

## Cytogenetical Relationships among Three Species of Chili Peppers, *Capsicum chinense*, *C. frutescens* and *C. baccatum*<sup>1)</sup>

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In order to elucidate cytogenetical relationships among three *Capsicum* species, *C. chinense*, *C. frutescens* and *C. baccatum*, intra- and inter-specific hybrids were examined cytologically.

In intraspecific hybrids of *C. baccatum* and *C. frutescens*, chromosome pairing at MI of PMCs was regular with 12 bivalents, and their pollen stainability was high. For the interspecific hybrids between *C. chinense* and *C. frutescens*, meiotic chromosome pairing was also very regular with 12 bivalents. The average was 0.06 univalents and 11.97 bivalents (9.81 rings and 2.16 rods). Their pollen stainability was relatively high. Therefore, it was suggested that *C. chinense* and *C. frutescens* are not two distinct species but a single species involving two different varieties as seen in *C. baccatum*.

For the interspecific hybrids of *C. baccatum* × *C. frutescens*, few univalents were observed at MI, and the average meiotic chromosome pairing was 0.87 univalents, 10.22 bivalents, 0.31 trivalents, 0.34 quadrivalents and 0.06 hexavalents.

Considering the present results and the geographical distribution of wild progenitors, it can be concluded that *C. baccatum* and *C. chinense*/*C. frutescens* complex were originally derived from a common ancestral species monophyletically, and that their geographical distribution occurred subsequently, resulting in reproductive isolation among them.

KEY WORDS : *Capsicum chinense*, *C. frutescens*, *C. baccatum*, interspecific hybrid, chromosome pairing, genome, cytogenetical relationship

### Introduction

Chili peppers are cultivated plants which originated in the New World. They belong to the genus *Capsicum* and comprise four different cultivated species, *C. annuum* L. var. *annuum*, *C. chinense* Jacq., *C. baccatum* L. var. *pendulum* (Willd.) Eshbaugh and *C. pubescens* Ruiz. and Pav. It is known that wild progenitors of these cultivated taxa occur naturally.

Of these species, *C. chinense* and its wild relative *C. frutescens* L. are sympatric and widespread in the lowlands of South America, particularly in the lowland area of Upper Amazon (PICKERSGILL 1971, ESHBAUGH 1975 and YAMAMOTO 1978). It is suggested that the above two species are closely related to each other and together they form a complex of cultivated and wild types comparable to that in *C. baccatum* (PICKERSGILL 1966, 1971 and YAMAMOTO 1978). *C. baccatum* var. *pendulum* is commonly cultivated in Peru and Bolivia. The geographical distribution of its wild progenitor, *C. baccatum* var. *baccatum*, is restricted to southern Peru and Bolivia (PICKERSGILL 1971, ESHBAUGH 1975 and YAMAMOTO 1978).

So far, the relationships among the species mentioned above have been investigated merely from the view point of cross compatibility (SMITH and HEISER 1957 and HIROSE, NISHI and TAKASHIMA 1960), or pollen stainability of F<sub>1</sub> hybrids (ESHBAUGH 1964, 1975,

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Table 1. The strains of *Capsicum* species used in the present study.

	Strain No.	Collection Locality
1. <i>C. chinense</i>	{ 3730	Bocana de Miriti, Colombia
	{ 3731	Bocana de Miriti, Colombia
2. <i>C. frutescens</i>	{ 3503	Miriflores, Colombia
	{ 3589	Recreo, Colombia
	{ 3944	Mitu, Colombia
3. <i>C. baccatum</i> var. <i>pendulum</i>	{ 3031	Salta, Argentina
	{ 3908	Santa Cruz, Bolivia
	{ 3909	Scaba, Bolivia
	{ 3985	Obentini, Peru
4. <i>C. baccatum</i> var. <i>baccatum</i>	{ 3888	Chahuares, Peru
	{ 3892	Chahuares, Peru
	{ 3905	La Merced, Bolivia

PICKERSGILL 1966, 1971, and YAMAMOTO 1978). Recently, JENSEN *et al.* (1979) and McLEOD *et al.* (1979) discussed the origin of chili peppers on the basis of analyses of allele frequency at a number of loci. However, the data available concerning meiotic chromosome pairing of  $F_1$  hybrids have not been reported yet except that of *C. frutescens*  $\times$  *C. annuum* by OHTA (1962).

Therefore, interspecific genome relationships among chili peppers have not been clarified. The present study was carried out to elucidate cytogenetical relationships among *C. chinense*, *C. frutescens* and *C. baccatum* on the basis of meiotic chromosome pairing of  $F_1$  hybrids. It is hoped that the new evidence from this field of study will shed more light on the problem of the origin of chili peppers.

### Materials and Methods

The materials used in the present study are listed in Table 1.

These strains were grown in pots in a glasshouse at the Plant Germ-plasm Institute, Kyoto University.

For the cytological examination, young anthers were fixed in acetic alcohol (1:3). Chromosome pairing was observed at first metaphase (MI) of pollen mother cells (PMCs) using the aceto-carmine squash method. Microphotographs were taken from temporary preparations. Pollen stainability was determined by the percentage of well-stained pollen grains by aceto-carmine.

### Results

#### *Intraspecific hybrids of C. baccatum*

Chromosome pairing at MI of PMCs in intraspecific hybrids of *C. baccatum* is presented in Table 2 and Fig. 1a, b. The average chromosome pairing in var. *pendulum*  $\times$  var. *pendulum*, var. *pendulum*  $\times$  var. *baccatum*, var. *baccatum*  $\times$  var. *pendulum* and var. *baccatum*  $\times$  var. *baccatum* was 0.04 univalents and 11.98 bivalents (9.16 rings and 2.82 rods), 12 bivalents (10.16 rings and 1.84 rods), 0.02 univalents and 11.99 bivalents (9.50 rings and 2.49 rods), and 12 bivalents (8.53 rings and 3.47 rods), respectively. All  $F_1$  hybrids analysed cytologically showed completely regular chromosome configuration

Table 2. Mean chromosome pairing and pollen stainability of hybrids used in the present study.

Hybrid No.	Cross Combination	No. of cells observed	Chromosome pairing								Pollen stainability
			I	II			III	IV	VI	VII	
				Ring	Rod	Total					
1. Intraspecific hybrids of <i>C. baccatum</i>											
var. <i>pendulum</i> × var. <i>pendulum</i>											
8154	3909 × 3031	50	0.04	9.16	2.82	11.98	—	—	—	—	97.4%
var. <i>pendulum</i> × var. <i>baccatum</i>											
76	3908 × 3892	50	—	10.32	1.68	12.00	—	—	—	—	88.9
212	3985 × 3892	80	—	10.69	1.31	12.00	—	—	—	—	89.2
215	3985 × 3888	29	—	9.28	2.72	12.00	—	—	—	—	83.1
220	3908 × 3892	39	—	9.51	2.49	12.00	—	—	—	—	85.2
	Average		—	10.16	1.84	12.00	—	—	—	—	—
var. <i>baccatum</i> × var. <i>pendulum</i>											
85	3888 × 3908	30	—	9.07	2.93	12.00	—	—	—	—	79.7
88	3892 × 3908	50	—	10.52	1.48	12.00	—	—	—	—	50.7
118	3888 × 3909	50	—	9.60	2.40	12.00	—	—	—	—	89.3
120	3892 × 3909	100	0.02	9.71	2.28	11.99	—	—	—	—	97.2
216	3888 × 3985	50	—	10.04	1.96	12.00	—	—	—	—	62.4
8150	3888 × 3031	30	0.07	6.47	5.50	11.97	—	—	—	—	—
	Average		0.02	9.50	2.49	11.99	—	—	—	—	—
var. <i>baccatum</i> × var. <i>baccatum</i>											
71	3905 × 3888	32	—	8.53	3.47	12.00	—	—	—	—	—
2. Intraspecific hybrids of <i>C. frutescens</i>											
3	3503 × 3589	60	0.06	10.02	1.95	11.97	—	—	—	—	81.7
5	3944 × 3589	50	0.04	10.18	1.80	11.98	—	—	—	—	81.9
	Average		0.06	10.09	1.88	11.97	—	—	—	—	—
3. Interspecific hybrids											
<i>C. chinense</i> × <i>C. frutescens</i>											
171	3731 × 3589	50	0.12	8.74	3.20	11.94	—	—	—	—	70.6
332	3730 × 3503	50	—	10.88	1.12	12.00	—	—	—	—	36.3
	Average		0.06	9.81	2.16	11.97	—	—	—	—	—
<i>C. baccatum</i> × <i>C. frutescens</i>											
286	3985 × 3503	25	0.08	4.12	6.72	10.84	0.08	0.44	0.04	—	0.0
485	3892 × 3589	50	1.64	2.92	6.78	9.70	0.54	0.24	0.04	0.02	11.6
486	3909 × 3589	34	0.32	3.74	6.79	10.53	0.15	0.41	0.09	—	15.3
	Average		0.87	3.45	6.77	10.22	0.31	0.34	0.06	—	—

Note : I, II, III, IV, VI and VII denote univalent, bivalent, trivalent, quadrivalent, hexavalent and heptavalent, respectively.

with 12 bivalents. About more than nine closely associated ring bivalents were detected in most cells (Fig. 1a, b). Univalents were found only rarely. The pollen stainability of certain hybrid combinations, for example Nos. 88 and 216, was relatively low (50.7% and 62.4%, respectively). But other combinations had very high pollen stainability (about more than 80%).

#### *Intraspecific hybrids of C. frutescens*

Intraspecific hybrids of *C. frutescens* showed normal chromosome pairing at MI of

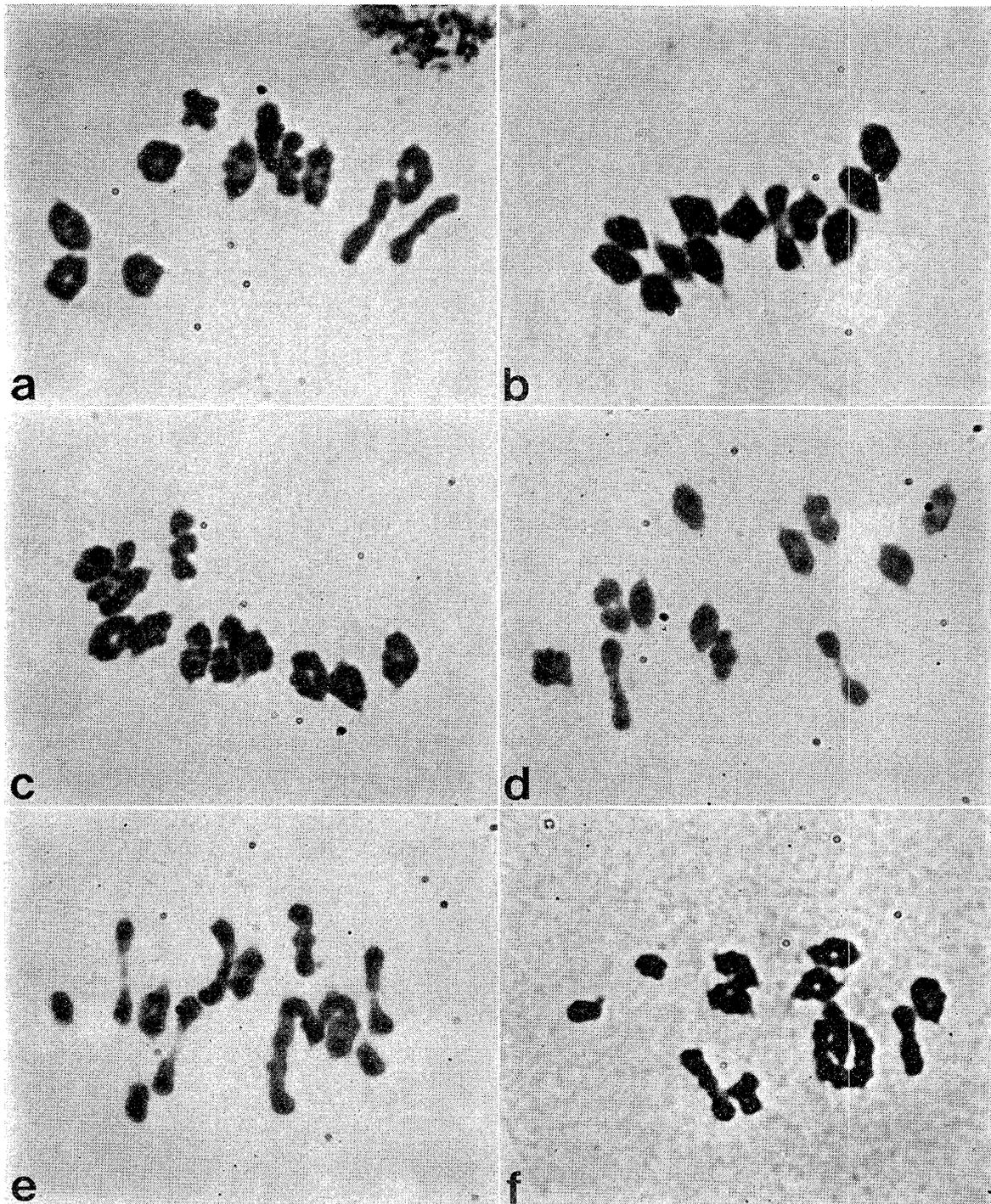


Fig. 1. Chromosome pairing at MI of  $F_1$  hybrids. a and b: Intraspecific hybrids of *C. baccatum*. From left to right, Hybrid Nos. 76 and 85. Both show 12 bivalents. c: Intraspecific hybrid of *C. frutescens* (No. 5) with 12 bivalents. d: *C. chinense*  $\times$  *C. frutescens* (No. 171) with 12 bivalents. e: *C. baccatum*  $\times$  *C. frutescens* (No. 485) with 2 univalents, 8 bivalents and 1 hexavalent. f: *C. baccatum*  $\times$  *C. frutescens* (No. 486) with 10 bivalents and 1 quadrivalent.

PMCs (Table 2 and Fig. 1c). The average was 0.06 univalents and 11.97 bivalents (10.09 rings and 1.88 rods). Moreover, the pollen stainability was very high (ca. 82%).

*Interspecific hybrids between C. chinense and C. frutescens*

Chromosome pairing at MI of PMCs and pollen stainability of interspecific hybrids between *C. chinense* and *C. frutescens* are shown in Table 2. For two interspecific

hybrid combinations examined, meiosis was very regular with 12 bivalents. For the cross 3731×3589 (No. 171), of 50 cells examined, univalents were observed only in 3 cells (6%). The average chromosome pairing was 0.06 univalents and 11.97 bivalents (9.81 rings and 2.16 rods). Of these two hybrids, one (No. 171) had rather high pollen stainability (70.6%), but the other showed relatively low pollen stainability (36.3%).

*Interspecific hybrids between C. baccatum and C. frutescens*

Three hybrid combinations of *C. baccatum*×*C. frutescens* were examined cytologically. As shown in Table 2 and Fig. 1e,f, almost identical pattern of chromosome pairing was observed. A low frequency of univalents, and a quadrivalent (or a hexavalent) formation were detected. The highest frequency of univalent formation per cell was 1.64 observed in the cross 3892×3589 (No. 485). In this case, of 50 cells examined, 7 cells (14%) exhibited 4 to 8 univalents (Table 3). For the cross 3985×3503 (No. 286), a quadrivalent was found in 11 out of 25 cells observed (44%). The average meiotic chromosome pairing was 0.87 univalents, 10.22 bivalents (3.45 rings and 6.77 rods), 0.31 trivalents, 0.34 quadrivalents and 0.06 hexavalents. The pollen stainability of these hybrids was very low. Its range was 0 to 15.3% (Table 2).

### Discussion

*C. baccatum* has been conventionally classified into two different species, *C. pendulum* Willd. and *C. microcarpum* Cav. Based on crossability and quantitative morphological analysis, however, ESHBAUGH (1964, 1970) suggested that these two species form a complex of cultivated and wild types. *C. pendulum* represents the cultivated type and *C. microcarpum* its wild progenitor. ESHBAUGH (1968, 1970) designated *C. pendulum* as *C. baccatum* var. *pendulum* (Willd.) Eshbaugh and *C. microcarpum* as *C. baccatum* L. var. *baccatum*. Hybrids between those two varieties investigated cytologically in

Table 3. Chromosome pairing at MI of interspecific hybrids *C. baccatum*×*C. frutescens*.

Chromosome pairing						No. of cells observed		
I	II	III	IV	V	VI	Hybrid No.		
						286	486	485
	12					11(44.0%)	9(26.5%)	7(14.0%)
2	11						4(11.8 )	3( 6.0 )
	10		1			11(44.0 )	14(41.2 )	4( 8.0 )
1	10	1				2( 8.0 )	3( 8.8 )	9(18.0 )
4	10							4( 8.0 )
	9			1		1( 4.0 )	3( 8.8 )	1( 2.0 )
2	9		1					5(10.0 )
	9	2					1( 2.9 )	4( 8.0 )
3	9	1						6(12.0 )
1	8				1			1( 2.0 )
2	8			1				1( 2.0 )
4	8		1					1( 2.0 )
1	8	1	1					2( 4.0 )
8	8							1( 2.0 )
4	5	2	1					1( 2.0 )

the present study showed normal chromosome pairing with 12 bivalents. Evidently, the genome of both varieties is completely homologous. The present study supports Eshbaugh's taxonomical treatment of these two taxa.

Most of the intraspecific hybrids of *C. baccatum* showed high pollen stainability. However, in certain hybrid combinations, for example, Nos. 88 and 216, a reduction in pollen stainability was observed. ESHBAUGH (1964) pointed out that the mechanisms which separated these two varieties distinctly were the tendency toward self-pollination in cultivated taxa and agricultural isolation by man.

In interspecific hybrids between *C. chinense* and *C. frutescens*, very regular chromosome associations with few univalents were found at meiosis. This suggests the high genome homology between these two species. Using morphological and genetical data, PICKERSGILL (1966, 1971) and YAMAMOTO (1978) already suggested that *C. chinense* and *C. frutescens* are closely related to each other and that together they form a complex of cultivated and wild types comparable to that seen in *C. baccatum*. However, meiotic chromosome behavior of F<sub>1</sub> hybrids between them has not been reported yet. On the basis of meiotic chromosome pairing of F<sub>1</sub> hybrids as clearly shown in Table 2 and Fig. 1d, the present study demonstrated that *C. chinense* and *C. frutescens* are not two distinct species but a single species involving two different varieties. Of two hybrids examined, the pollen stainability of one combination (3730×3503) was relatively low (36.3%). This might indicate slight chromosomal differentiation between these two taxa.

As shown in Tables 2 and 3 and Fig. 1e,f, few univalents were observed in the hybrids between *C. baccatum* and *C. frutescens*. The highest frequency of univalent formation per cell was 1.64 as observed in 3892×3589. This evidence indicates that *C. baccatum* and *C. chinense*/*C. frutescens* complex share a common genome with each other. At the same time a quadrivalent (or a hexavalent) was detected at MI of PMCs in these interspecific hybrids. Thus, *C. baccatum* differs from *C. chinense*/*C. frutescens* complex by one reciprocal translocation. The pollen stainability of three interspecific hybrids was 0 to 15.3%, showing considerably high sterility.

The *C. chinense*/*C. frutescens* complex is widespread in South America, particularly in Colombian Amazon Basin. On the other hand, wild *C. baccatum* var. *baccatum* was found mainly in southern Peru and Bolivia (PICKERSGILL 1971, ESHBAUGH 1975 and YAMAMOTO 1978). Thus the wild progenitors, *C. frutescens* and *C. baccatum* var. *baccatum* are distributed in different areas and clearly isolated from each other.

Judging from these evidences, it is concluded that *C. chinense*/*C. frutescens* complex and *C. baccatum* were originally derived from a common ancestral species monophyletically, and that their geographical distribution occurred subsequently and finally reproductive isolation such as hybrid sterility resulted among them. The assumption of the monophyletic origin of these taxa may be supported by the fact that they show parallel variation in fruit color, size, shape and pungency.

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トウガラシの3種, *Capsicum chinense*, *C. frutescens* 及び *C. baccatum* 間の  
細胞遺伝学的類縁関係に就いて

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トウガラシは新世界に起原した栽培植物で、4つの栽培種を含んでいる。*C. chinense* は、アマゾン低地で広く栽培されており、同所的に分布している *C. frutescens* がその祖先野生型である。*C. baccatum* は、栽培型と野生型の2つの variety から成り、栽培型 var. *pendulum* は、ペルー及びボリビアで主に栽培されており、祖先野生型 var. *baccatum* は、ボリビア低地から高地にかけて自生している。筆者らは、南米で収集したこれらの種の系統間の類縁関係を明らかにするため種内及び種間雑種を作出し、その染色体対合を観察した。その結果、*C. baccatum* の種内雑種はすべて 12" の正常な染色体対合と高い花粉稔性を示した。又、*C. frutescens* の種内雑種、及び *C. chinense* と *C. frutescens* との種間雑種も 12" の対合と高い花粉稔性を示した。このことは、この両種の形態的類似性と考え併せて、*C. chinense* と *C. frutescens* は、異なる種ではなく、ひとつの種と考えるべきであることを示唆している。*C. baccatum* と *C. frutescens* との種間雑種では、多価染色体が観察された。又、一価の出現頻度が低く、これらの二種は、共通ゲノムを有すると結論された。この雑種の稔性は、極めて低く、*C. baccatum* と *C. chinense*/*C. frutescens* との間には、生殖隔離が発達している。

本研究結果と野生型の地理的分布を考えると、これらの種は、元々或る共通の祖先種から一元的に起原し、その後地理的に隔離され、生殖隔離を生じたものと結論される。