm) and *B. fruticulosa* is variable in height. *B. spinescens* has thick leaves with well developed cuticles that may be an adaptive character to its habitat on the coastal rocky cliffs. Takahata and

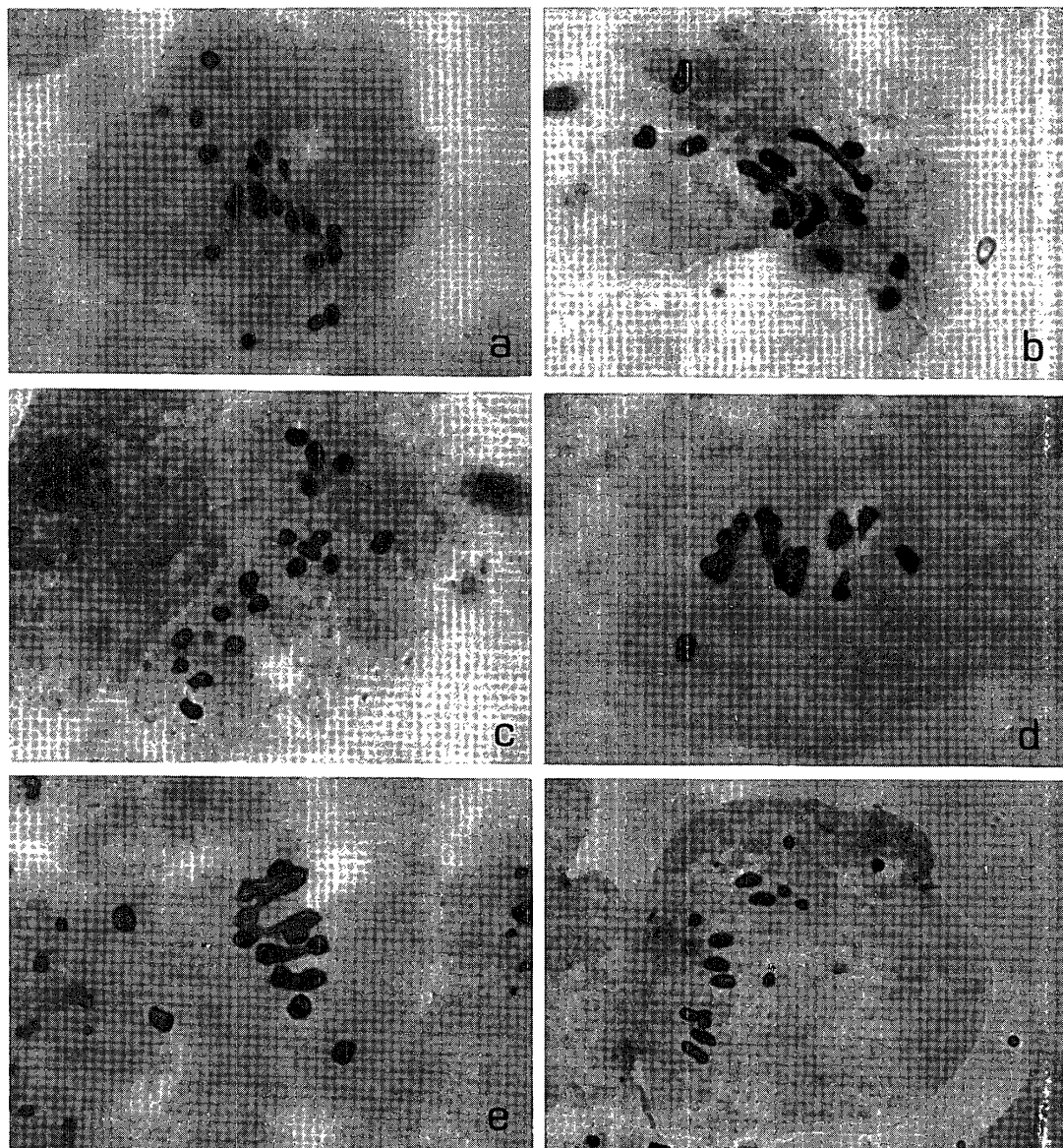


FIG. 2. Chromosomes at Metaphase I in inter-cytodeme hybrids.

- a. *B. barrelieri* (Ba-106) × *B. campestris* (C-333): 5II+10I.
- b. *B. barrelieri* (Ba-109) × *B. fruticulosa* (Fr-104): 4II+10I.
- c. *B. fruticulosa* (Fr-503) × *B. campestris* (C-333): 18I.
- d. *B. maurorum* (Ma-5) × *B. nigra* (Ni-141): 7II+2I.
- e. *Es. varium* (VARIM-2) × *B. nigra* (Ni-116): 5II+5I.
- f. *Es. gallicum* (GALCM-1) × *Es. nasturtiifolium* (NASTF-3): 8II+7I.

Tsunoda (12) pointed out that *B. spinescens* and *B. fruticulosa* growing in the coastal regions are more tolerant to salt than *B. maurorum* which is inland. The present experiment revealed that the hybrids between *B. spinescens* and other species showed greater meiotic irregularities and lower pollen fertilities as compared with the hybrids between *B. fruticulosa* and *B. maurorum*. In this cytodeme, the three species are now under differentiation ecologically, physiologically

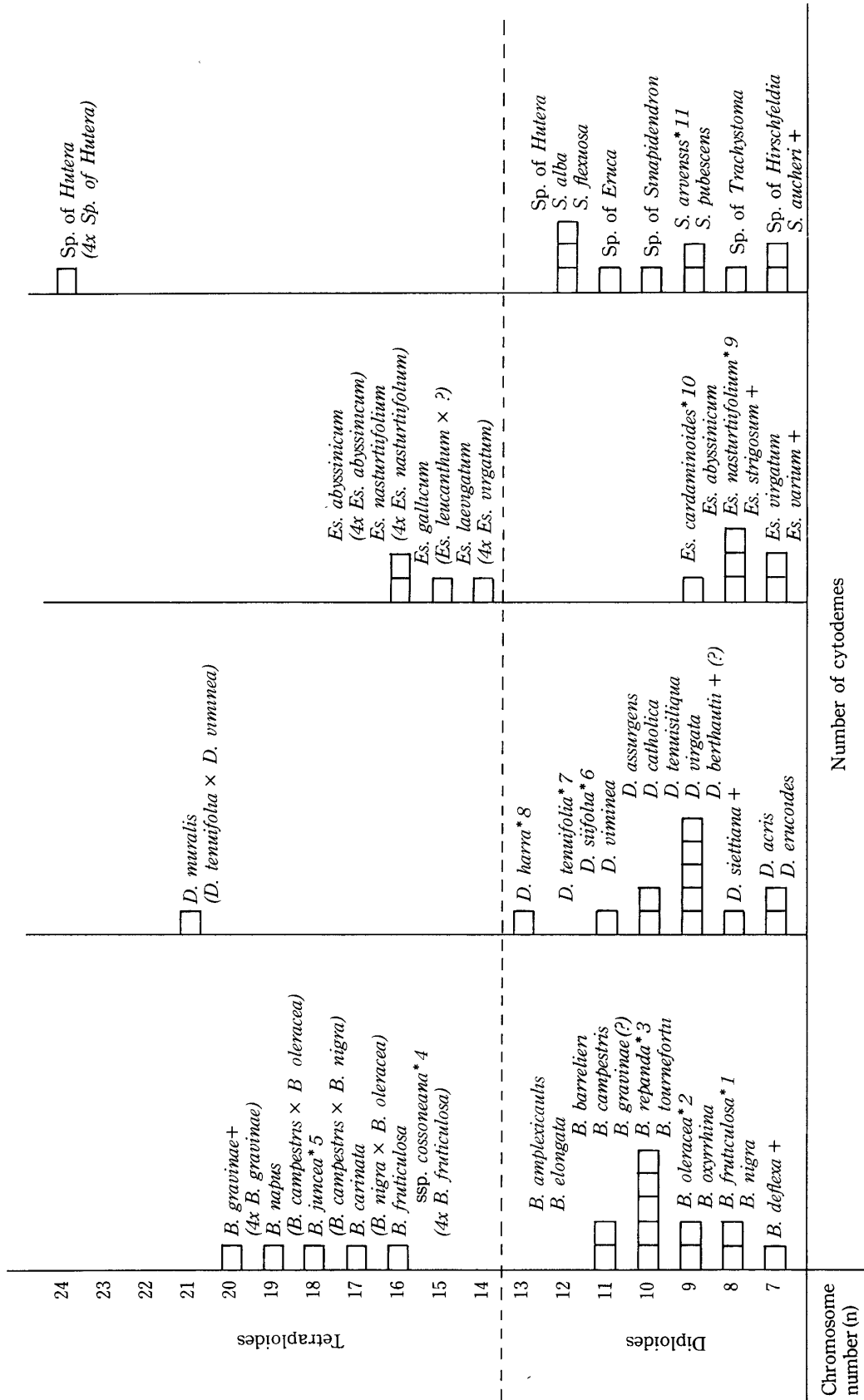


Fig. 3. Cytodesemes in subtribe *Brassicinae* made from Harberd's (5) and the present results (indicated by +mark). Genera are separately presented in four columns; *Brassica*, *Diplotaxis*, *Erucastrum* and others from left to right. One square presents one cytodeseme. Parenthesis for tetraploids indicates their supposed parents. *1 includes *B. maurorum* and *B. villosa*. *2 includes *B. alboglabra*, *B. montana*, *B. rupestris*, *B. insularis*, *B. cretica*, *B. incana*, *B. macrocarpa* and *B. villosa*. *3 includes *B. desnotiesii*, *B. nudicaulis*, *B. saxatilis* and provisionally *B. gravinae*(?). *4 includes *B. fruticulosa* ssp. *mauritanica* and ssp. *radicata*. *5 includes *B. integrifolia* and *B. cernua*. *6 provisionally includes *D. berthautii* (?). *7 includes *D. cretacea* and *D. pitardiana*. *8 includes *D. crassifolia*. *9 includes *Es. leucanthum*. *10 includes *Es. canariense*. *11 includes *S. allioni* and *S. turgida*.

and morphologically and their chromosomal differentiation is also in progress.

Although chromosomal differentiation is occurring in certain cytodemes, when we compare between intra- and inter-cytodeme hybrids, their bivalent formation revealed clear difference. Meiotic irregularity was prominent in the inter-cytodeme hybrids. The cytodemes classified by Harberd (4, 5) are accepted in general.

B. fruticulosa ssp. *mauritanica* and ssp. *radicata* can be classified into the *B. cossoneana* cytodeme. *B. desnottesii* can be a member of the *Brassicaria* cytodeme.

In view of chromosome numbers, there has been some confusion concerning the classification of *Es. laevigatum* as follows; *Es. laevigatum*, $n=14$ (4), $n=7$ (1), var. *pseudosinapis*, $n=14$ (3), ssp. *glabrum* (LEVGT-4), $n=8$ (3), ssp. *littoreum*, $n=16$ (3) and ssp. *littoreum* var. *brachycarpum*, $n=24$ (3). This species, including LEVGT-4, should be re-examined precisely from taxonomical and cytogenetical views. Besides this species, *D. berthautii* was also left for further studies, because our material ($n=9$) was different in chromosome number from that ($n=10$) of Harberd's (5).

B. gravinae ($n=20$) seems to be an autotetraploid of *B. gravinae* ($n=10$) reported by Harberd (5), due to its tetraploidal chromosome number and the larger size of its morphological characters. *B. gravinae* ($n=10$) was provisionally included in *Brassicaria* cytodeme (5). This species was unsuccessful in attempts to hybridize it with *B. repanda*, and it is possible it belongs to the other cytodemes than *Brassicaria*.

Because of the failure of hybridization and their morphological traits, we have speculated that seven species are independent from the cytodemes described by Harberd (5). The seven species are as follows; *B. deflexa* ($n=7$), *B. gravinae* ($n=20$), *D. berthautii* ($n=9$), *D. siettiana* ($n=8$), *Es. strigosum* ($n=8$), *Es. varium* ($n=7$) and *S. aucheri* ($n=7$). Combining the present speculation with the existing cytodemes, the cytodemes in the subtribe *Brassicinae* are summarized in Fig. 3.

Of 18 species combinations of inter-cytodeme hybrids, 14 may be the newly described hybrids by the present experiment. All the hybrids formed a certain amount of bivalents in the PMC without exception, though the frequency varied among species combinations. The bivalent frequency of hybrids has been used as an indicator of the genetic relationships of parental species by many researchers. On the other hand, similarity of morphological characters has also been taken as another indicator for the genetic relations (11). An interesting point would be the correlations between cytogenetical and morphological relations. This will be reported elsewhere by compiling the other data of cytogenetics.

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