Species Differentiation in *Solanum*, Sect. *Tuberarium*

IX. Genomic Relationships between Three Mexican Diploid Species

Motokazu Matsubayashi* and Shûji Miso**

* Laboratory of Plant Breeding, Faculty of Agriculture, Kobe University, Kobe 657

Genomic relationships between three Mexican diploid *Solanums* (2n=24), *S. bulbocastanum* (blb), *S. jamesii* (jam) and *S. sambucinum* (smb), were studied on the basis of meiotic chromosome pairing and pollen fertility in the hybrids and amphidiploids induced from them.

The F1 hybrids, *smb*×*jam*, had as regular meiosis and as high pollen fertility as those found in their parents, thus suggesting that the parental genomes are closely related to each other. A similar situation was also found in the F1 hybrids from *smb*×*blb*, though there were a slight reduction in the amount of chromosome pairing and a rare occurrence of chromatid bridges. The F1 hybrids, *jam*×*blb*, differed from the above two instances, not only by showing multiple associations in 23~51% of the metaphase I cells examined and more frequent occurrences of chromatid bridges and laggards but also by having a reduced pollen fertility (37~52%).

On the other hand, while the *smb*×*jam* amphidiploids formed as many multivalents as in their parental autotetraploids, the *smb*×*blb* amphidiploids showed, like the *jam*×*blb* ones, a significant reduction in multivalent frequency as compared with their parental autotetraploids. This may be interpreted as due to a higher differential affinity existing between some chromosomes of the parental genomes in the latter two instances.

From these results, it is concluded that the genomes of *smb* and *jam* are highly homologous and that the *blb* genome is distantly related to, though not distinct from, those of the above two species.

**Introduction**

With regard to the genomic relationships in diploid tuberous *Solanums*, cytogenetic studies have so far been made, their subjects being in most cases placed on South American species. Consequently, our knowledge of Mexican and Central American diploid species in this respect is still meager, despite their possessing the germ plasm useful for potato breeding, though a small amount of the work has been made by several earlier workers (Magoon et al. 1958; Sano 1962; Matsubayashi 1964; Mark 1968) with some diploid species belonging to the taxonomic series Bulbocastana, Cardiophylla, Morelliformis and Pinnatisecta. Especially, nothing is yet known about the genomic relationship between the two first-mentioned series.

The present study, from such a point of view, has been undertaken to clarify the genomic affinity between each of the series Bulbocastana, Cardiophylla and Pinnatisecta, using the species each representing these three series. In all cases the genomic affinities were critically discussed in the light of the information obtained by employing ‘amphidiploid method’ as described later. In this paper there will be outlined the results thus obtained.

Received September 25, 1976
Materials and Methods

Two or three clones of the following material each were used in this study: diploid F1 hybrids (2n=24) from *Sambucus* (CAR, 2n=24)*×* *S. bulbocastanum* (BUL, 2n=24)*, *S. sambucina×S. jameisi* (PIN, 2n=24)*, and *S. jameisi×S. bulbocastanum*; amphidiploids (2n=48) induced from these hybrids; and autotetraploids (2n=48) induced from the parental species of the above hybrids. Of these, hybrids and their parental species had been supplied by the late Dr. H.J. Tixeoreus, Institute of Agricultural Plant Breeding, Wageningen, and the others were all obtained following colchicine treatments in this laboratory. All the plants used were potted and grown in the glasshouse during all the growth period from March to July.

Cytological observations were made in microsporocyte squashes stained by a modified acetocarmine technique (MATSUBAYASHI 1963). Besides the first metaphase configurations, diakinetiic ones were also analyzed to distinguish multivalent formation from secondary associations, and the 't' test was done for the significance in the differences between the materials in their mean chromosome pairing frequencies. Pollen fertility was estimated by the percentage of normal-shaped grains stained with acetocarmine.

Results

Meiotic behavior and pollen fertility in parental species

Of the three parental species used, *S. bulbocastanum* and *S. jamesii* both showed

<table>
<thead>
<tr>
<th>Material</th>
<th>Clone</th>
<th>No. of cells observ.</th>
<th>Mean (±S.E.) and range (Italic) in number per cell of</th>
<th>Frequency of cells with 12e (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. sambucina×S. jameisi</em></td>
<td>1</td>
<td>40</td>
<td>0 0 11.95±0.03 (11–12) 0.10±0.07 (0–2) 95.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>27</td>
<td>0 0 11.93±0.05 (11–12) 0.15±0.10 (0–2) 92.6</td>
<td></td>
</tr>
<tr>
<td><em>S. sambucina×S. bulbocastanum</em></td>
<td>1</td>
<td>30</td>
<td>0 0 11.90±0.06 (11–12) 0.20±0.11 (0–2) 90.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>26</td>
<td>0 0 11.81±0.08 (11–12) 0.39±0.16 (0–2) 80.8</td>
<td></td>
</tr>
<tr>
<td><em>S. jamesii×S. bulbocastanum</em></td>
<td>1</td>
<td>28</td>
<td>0.64±0.09 (0–1) 10.71±0.18 (10–12) 0 35.7 (64.3)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>31</td>
<td>0.29±0.08 (0–1) 11.39±0.17 (10–12) 0.07±0.07 (0–2) 67.7 (29.0)**</td>
<td></td>
</tr>
</tbody>
</table>

* Tetrapartite associations probably resulting from segmental interchanges and their derivative tripartite ones.
** Percentage frequency of cells with multiple associations.

* BUL, CAR and PIN indicate the taxonomic series in the section Tubera, *i.e.* Bulbocastana, Cardiophylla and Pinnatisecta, respectively.
regular meiotic behavior, forming 12 bivalents in 94~97% of the cells at metaphase I, with a rare occurrence of a few univalents, and showing 12~12 separation in almost all cells at anaphase I. All clones of these species had pollen fertility as high as 90%. *S. sambucinum* differed from the above species in possessing slightly irregular meiosis. In this instance, 76~84% of the metaphase I cells formed 12 bivalents and the rest had 2~4 univalents, and laggards occurred occasionally at anaphase I. Pollen fertility ranged from 78 to 86% among the clones.

**Meiotic behavior and pollen fertility in F₁ hybrids**

The results obtained in the F₁ hybrids concerned are summarized in Tables 1, 2 and 3. The F₁ hybrids from *S. sambucinum × S. jamesii* showed 12 bivalents, respectively, in 93~95% of the cells at diakinesis and 88~91% at metaphase I (Fig. 1-a). Occasionally,

Table 2. Chromosome associations at metaphase I in the diploid F₁ hybrids involving three Mexican diploid *Solanums*.

<table>
<thead>
<tr>
<th>Material</th>
<th>Clone</th>
<th>No. of cells observ.</th>
<th>Mean (±S.E.) and range (Italic) in number per cell of</th>
<th>Frequency of cells with 12ₙ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. sambucinum × S. jamesii</em></td>
<td>1</td>
<td>81</td>
<td>0 0 11.88±0.04 (11<del>12) 0.25±0.07 (0</del>2) 87.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>64</td>
<td>0 0 11.91±0.04 (11<del>12) 0.19±0.07 (0</del>2) 90.6</td>
<td></td>
</tr>
<tr>
<td><em>S. sambucinum × S. bulbocastanum</em></td>
<td>1</td>
<td>60</td>
<td>0 0 11.85±0.06 (11<del>12) 0.30±0.09 (0</del>2) 85.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>70</td>
<td>0 0 11.79±0.06 (10<del>12) 0.43±0.11 (0</del>4) 81.4</td>
<td></td>
</tr>
<tr>
<td><em>S. jamesii × S. bulbocastanum</em></td>
<td>1</td>
<td>51</td>
<td>0.31±0.06 (0<del>1) 0.20±0.06 (0</del>1) 10.77±0.13 (9<del>12) 0.63±0.13 (0</del>4) 31.4 (51.0)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>86</td>
<td>0.38±0.07 (0<del>1) 0.09±0.03 (0</del>1) 10.98±0.11 (9<del>12) 0.23±0.06 (0</del>4) 48.2 (47.1)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>77</td>
<td>0.29±0.05 (0<del>1) 0.03±0.02 (0</del>1) 11.27±0.10 (10<del>12) 0.23±0.07 (0</del>2) 58.4 (23.3)**</td>
<td></td>
</tr>
</tbody>
</table>

*, ** See Table 1.

Table 3. Chromosome behaviors at anaphase I and metaphase II in the diploid F₁ hybrids involving three Mexican diploid *Solanums* and their pollen fertility.

<table>
<thead>
<tr>
<th>Material</th>
<th>Clone</th>
<th>Anaphase I</th>
<th>Metaphase II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cells with laggards (%)</td>
<td>No. of laggards per cell</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td><em>S. sambucinum × S. jamesii</em></td>
<td>1</td>
<td>4.0</td>
<td>0.04  0~1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.1</td>
<td>0.02  0~1</td>
</tr>
<tr>
<td><em>S. sambucinum × S. bulbocastanum</em></td>
<td>1</td>
<td>9.3</td>
<td>0.09  0~2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.8</td>
<td>0.06  0~1</td>
</tr>
<tr>
<td><em>S. jamesii × S. bulbocastanum</em></td>
<td>1</td>
<td>11.1</td>
<td>0.13  0~2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15.2</td>
<td>0.21  0~2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8.3</td>
<td>0.08  0~1</td>
</tr>
</tbody>
</table>

* Chromosomes scattered outside the metaphase II plates.
** Frequency of metaphase II plates consisting of 12 chromosomes.
there were 11 bivalents and 2 univalents. Of 12 bivalents, however, 2 or 3 were heteromorphic and they occurred in every metaphase plate. At anaphase I, almost all of the cells showed regular chromosome separation (Fig. 1-c) and there were found laggards in

Fig. 1. Meiotic configurations in Mexican diploid *Solanum* hybrids (ca. x1500). a. Diakinesis in F₁ hybrid, *smb* × *jam*, 12ₘ; b. Metaphase I in F₁ hybrid, *smb* × *blb*, 12ₘ. Arrows indicate heteromorphic bivalents; c. Anaphase I in F₁ hybrid, *smb* × *jam*, showing regular chromosome separation; d. Metaphase I in F₁ hybrid, *smb* × *blb*, 11ₘ + 2ₘ; e & f. Metaphase I in F₁ hybrids, *jam* × *blb*, there being seen 1ₘ + 1₀ and 1ₘ + 1₀ + 1₁, respectively; g & h. Anaphase I in F₁ hybrids, *jam* × *blb*, showing two laggards and dicentric bridge being accompanied by acentric fragment, respectively.
Species Differentiation in *Solanum*, Sect. *Tuberarium* 245

a few cells but no chromatid bridges. About 98% of metaphase II plates were numerically balanced ones consisting of 12 chromosomes. In these meiotic features the hybrids did not differ from their parental species. All the hybrids were as pollen fertile as their parental species themselves (Fig. 2-a).

The F₁ hybrids from *S. sambucinum* × *S. bulbocastanum* behaved cytologically in nearly similar manner to that of the hybrids mentioned above, although they showed a slight reduction in chromosome pairing frequency (Fig. 1-d), there being found 12 bivalents in 81~90% of the cells at diakinesis and in 81~85% of metaphase I cells. Similarly, heteromorphic bivalents were also present in every cell (Fig. 1-b). Chromosome behaviors at the subsequent stages and pollen fertility were not significantly differed from those in the above *S. sambucinum* × *S. jamesii* hybrids (Fig. 2-b), even though at anaphase I there were laggards in a slightly higher frequency and a rare occurrence of chromatid bridges.

On the other hand, the F₁ hybrids obtained from *S. jamesii* × *S. bulbocastanum* were characterized by the meiotic features not found in the preceding two instances. In the present instance, as shown in Figs. 1-e and f, either a tetrapartite configuration, probably resulting from segmental interchanges, or its derivative tripartite one was observed one by one in each cell, where present, with the frequencies as high as 64 and 29% of the cells examined at diakinesis in the clones 1 and 3, and as 49, 48 and 31% at metaphase I in the clones 1, 2 and 3, respectively. Such multiple configurations have been reported by Magoon et al. (1958) for diploid hybrids from *S. pinnatisectum* × *S. bulbocastanum* and also by Marks (1968) for that from *S. morelliforme* × *S. clarum*. Of the tetrapartite associations found in the present study, O-shaped configuration was the most frequent, followed by N- and U-shaped ones and X-shaped one was of rare occurrence. At metaphase I, the tripartite configurations were occasionally present, which resulted probably from the failure of tetrapartite association, and unpaired chromosomes appeared with the frequency of 2 to 4 per cell. Consequently, the frequency of cells with 12 bivalents was between 31 and 58% of the total number of cells examined. Of the paired chromosomes 2 pairs at least were heteromorphic and they were always found in every cell. At

![Fig. 2. Pollen grains of Mexican diploid *Solanum* hybrids (ca. x300).](image)

a. *smb* × *jam*;
   b. *smb* × *blb*, arrow indicates an unreduced grain;
   c. *jam* × *blb.*
Fig. 3. Meiotic metaphase I configurations in amphidiploids induced from Mexican diploid *Solanum* hybrids and their parental autotetraploids (ca.×1500).

a. *jam*×*blb* amphidiploid, 1<sub>W</sub>+22<sub>H</sub>; b. *smb*×*blb* amphidiploid, 2<sub>W</sub>+20<sub>H</sub>; c. *smb*×
*jam* amphidiploid, 4<sub>W</sub>+16<sub>H</sub>; d. Autotetraploid of *jam*, 6<sub>W</sub>+2<sub>H</sub>+8<sub>g</sub>+2<sub>i</sub>.

anaphase I, 1 or 2 laggards per cell were found in 8~15% of the cells and dicentric bridges with acentric fragments occurred in occasional cells (Fig.1-g and h). About 80% of the metaphase II plates were numerically balanced including 12 chromosomes, and 37~52% of pollen grains were stainable (Fig.2-c).

**Multivalent formation in tetraploids induced from hybrids and their parental species**

`Amphidiploid method` as suggested by Stephens (1950) was employed to determine whether cryptic structural differences exist between the chromosomes of the species used, and, for this, meiotic analyses were made both on amphidiploids induced from the F<sub>1</sub> hybrids and autotetraploids from the parental species. In Table 4 the results thus obtained are given as comparisons between the tetraploid materials used for their multivalent frequencies per cell and the data presented in this table are graphed in Fig.4 for the convenience of comparison in regard to the variations in multivalent frequency. The amphidiploids from *S. sambucinum*×*S. jamesii* had a maximum of 5 multivalents per cell, 3 being the most frequent (Fig.3-c). In this respect, the amphidiploids were comparable to autotetraploids of *S. sambucinum*, though different from those of *S. jamesii* (Fig.3-d). The amphidiploids from *S. sambucinum*×*S. bulbocastanum* differed significantly from either of the parental autotetraploids showing a decreased frequency of multivalent formation,
Table 4. Comparison of multivalent frequencies in the amphidiploids and their parental autotetraploids.

<table>
<thead>
<tr>
<th>Material</th>
<th>No. of cells observed</th>
<th>Freq. of cells with multivalents of</th>
<th>Mean freq. per cell (±S.E.)</th>
<th>Multivalent coefficient*</th>
<th>$t = \frac{D^{**}}{E_D}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental autotetraploids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. jamesii</em></td>
<td>54</td>
<td>0 1 5 9 19 18 2</td>
<td>4.00 (±0.15)</td>
<td>32.6</td>
<td></td>
</tr>
<tr>
<td><em>S. bulbocastanum</em></td>
<td>48</td>
<td>1 1 6 14 15 11 0</td>
<td>3.55 (±0.17)</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td><em>S. sambucinum</em></td>
<td>48</td>
<td>0 3 11 13 14 7 0</td>
<td>3.23 (±0.17)</td>
<td>26.1</td>
<td></td>
</tr>
<tr>
<td>Amphidiploids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. sambucinum × S. jamesii</em></td>
<td>50</td>
<td>0 4 7 25 11 3 0</td>
<td>3.04 (±0.14)</td>
<td>24.1</td>
<td>0.950 (0.4~0.3) &lt; 0.001</td>
</tr>
<tr>
<td><em>S. sambucinum × S. bulbocastanum</em></td>
<td>45</td>
<td>3 7 16 15 4 0 0</td>
<td>2.20 (±0.16)</td>
<td>17.3</td>
<td>4.402 (0.001) &lt; 0.001</td>
</tr>
<tr>
<td><em>S. jamesii × S. bulbocastanum</em></td>
<td>41</td>
<td>6 11 17 7 0 0 0</td>
<td>1.61 (±0.15)</td>
<td>12.7</td>
<td>11.274 (&gt;0.001) &lt; 0.001</td>
</tr>
</tbody>
</table>

* Percentage of the total number of chromosomes taking part in the formation of multivalents (quadrivalents + trivalents).

** Calculated for testing the significant difference between multivalent means in the amphidiploids and their respective parental autotetraploids, the P values each being italicized in parentheses.

its modal frequency per cell being 2 (Fig.3-b). Such a situation was more conspicuously found in the amphidiploids from *S. jamesii × S. bulbocastanum*. In this instance there was only a mean frequency of 1.6 multivalents per cell, with a maximum at 3 (Fig.3-a). This value was of significantly low order as compared not only with those of their parental autotetraploids but also with those of the preceding two instances of amphidiploids.

**Discussion**

As Swaminathan and Howard (1953) have suggested, species differentiation at diploid level may have taken place in the ways as follows: first, changes in the gene constitution without any chromosome structural changes, *i.e.* 'multiple gene substitution' proposed by Harland (1936) and Stephens

Fig. 4. Graph showing comparisons of multivalent frequencies between the tetraploid materials induced from Mexican diploid *Solanum* hybrids and their parental species.
(1950); secondly, changes in the chromosomes too small to affect meiotic pairing in species hybrids, i.e. 'cryptic structural differentiation' proposed by Stebbins (1947); thirdly, changes in the chromosomes which are so gross that there is a little or little meiotic pairing in species hybrids; and fourthly, the formation of sterility barriers between the species, correlated with or quite independent of any of the preceding three changes.

It has been generally accepted that diploid tuberous Solanum species, in a great majority of the cases, differ genomically from one another only at such levels as given either in the first or at most in the second of the four cases mentioned above (Howard and Swaminathan 1952; Swaminathan and Howard 1953). However, it has thereafter been reported that the chromosome structural differences detectable by the usual observations, which fall under the third category, existed between the genomes of certain diploid species (Magoon et al. 1958; Marks 1968). A similar case was also encountered in the present study.

The diploid F₁ hybrids, S. sambucinum × S. jamesii, were quite similar to either of their parents in cytological behavior and pollen fertility, suggesting that the parental genomes are highly homologous. Similar meiotic features were also observed in the diploid F₁ hybrids, S. sambucinum × S. bulbocastanum, although there was an occasional occurrence of meiotic irregularities such as dicentric bridges and chromosome lagging. The diploid F₁ hybrids from S. jamesii × S. bulbocastanum, however, differed from the above two instances not only in possessing, besides the irregularities just mentioned, frequent multiple associations but also in showing a considerable reduction both in the amount of meiotic pairing and in pollen fertility. These facts seem apparently to suggest that S. sambucinum is closely related to S. jamesii and also to S. bulbocastanum, and the latter two species are somewhat distantly related to each other. This view, however, has a syllogistical contradiction.

In such a case, a solution to the problem may be provided by applying 'amphidiploid method' to the materials concerned. This method is employed as a tool for determining, at amphidiploid level, the degree in the differential affinity which causes each chromosome to pair with its identical partner, leading thus to preferential pairing between the chromosomes derived from the same parental genome. In tuberous salanum, some diploid species have so far been investigated by this method (Swaminathan 1953; Swaminathan and Howard 1953; Sano 1962; Matsubayashi 1964), and a significant reduction in multivalent frequency has been reported to be observed in amphidiploids derived from certain diploid species hybrids as compared with their parental autotetraploids. A similar situation was also found in the present materials (Table 4 and Fig.3). The amphidiploid S. sambucinum × S. jamesii had as high a multivalent frequency as the autotetraploids from the parental species, whereas the amphidiploids induced from S. sambucinum × S. bulbocastanum and S. jamesii × S. bulbocastanum both differed from their respective parental autotetraploids in possessing the significantly reduced frequencies of multivalent formation. A point worthy to be noted here is that while the S. sambucinum × S. bulbocastanum hybrids show a fairly regular meiotic pairing at diploid level as stated previously, they have so low multivalent
frequency as just mentioned at amphidiploid level. This fact implies that the degree of
genome homology between these two species is not sufficiently close to show as high a
multivalent frequency in their amphidiploids as in the autotetraploids. In this respect,
the above two species may be considered to be as distantly related to each as *S. jamesii*
and *S. bulbocastanum* are to each other. This view seems to be in line with the suggestion
of Magoon *et al.* (1958).

Thus, the conclusion reached in this study may be summed up as follows: 1) *S. sambuci-
cinum* and *S. jamesii* have a very similar genome in common, and 2) the genome of
*S. bulbocastanum* is distantly related to those of the above two species to such a degree
that the detectable structural differences exist in some chromosomes of their genomes.

Taking into consideration the results presented in this study as well as those reported
by other workers, it is probably considered that, apart from *S. verrucosum*, almost all
Mexican and Central American diploid *Solanums* have a more or less similar genome in
common. Likewise, most, or possibly all, of the available evidence to date have pointed
out that South American diploid *Solanums* are also related to one another by their
possessing in common a more or less similar genome. The problem for further investiga-
tions, therefore, should be to determine how diploid *Solanums* in both these continents
are related genonomically to each other.

**Literature Cited**


Howard, H.W. and M.S. Swaminathan 1952. Species differentiation in the section *Tuberarium* of
*Solanum* with particular reference to the use of interspecific hybridization in breeding. Euphy-

section *Tuberarium*. Amer. J. Bot. 45: 207–221.


Matsumatashi, M. 1963. Prolonged direct staining of acetocarmine squashes of *Solanum* microsporo-
cytes. Stain Tech. 38: 265–266.

———. 1964. Genome relationships between the series Bulbocastana, Cardiophylla and Pinnati-

II. Poliploides artificiales: autotetraploides y an diploides. Anales Inst. Nac. Invest. Agron. 11:
159–187.

Stebbins, G.L. 1947. Types of polyploids: their classification and significance. Advances in Genetics
1: 403–429.


Swaminathan, M.S. 1953. Studies on the inter-relationships between taxonomic series in the section

———. and H.W. Howard 1953. The cytology and genetics of the potato (*Solanum tuberosum*)
パレイノ近縁種における種の分化

IX. メキシコ産2倍性近縁種相互間のゲノム類縁関係

松林 元一・三十尾修司
（神戸大学農学部）

メキシコ産パレイノ近縁種のうち、Bulbostanana, CardiophyllaおよびPinnatisecta3群相互間におけるゲノム類縁関係を明らかにするため、各群よりそれぞれ代表的な2倍種（2n＝24）、S. bulbostanana（blb）、S. sambucinum（smb）およびS. jamesii（jam）を選び、それらのF1雑種における染色体の対合行動と花粉稔性を調べた。さらに各雑種から育成された複2倍体とそれら両親の同質4倍体につき、各個染色体の形成頻度を比較して、各種間における染色体の微細な構造的差異を検討した。

smb×jam のF1雑種は、何れの系統もM-Iで大部分の細胞が規則的に12Aを形成し、90% 前後の高い花粉稔性を示した。また smb×blb のF1雑種では、これに比べて、やや対合量の低下や穢に染色体橋が認められたのが、本質的には上記の雑種と変わりないようであった。しかしに jam×blb のF1雑種は、前記2つの場合と異なって、系統によって23～51%の細胞が4連あるいはそれから派生した3連染色体を形成し、A-Iでは途端染色体や染色体橋がしばしばみられ、花粉稔性も37～52%という低率であった。これらの事実は、前記の試験3種の相互間におけるゲノムの相同関係の成立にとって論理的に矛盾する。そこで各雑種から育成された複2倍体の多価対合頻度を、それぞれの両親の同質4倍体のそれと比較して、各ゲノム間における染色体の‘differential affinity’を検討した。その結果 smb×blb の複2倍体は smb×jam の複2倍体とは有意に異なり、 jam×blb の複2倍体に近似の値を示すことがわかった。

以上の結果から次の見解が導かれる。smb と jam のゲノムは互いに高い相同関係にあるが、blb のゲノムはこれら両種のゲノムと同一カテゴリーに属するといえ、それらの染色体の若干には転座や逆位などによる構造的差異が存在する。