

## CYTOGENETIC STUDIES IN SPERGULARIA

### II. An Attempt to Discover Cytogenetic Relationships of Some Species\*

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THE present account deals with some of the results of a programme of interspecific hybridisation carried out in the genus *Spergularia*. The study has involved eleven species but for brevity the present paper is restricted to hybrids amongst the six species listed below which can suitably be considered apart from the others.

	2n	Distribution	Habit
<i>S. fimbriata</i> Boiss.	18	S.W. Spain, S. Portugal, Morocco, Canary Is.	Robust perennial.
<i>S. rupicola</i> Lebel ex Le Jolis	36	Atlantic coast of Europe to c58° N. in Scotland.	Robust perennial.
<i>S. purpurea</i> (Pers.) G. Don ( <i>S. longipes</i> Rouy)	18, 36	Mediterranean region, N. Africa.	Slender annual.
<i>S. nicaeensis</i> (Sarato) Burnat	36	Mediterranean region.	Short-lived perennial.
<i>S. bocconii</i> (Scheele) Aschers. & Graebn.	36	S.W. Europe and Mediterranean region.	Slender annual or short-lived perennial.
( <i>S. atheniensis</i> (Heldr. & Sart.) Aschers. & Schweinf.)			
<i>S. heldreichii</i> (Foucaud) Simon & Monnier	36	Mediterranean region extending to the Atlantic coast of France.	Slender annual.

Descriptions of all species are given in *Flora Europaea* Vol. 1. They are all morphologically quite distinct and differ from each other in a number of characters.

The six species are only a small part of the twenty or so species which occur in the Old World, but they cover much of the variation to be found in the genus, ranging from large robust perennials such as *S. fimbriata* and *S. rupicola* to small annuals represented by *S. purpurea*, *S. bocconii* and *S. heldreichii*. Diploid species ( $2n=18$ ) are represented by *S. fimbriata* and tetraploids ( $2n=36$ ) by *S. rupicola*, *S. nicaeensis*, *S. bocconii* and *S. heldreichii*, whilst *S. purpurea* includes both diploid and tetraploid races. The two cytological races of *S. purpurea* will be referred to as *S. purpurea* (2x) and *S. purpurea* (4x).

The hybrids were produced primarily to try to obtain information concerning specific interrelationships by observation of meiotic pairing. Since the present paper is devoted to this subject it deals only with hybrids which have been successfully grown to flowering. The absence of some hybrid com-

\*Some of the results in this paper are taken from a thesis accepted for the degree of Ph.D. in the University of Liverpool.

binations (e.g. hybrids between diploid and tetraploid *S. purpurea*) and the small numbers of plants in others are the result of the operation of various barriers to hybridisation, most important of which is seed incompatibility.

#### MATERIALS AND METHODS

The plants used as parents were obtained from field collections made by the writer and by other collectors (particularly Dr. P. Monnier of the University of Montpellier) and from seeds sent by various botanic gardens. The source of all material is mentioned in Table 1 and has been given in more detail previously (Ratter, 1964).

In both *S. nicaeensis* and *S. bocconii* two different provenances have been used in the hybridisations. In both cases intraspecific hybrids between them have shown normal morphology, chromosome pairing at meiosis, and fertility. It seems reasonable, therefore, to consider the provenances of these species comparable when considering interspecific hybrids which involve them.

All plants used in the hybridisations were grown in a cool greenhouse in pots of John Innes Potting Compost No. 1. A rather "drawn" appearance characterised the greenhouse plants and therefore, to obtain specimens of more natural growth habit for morphological study, a proportion of the plants of species and synthetic hybrids were grown in an outdoor plot.

In *S. rupicola*, *S. nicaeensis*, *S. bocconii* and *S. heldreichii* autogamy is probably the normal breeding system. The flowers are more or less homogamous and are self-pollinated by the spreading stigmas touching the anthers. In these species emasculation of flowers to be used in controlled pollinations was therefore necessary and was carried out one day prior to anthesis using a pair of fine forceps. In *S. fimbriata* and *S. purpurea* emasculation can be accomplished at anthesis since in these species the stigmas at first stand upright away from the anthers and only later reflex. In practice the latter two species were used little as female parents since their seed-setting under greenhouse conditions was never good. No special precautions were taken to protect emasculated flowers from accidental pollination since insects were very rare in the greenhouse. Less than 5% of a trial batch of over 300 unpollinated emasculated flowers developed into fruits and the seed from these all proved to be selfs of the parental species, no doubt resulting from faulty emasculation. Controlled pollination was carried out by touching detached stamens of the male parent on the stigmas of the female parent until there was a visible coating of pollen.

Meiosis was studied in pollen mother-cells which were fixed in 3:1 ethanol: acetic acid and stained using the iron-acetocarmine squash method. Flower buds to be used for preparations were stored in fixative in a "deep-freeze" at -10°C and even after five years showed no deterioration.

Pollen fertility was estimated by observing the percentage of grains which were well formed and stained deeply with iron acetocarmine.

#### RESULTS

##### *F<sub>1</sub>* hybrids

Fourteen different interspecific *F<sub>1</sub>* hybrids have been successfully synthesised and grown to flowering. Table 1 (p. 215) summarises information of parentage, meiotic pairing, pollen fertility and seed fertility.

The hybrids are intermediate between their parental species in most characters and no matrocliny has been observed where reciprocal hybrids have been produced. They are mostly very vigorous and are usually larger than their parents, for instance some of the perennials such as *S. nicaeensis* *x* *fimbriata* and *S. nicaeensis* *x* *rupicola* when grown in the outdoor plot rapidly produce large tussocks up to one metre in diameter whilst their parents remain considerably smaller. On the other hand *S. rupicola* *x* *purpurea* (4x) and *S. nicaeensis* *x* *purpurea* (4x), which are by far the most fertile of the hybrids, are much smaller and less robust plants than their maternal parents. A characteristic feature of the hybrids is that flowering in the absence or only sparse occurrence of fruiting is much prolonged so that the inflorescences become very long and straggling. The stamens of most hybrids are abnormally small but otherwise the plants show no obvious malformations.

In pollen mother cells the usual pattern occurs with bivalents and trivalents arranged on the equatorial plate at first meiotic metaphase and univalents, when present, scattered through the cell (Plate 21a, c, d). As in the parental species there are only one or two chiasmata per bivalent. At first meiotic anaphase and telophase the univalents can be seen as laggards which usually divide equationally and then travel to the poles (Plates 22c, 24a). Exclusion of lagging chromosomes from the daughter nuclei appears to be rare in all of the hybrids and polyspory has not been seen. A few aberrant pollen mother cells have been observed. An octoploid pollen mother cell ( $2n=72$ ) was observed in a squash of an anther of *S. nicaeensis* *x* *rupicola* which also contained many normal tetraploid pollen mother cells. The octoploid cell contained 29 bivalents and 14 single chromosomes aligned in opposing pairs; the latter were obviously precociously separated bivalents. A group of three high polyploid pollen mother cells occurred in a squash of an *S. bocconii* *x* *heldreichii* anther where the other pollen mother cells were of normal tetraploid number. Occasional binucleate pollen mother cells have been seen in *S. bocconii* *x* *rupicola*.

Pollen fertility, as determined by morphology and staining in acetocarmine, ranges from about 50% in *S. rupicola* *x* *purpurea* (4x) and *S. nicaeensis* *x* *purpurea* (4x) to nil in most of the other hybrids (see Table 1). Production of seed is more or less correlated with pollen fertility, and ranges from about 5% of normal seed production of the female parent in *S. rupicola* *x* *purpurea* (4x) and *S. nicaeensis* *x* *purpurea* (4x) to much below 1% in *S. bocconii* *x* *nicaeensis* (Denia x Hérault provenances)\* and *S. nicaeensis* *x* *purpurea* (2x), and to nil in the other hybrids.

#### *F*<sub>2</sub> hybrids

Only two of the possible four *F*<sub>2</sub> hybrid types have so far been grown; these are *S. bocconii* *x* *nicaeensis* *F*<sub>2</sub> and *S. rupicola* *x* *purpurea* (4x) *F*<sub>2</sub>. Preliminary investigations of meiosis, fertility and morphology have been made, but the meiotic studies have as yet been restricted to a few plants (see Table 2, p. 223).

\*Since this was written another batch of plants of *S. bocconii* *x* *nicaeensis* (Denia x Hérault) has been grown. This latter batch showed nearly 50% seed fertility in all plants. This remarkable difference in fertility is presumably due to some difference in environmental conditions, perhaps to the amount of fine weather occurring during the flowering period.

In *S. boconii* x *nicaeensis* seed was produced by  $F_1$  plants of Denia x Hérault collection parentage only.\* The  $F_2$  generation shows a series of segregates from *boconii*-like forms through intermediates to *nicaeensis*-like forms. Most of the  $F_2$  generation show a 5–30% fertility in both pollen and seed production, as compared to a much lower than 1% fertility in the  $F_1$ , but completely sterile plants do occur. Three plants were chosen for initial cytological investigation, two were morphologically close to the two parental species and one was intermediate. The *boconii*-like plant and the intermediate plant had  $2n=36$  with fairly regular production of  $18_{11}$  whereas the *nicaeensis*-like plant had  $2n=40$  with production of usually  $18_{11} 4_1$  or  $19_{11} 2_1$ . Another *nicaeensis*-like plant was then investigated and this was of normal tetraploid number ( $2n=36$ ) and showed fairly regular production of  $18_{11}$ . The *boconii*-like plant and the *nicaeensis*-like plant with  $2n=36$  showed about 30% pollen and seed fertility, whilst the intermediate plant and the *nicaeensis*-like plant with  $2n=40$  were considerably less fertile (see Table 2 for details).

*S. rupicola* x *purpurea* (4x)  $F_2$  generation also shows segregation towards the parental species but no plants have been grown which approach the parents nearly so closely as in *S. boconii* x *nicaeensis*  $F_2$ . No doubt this is because the parental species are much more dissimilar than are *S. boconii* and *S. nicaeensis*. Some of the plants show signs of extreme unbalance, e.g. tendency to chlorosis, extreme compactness of form, lack of vigour and failure to produce normal flowers or to flower at all. The fertility varies from slightly higher than in the  $F_1$  to nil; in no case is fertility as high as in many of the  $F_2$  plants of *S. boconii* x *nicaeensis*. Three slightly fertile plants were investigated cytologically, one tending towards *S. purpurea* morphology another towards *S. rupicola* and the third being more or less intermediate. The *purpurea*-like plant had  $2n=39$  with high pairing at meiosis (e.g.  $18_{11} 3_1, 2_{111} 15_{11} 3_1, 2_{111} 14_{11} 8_1$ ), the *rupicola*-like plant had  $2n=38$  with  $18_{11} 2_1$  or in some cells rather more univalents, whilst the intermediate plant had  $2n=36$  and usually a regular formation of  $18_{11}$ , although sometimes  $17_{11} 2_1$  or  $16_{11} 4_1$ . Obvious secondary association of bivalents could be seen in some of the pollen mother cells of these hybrids. The most fertile of the three plants was the *rupicola*-like specimen with  $2n=38$  which had c. 40% pollen fertility and c. 5% seed fertility. The other two plants were very considerably less fertile (see Table 2, p. 223).

#### DISCUSSION

Before discussing the cytological results it is worth devoting a paragraph to morphological aspects. The  $F_1$  hybrids have shown almost perfect intermediacy between their parents, apart, of course, from the character of the long straggling inflorescences which is associated with their sterility. The vigour of most of the hybrids is probably associated at least in part with the saving of heavy nutrient expenditure in fruiting and it is interesting that the two most fertile  $F_1$  hybrids, *S. rupicola* x *purpurea* (4x) and *S. nicaeensis* x *purpurea* (4x) are also the least vigorous. The only example of disharmony in development observed in the  $F_1$  appears to be in the abnormally small stamens of most of the hybrids. Similar abnormalities of the stamens of

\*A batch of this hybrid involving the Barcelona collection of *S. nicaeensis* which was grown since this was written has shown a very slight fertility.

hybrids have been recorded by other workers in very diverse families, e.g. in *Paeonia* (Saunders & Stebbins, 1938) and in *Elymus condensatus* *x* *glaucus* (Stebbins & Walters, 1949). In the  $F_2$  of *S. rupicola* *x* *purpurea* (4x) dis-harmony affecting vegetative and floral structures occurs in some plants.

#### (a) Cytology of the parental species

The cytology of the parental species has already been described (Ratter, 1964) but a brief resumé of relevant points is necessary before cytology of the hybrids can be discussed. The chromosomes in all species are small ( $0.75-2.5\mu$ ) and usually only one or two chiasmata per bivalent occur. Regular formation of bivalents is normal for both diploid and tetraploid species and quadrivalents have been observed in only three of the tetraploid species, *S. rupicola*, *S. purpurea* (4x) and *S. nicaeensis*, where they occur in only 4-7% of the pollen mother cells. The tetraploid species therefore exhibit almost completely cytologically diploid behaviour, but this does not necessarily mean that they are strict allotetraploids since multivalent formation would be inhibited by the low chiasma frequency (or indeed could be suppressed genetically). *S. purpurea* is notable since it contains diploid and tetraploid races which are morphologically very similar. It seems reasonable to suspect on morphological grounds that the tetraploid race is autopolyploid, but cryptic allopolyploidy cannot be discounted, particularly since quadrivalents are of such rare occurrence.

#### (b) Cytology of the hybrids

Figure 1 p. 208 represents the pattern of hybridisation and summarises the most frequent meiotic configurations in the hybrids. The triploid and tetraploid hybrids may be conveniently considered separately.

##### *Triploid hybrids*

The hybrids at triploid level form a suitable starting point for the consideration of the significance of chromosome pairing. The configurations observed in most of the pollen mother cells of the hybrids between the diploid *S. fimbriata* and the tetraploid species *S. rupicola*, *S. purpurea* (4x), *S. nicaeensis*, *S. bocconii* and *S. heldreichii* approximate to, or at least approach,  $9_{11} 9_1$ , although trivalents are also often present. A configuration of  $9_{11} 9_1$  is explicable in the following ways: 1) Allosyndesis is occurring between genomes of the two parental species of the hybrid, i.e. the parents possess a single common genome which is pairing in the hybrid whilst the other genome of the tetraploid parent remains unpaired. 2) Autosyndesis is occurring between the two genomes of the tetraploid parent whilst the *fimbriata* genome remains unpaired.

Amongst this series of hybrids the two types of pairing are not necessarily mutually exclusive; some might exhibit type 1 and others type 2. The occurrence of cells containing  $11_{11} 5_1$ ,  $10_{11} 7_1$ ,  $1_{111} 9_{11} 6_1$ ,  $2_{111} 8_{111} 5_1$  etc., i.e. numbers higher than can be explained by simple associations of genomic sets, probably indicates that some random pairing may also occur.

The hybrids of *S. nicaeensis* and *S. bocconii* with *S. purpurea* (2x) show similar configurations to those occurring in the *fimbriata* hybrids and the possible explanations are again the same.

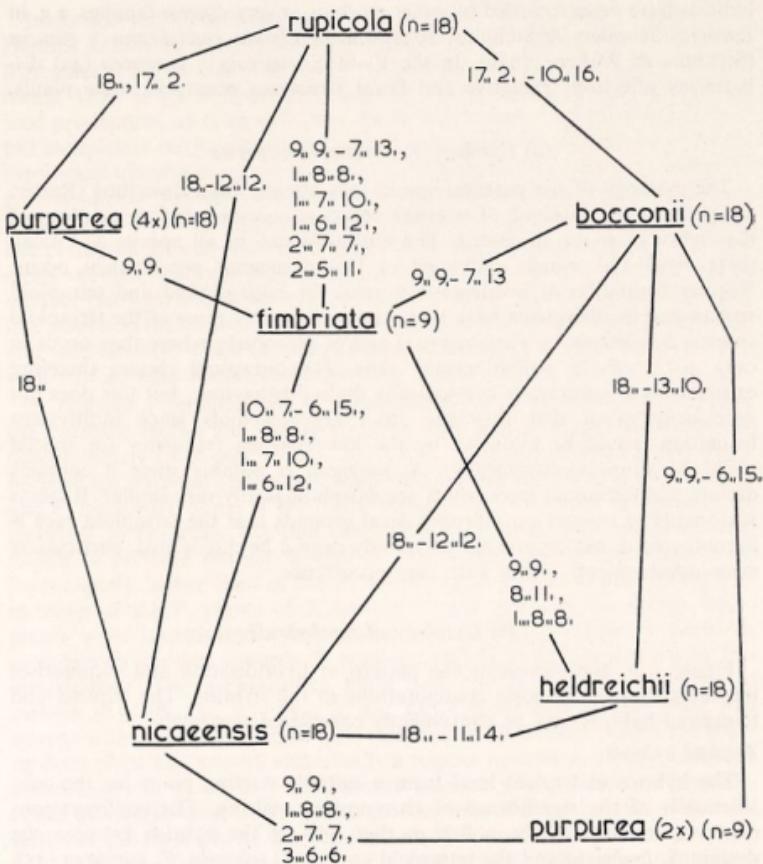


FIG. 1. Pattern of hybridisation and summary of the most frequent meiotic configurations in the hybrids.

The evidence available at present is insufficient to resolve conclusively for any of these hybrids which explanation of pairing is correct but the following discussion attempts to put forward relevant information bearing on the problem.

The occurrence of trivalents in a varying percentage of the pollen mother cells of all seven of the triploid hybrids is significant. The maximum number of trivalents observed per cell is five in *S. nicaeensis* x *purpurea* (2x), four in *S. rupicola* x *fimbriata*, *S. bocconii* x *fimbriata* and *S. heldreichii* x *fimbriata*, three in *S. purpurea* (4x) x *fimbriata* and *S. nicaeensis* x *fimbriata* and two in *S. bocconii* x *purpurea* (2x). The percentage of pollen mother cells containing trivalents varies from about 70% in *S. nicaeensis* x *purpurea* (2x) to 5% in *S. purpurea* (4x) x *fimbriata*. The trivalents are probably the result of the association of corresponding chromosomes of both genomes of

the tetraploid parent with the equivalent *fimbriata* or *purpurea* (2x) chromosome. Their presence therefore seems to reveal a capacity for both auto- and allosyndesis (since both types of association occur in them). Another possible explanation for the occurrence of trivalents is that translocations in a common genome shared by the diploid and tetraploid parents might lead to the formation of trivalents if the low chiasma frequency prevented quadrivalent formation. Similarly it is possible to postulate an entirely autosyndetic trivalent caused by structural heterozygosity of the two genomes of the tetraploid parent and failure of quadrivalent formation. The fact, however, that so many trivalents have been observed (270 in all) but not a single quadrivalent seems to prove conclusively that they originate, as first suggested, by normal association of the equivalent chromosomes of the three genomes present, and not as a result of structural heterozygosity.

Although occurrence of trivalents reveals the existence of some capacity for both auto- and allosyndesis this does not tell us which type of association normally occurs in bivalents in pollen mother cells of the triploid hybrids. Trivalents do indicate, however, the presence of fairly widespread homologies since, for example, the *fimbriata* genome is sufficiently similar to both genomes of the tetraploids *S. rupicola*, *S. purpurea* (4x), *S. nicaeensis*, *S. bocconii* and *S. heldreichii* to produce fair numbers of trivalents in occasional pollen mother cells. If chiasma frequency were higher the expression of this homology in number of trivalents per pollen mother cell might be greater.

Consideration of the case where there are hybrids of both diploid species with two different tetraploid species, i.e. the hybrids of *S. fimbriata* and *S. purpurea* (2x) with *S. nicaeensis* and *S. bocconii*, is deferred until after the following discussion of the tetraploid hybrids.

#### Tetraploid hybrids

The seven tetraploid  $F_1$  hybrids (see Fig. 1) are all characterised by a degree of pairing much in excess of that to be expected if the parents were a series of allopolyploids related to each other by sharing single common diploid ancestral genomes (i.e. 9<sub>11</sub> 18<sub>1</sub>). In fact in most of these hybrids pollen mother cells have been observed showing complete bivalent formation and the average degree of pairing is fairly high. Odd trivalents and quadrivalents occur in a fairly low percentage of the cells in all hybrids except *S. rupicola* x *purpurea* (4x) and *S. bocconii* x *rupicola* where about 20% of pollen mother cells contain these associations. It seems reasonable to suppose that trivalents are a result of failure of quadrivalent formation due to low chiasma frequency, particularly in *S. rupicola* x *purpurea* (4x) where both types of association have been observed, but on the other hand they could be the result of homology of only three of the four genomes present.

Only in the two hybrids where information from  $F_2$  generations is available is there any evidence of whether the observed pairing is due to autosyndesis or to allosyndesis. In *S. bocconii* x *nicaeensis* the occurrence of fairly fertile tetraploid  $F_2$  plants which approach the morphology of both parental species suggests that allosyndesis occurs in the  $F_1$ , since it appears that only primary segregation following allosyndesis could account for their origin. It seems improbable that the alternative process of secondary, segregation, i.e. "shuffling" of the two genomes donated by each tetraploid parent following autosyndesis, could produce such variation. This argument

indicates that the whole chromosome complements of *S. bocconii* and *S. nicaeensis* possess a considerable degree of homology. That they are not completely homologous seems to be shown by the frequent failure of a few of the chromosomes to pair and probably by the fertility of less than 1%. (But see footnote on p. 205).

In the  $F_2$  of *S. rupicola x purpurea* (4x) segregation towards the parental species also occurs but in a less pronounced form. Preliminary cytological investigation of three representative  $F_2$  plants has shown that two were aneuploid, one with two and the other with three extra chromosomes ( $2n=38$  & 39) showing at meiosis about  $18_{11} 2_1$  and  $18_{11} 3_1$  respectively, whilst the other was a normal tetraploid with usually  $18_{11}$ . Here again allosyndesis in the  $F_1$  seems likely although it is possible that much of the segregation effect in the  $F_2$  plants studied might be caused by possession of extra chromosomes. Clearly much more investigation of these hybrids is necessary. If allosyndesis is occurring in *S. rupicola x purpurea* (4x)  $F_1$  it is interesting since *S. purpurea* (4x) has been suspected as an autopolyploid.

#### *Reconsideration of a group of triploid hybrids*

The hybrids of *S. fimbriata* and *S. purpurea* (2x) with *S. nicaeensis* and *S. bocconii* can be discussed now that the pairing of *S. bocconii x nicaeensis* has been considered. In all four of these triploid hybrids the meiotic configurations are approximately similar. That hybrids involving another species and *S. nicaeensis* or *S. bocconii* should show similar meiotic configurations is not surprising since, as already discussed, the chromosome complements of both these species appear to be very similar. This applies equally whether auto- or allosyndesis is occurring in the hybrids. Whether or not the genomes of *S. fimbriata* and *S. purpurea* (2x) are also sufficiently similar to pair is not known as attempted synthesis of the diploid hybrid has been unsuccessful.

#### *Conclusions*

Since it is impossible to distinguish between auto- and allosyndesis in most of the hybrids the information at present available does little to elucidate the relationships of the species. An exception is the case of *S. bocconii* and *S. nicaeensis* where apparent allosyndesis in their hybrid accompanied (at least in one combination of parental provenances) by some, fertility seems to show that the species are at an ecospecific level of differentiation with limited capacity for gene exchange\*. The same is possibly true for *S. rupicola* and *S. purpurea* (4x) but much more evidence is required here. The situation with regard to pairing in other hybrids can be explained very neatly by autosyndesis, which would indicate that the tetraploid species are autopolyploids or segmental allotetraploids. But on the other hand the occurrence of trivalents in the triploid hybrids has revealed some rather widespread homologies between species and, therefore, the possibility that allosyndesis is the main type of pairing involved is just as great.

The investigation has demonstrated that hybrid sterility is a strong barrier to gene exchange amongst the species studied. This sterility varies from 100%

\*Whether this slight potentiality for gene exchange is of any importance in the wild is not yet known.

in most  $F_1$  hybrids (see Table 1) to 95% (5% of normal seed production for the female parent) in others. The frequent occurrence in the hybrids of partial failure of pairing even when the parents are of the same chromosome number probably indicates that sterility is of the chromosomal type.

Future research may produce results which will elucidate the problems. It is hoped that further information may be obtained from the  $F_2$  hybrids of *S. nicaeensis*  $\times$  *purpurea* (2x) and *S. nicaeensis*  $\times$  *purpurea* (4x) and by more detailed investigation of the  $F_2$  hybrids of *S. bocconii*  $\times$  *nicaeensis* and *S. rupicola*  $\times$  *purpurea* (4x). Hybrids involving as yet uncollected species might also yield useful results.

#### SUMMARY

Fourteen different  $F_1$  interspecific hybrids have been successfully synthesised amongst the species listed on page 203. Details of the hybrids are given in Table 1 and the pattern of hybridisation is summarised in Figure 1.  $F_2$  hybrids of *S. bocconii*  $\times$  *nicaeensis* and *S. rupicola*  $\times$  *purpurea* (4x) have also been grown and data concerning them is given in Table 2.

The  $F_1$  hybrids are intermediate in morphology between their parents and are mostly very vigorous. Characteristic features are the long straggling inflorescences and in most hybrids the abnormally small stamens. Meiotic irregularities occur to a greater or lesser degree and fertility varies from nil in most of the hybrids to 5% of normal seed set for the female parent in others.

$F_2$  hybrids of *S. bocconii*  $\times$  *nicaeensis* show segregation towards both parents and in most plants a fair degree of fertility occurs. Cytological study has shown that plants tending towards either parent or of intermediate morphology can have a normal tetraploid number and show regular pairing at meiosis. In *S. rupicola*  $\times$  *purpurea* (4x)  $F_2$  a less marked segregation towards the parental species occurs and some of the plants show signs of genetic unbalance. Initial cytological work has shown a tetraploid and two hypertetraploids in the  $F_2$  of this cross.

In most of the hybrids it is impossible to distinguish between auto- or allosyndesis in meiotic pairing and hence evidence of specific interrelationships is not available from this source. In *S. bocconii*  $\times$  *nicaeensis*, however, evidence from segregation in the  $F_2$  seems to indicate that allosyndesis occurs in the  $F_1$ , and it seems that *S. bocconii* and *S. nicaeensis* are at an ecospecific level of differentiation with a potential capacity for gene exchange across a partial sterility barrier.

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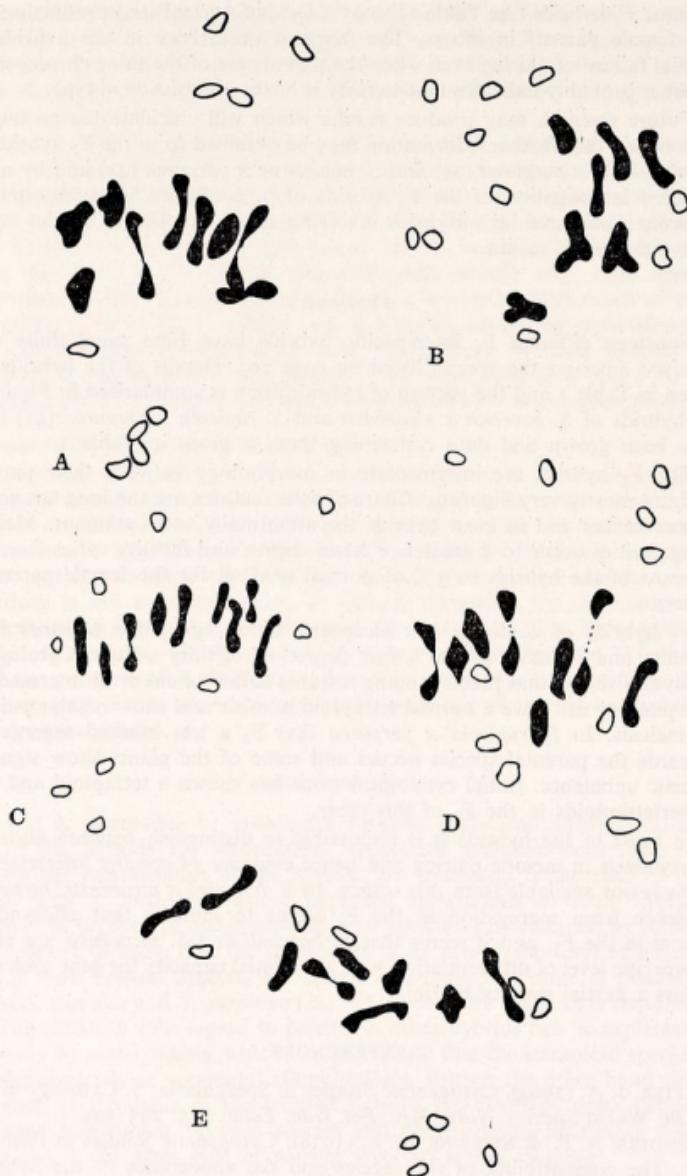


FIG. 2. Explanatory diagrams of Plate 21 A-E,  $\times$  approximately 3,000.

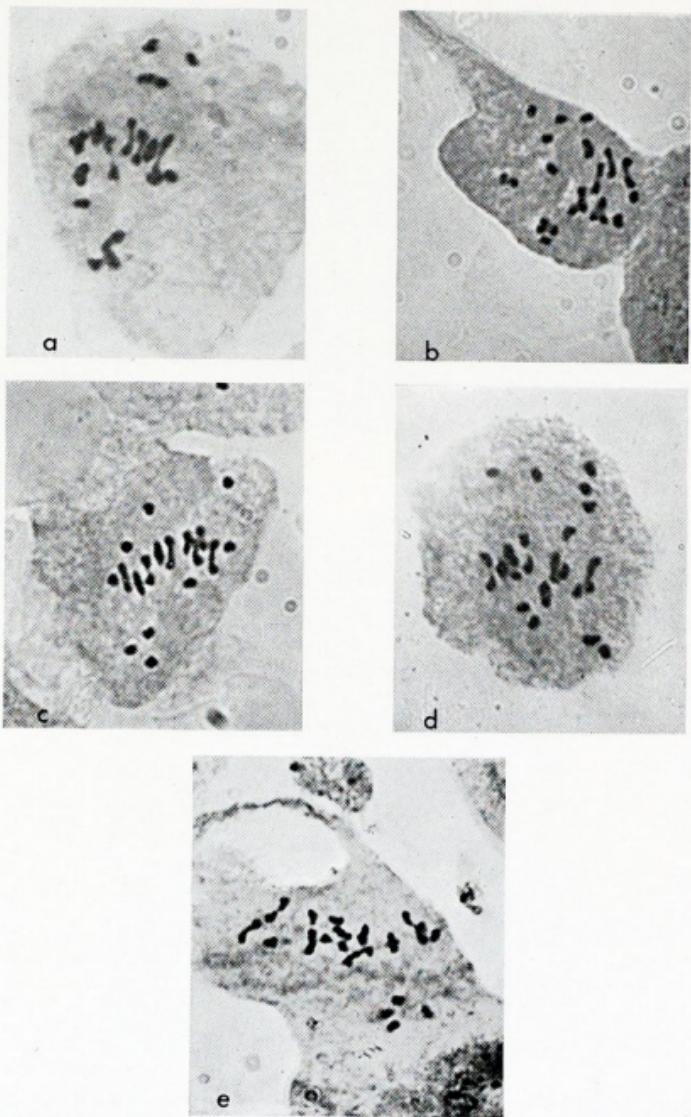


PLATE 21. Pollen mother cells. 1st meiotic metaphase,  $\times 1,500$ .  
 a) *S. rupicola* x *fimbriata* 9<sub>11</sub> 9<sub>1</sub>; b) *S. rupicola* x *fimbriata* 3<sub>111</sub> 4<sub>11</sub> 10<sub>1</sub>; c) *S. purpurea* (4x)  
*x fimbriata* 9<sub>11</sub> 9<sub>1</sub>; d) *S. nicaeensis* x *fimbriata* 8<sub>11</sub> 11<sub>1</sub>; e) *S. nicaeensis* (2x) 9<sub>1</sub> 9<sub>1</sub>.

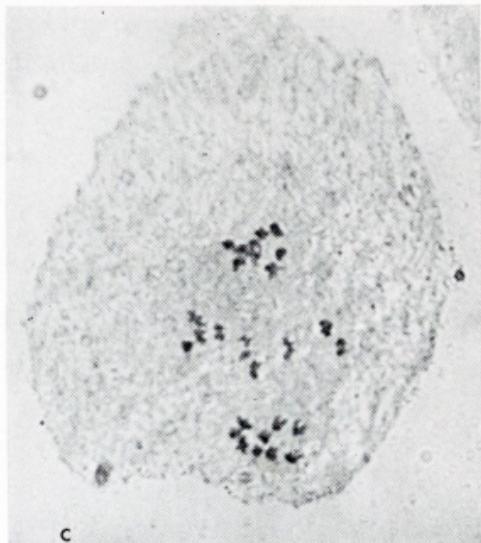
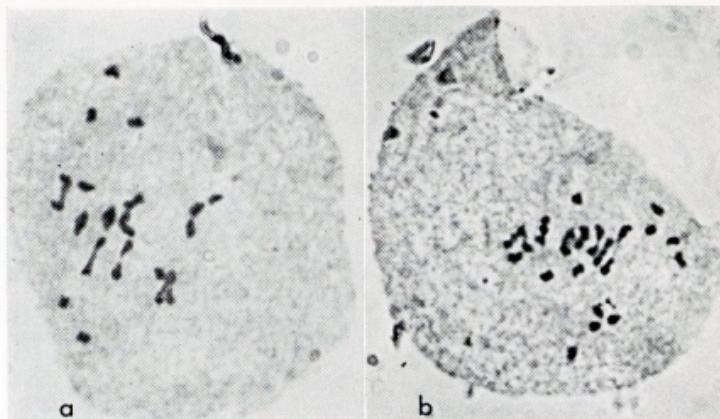


Plate 22. Pollen mother cells  $\times 1,500$  a) *S. bocconii*  $\times$  *fimbriata* 1st meiotic metaphase 8<sub>11</sub> 11<sub>1</sub>. The two univalents lying close to the bivalent on the extreme right are probably the result of separation of a bivalent, thus the configuration was probably originally 9<sub>11</sub> 9<sub>1</sub>; b) *S. bocconii*  $\times$  *purpurea* (2x) 1st meiotic metaphase 1<sub>111</sub> 6<sub>11</sub> 12<sub>1</sub>; c) *S. bocconii*  $\times$  *purpurea* (2x) 1st meiotic telophase showing 9 chromosomes at each pole and 9 lagging univalents undergoing mitotic division on the equator.

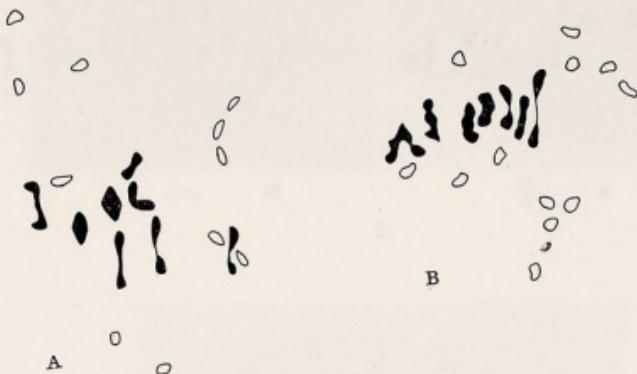


FIG. 3. Explanatory diagrams of Plate 22 A & B  $\times$  approximately 2,500.

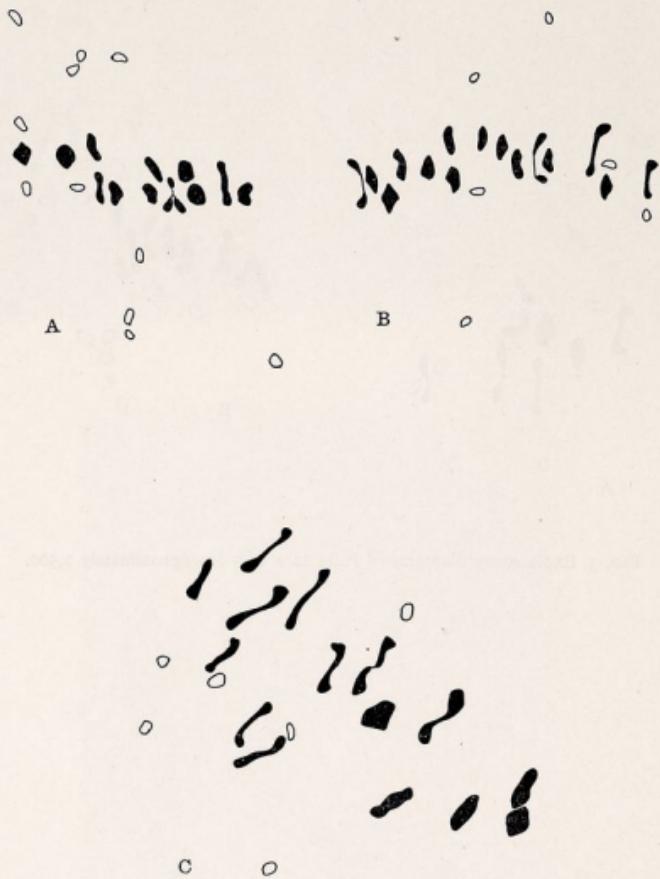


FIG. 4. Explanatory diagrams of Plate 23 A, B & C × approximately 2,500.

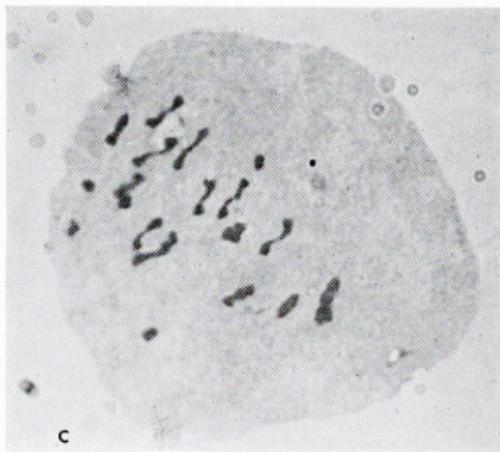
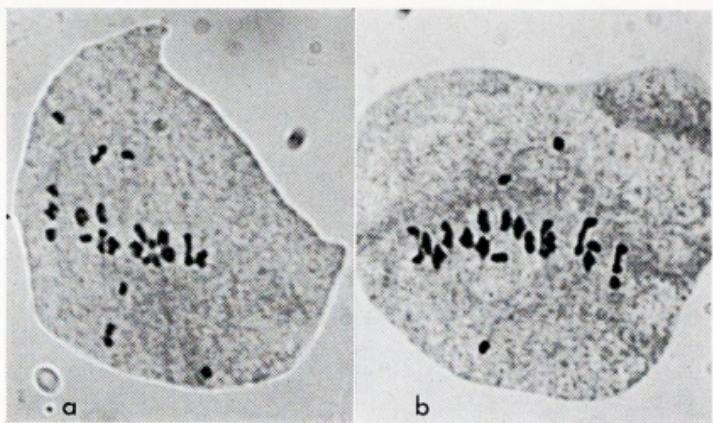


Plate 23. Pollen mother cells at 1st meiotic metaphase,  $\times 1,500$ . a)  $S. nicaeensis \times rupicola$   
 $1_{111} 11_{11} 11_1$  b)  $S. bocconii \times nicaeensis$  15<sub>11</sub> 6<sub>1</sub>; c)  $S. bocconii \times rupicola$  15<sub>11</sub> 6<sub>1</sub>.

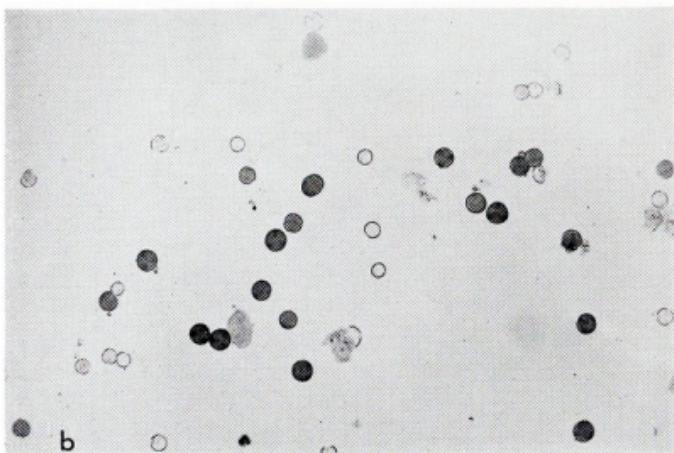
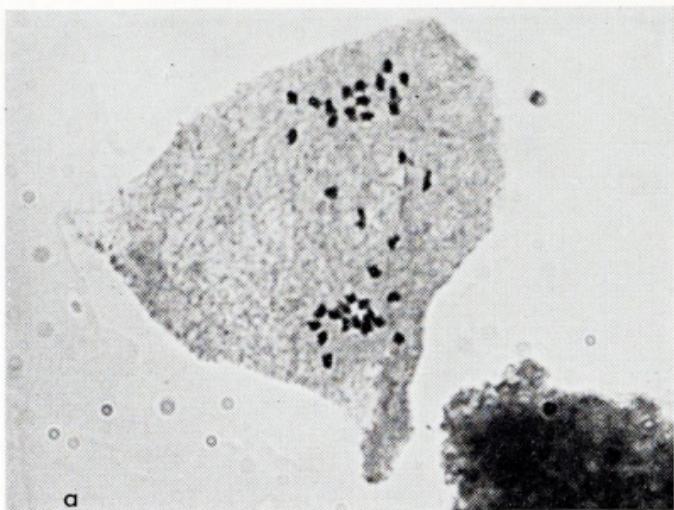


Plate 24. a) *S. bocconii x nicaeensis* Pollen mother cells at 1st meiotic telophase showing lagging univalents,  $\times 1,500$ ; b) *S. rupicola x purpurea (4x)* Pollen showing good and abortive grains,  $\times 175$ .

TABLE I  
*F<sub>1</sub> Interspecific Hybrids*

Hybrid combination and source of parents	2n	No. of plants	Pairing at M1			Notes	
			Configura- tion		No. of PMC		
			111	11	1		
S. rupicola x fimbriata ♀ ♂ Hilbre Is., Cheshire	27	7	10	7	4	Very vigorous robust perennial with thick woody rootstock. 3 plants have been growing in 6" pots for the last 6 years. Perfectly intermediate between parents, apart, of course, from the long straggling cymes. Pollen fertility 0%. Seed fertility 0%. (Seed fertility is expressed as a percentage of that normal for the female parent).	
			9	9	12		
			8	11	9		
			7	13	12		
			6	15	2		
			5	17	1		
			4	18	1		
			1	9	6		
			1	8	8		
			1	7	10		
			1	6	12		
			1	5	14		
			1	4	16		
			2	8	5		
			2	7	7		
			2	6	9		
			2	5	11		
			3	6	6		
			3	5	8		
			3	4	10		
			4	3	9		
			Aneuploid cells				
			9	8	1		
			1	6	11		
			2	8	6		
			Total			91	
S. purpurea (4x) x fimbriata ♀ ♂ Coimbra, Portugal	27	9	10	7	2	Fairly vigorous annual or short-lived perennial. None of the plants lived for more than 3 years. The hybrid is intermediate between the robust perennial <i>S. fimbriata</i> and the slender annual <i>S. purpurea</i> . The rootstock is halfway between the thick woody structure of <i>S. fimbriata</i> and the slender taproot of <i>S. purpurea</i> . Pollen fertility 0%. Seed fertility 0%.	
			9	9	48		
			8	11	2		
			7	13	1		
			1	9	6		
			1	8	8		
			3	6	6		
			Aneuploid cells				
			9	8	2		
			8	8	1		
			Total			59	

TABLE I—contd.

Hybrid combination and source of parents	2n	No. of plants	Pairing at M1			Notes	
			Configura- tion		No. of PMC		
			111	11			
S. nicaeensis x fimbriata ♀ ♂ Hérault, Rabat, France Morocco	27	32	10	7	2	Very vigorous robust per-	
			9	9	3	ennial with thick woody	
			8	11	17	rootstock. Intermediate	
			7	13	11	between its parents.	
			6	15	5	The petal colour is a	
			5	17	1	uniform pink as in the	
			1	9	6	parents.	
			1	8	2	Pollen fertility 0%.	
			1	7	5	Seed fertility 0%.	
			1	6	12		
			2	6	9		
			3	6	6		
			3	5	8		
			Aneuploid cells				
			8	12	1		
			7	12	1		
			1	5	13		
			2	5	10		
			Total			60	
♀ ♂ Barcelona, Rabat, Spain Morocco	27	8	10	7	5	These plants differ notice-	
			9	9	4	ably from those in which	
			8	11	10	the Hérault provenance of	
			7	13	7	S. nicaeensis was the	
			6	15	7	female parent only in	
			5	17	1	flower colour. The Bar-	
			3	21	1	celona provenance of S.	
			1	9	6	nicaeensis has white petals	
			1	8	4	and the hybrid shows an	
			1	7	10	unusual pattern with pink	
			1	6	12	centre and white margins.	
			1	5	14	Pollen fertility 0%.	
			2	7	7	Seed fertility 0%.	
			2	6	9		
			2	5	11		
			2	4	13		
			Total			50	

TABLE I—contd.

Hybrid combination and source of parents	2n	No. of plants	Pairing at M1			Notes
			Configura- tion	No. of PMC	111 11 1	
S. <i>bocconii</i> x <i>fimbriata</i> ♀ ♂ Par, Rabat, Cornwall Morocco	27	52	10 7 2 9 9 22 8 11 15 7 13 7 6 15 2 1 8 3 1 7 10 2 1 6 12 1 2 7 7 3			Vigorous annual or short-lived perennial. As in <i>S. purpurea</i> (4x) x <i>fimbriata</i> an intermediate between a perennial and an annual parent. Pollen fertility c. 0.1%. Seed fertility 0%.
			1 5 13 1			
			—			
			Total 63			
			One PMC occurred in which at least 4 trivalents were present, but otherwise the figure was not good enough for accurate analysis.			
S. <i>heldreichii</i> x <i>fimbriata</i> ♀ ♂ Tangier Rabat, Morocco	27	7	11 5 2 10 7 3 9 9 14 8 11 6 7 13 2 1 9 6 2 1 8 8 8 1 7 10 3 1 6 12 2 2 8 5 1 2 7 7 2 2 5 11 1 3 8 2 1 4 4 7 1			Vigorous short-lived perennial. A hybrid between an annual and a perennial species but with somewhat stouter rootstock than either <i>S. purpurea</i> (4x) x <i>fimbriata</i> or <i>S. bocconii</i> x <i>fimbriata</i> . Pollen fertility c. 0.1%. Seed fertility 0%.
			Aneuploid cell			
			1 7 9 2			
			—			
			Total 50			

TABLE I—*contd.*

Hybrid combination and source of parents	2n	No. of plants	Pairing at M1			Notes	
			Configura- tion	No. of PMC	111 11 1		
S. nicaeensis x purpurea (2x) ♀ ♂ Hérault, Marrakech, France Morocco	27	22	10	7	2	Vigorous annual or short-lived perennial. Intermediate between its parents. Pollen fertility >0.1%. Seed fertility >0.1%.	
			9	9	11		
			8	11	2		
			7	13	2		
			1	9	6		
			1	8	8		
			1	7	10		
			2	8	5		
			2	7	7		
			2	6	9		
			3	6	6		
			3	4	10		
			4	5	5		
			5	4	4		
			Aneuploid cells				
			8	12	1		
			3	5	10		
			—	—	—		
			Total	59	—		
S. bocconii x purpurea (2x) ♀ ♂ Denia, Marrakech, Spain Morocco	27	24	9	9	15	Vigorous annual. Intermediate between parents, both of which are annuals. Pollen fertility c. 0.1%. Seed fertility 0%.	
			8	11	5		
			7	13	11		
			6	15	5		
			5	17	1		
			1	8	8		
			1	7	10		
			1	6	12		
			2	8	5		
			2	7	7		
			Aneuploid cell				
			9	10	1		
			—	—	—		
			Total	51	—		

TABLE I—contd.

Hybrid combination and source of parents	2n	No. of plants	Pairing at M1			Notes
			Configura- tion	No. of PMC	111 11 1	
<i>S. rupicola</i> x <i>purpurea</i> (4x)	36	3				
♀                   ♂			18	27		Short-lived perennial. Much less robust than <i>S. rupicola</i> .
Hilbre Is.,           Coimbra,			17	2	8	Intermediate between parents.
Cheshire           Portugal			16	4	2	Pollen fertility c. 50%.
			15	6	3	Seed fertility c. 5%.
			14	8	1	
			13	10	1	
			12	12	1	
			1	16	1	
			1	14	5	
			1	13	7	
			1	12	9	
			1	11	10	
			2	14	2	
			2	10	10	
			2	9	12	
			16	—	I <sub>IV</sub> I	
			15	2	+ I <sub>IV</sub> I	
			13	6	+ I <sub>IV</sub> I	
			Aneuploid cell			
			1	14	6	1
					—	
				Total	59	
<i>S. nicaeensis</i> x <i>rupicola</i>	36	7				
♀                   ♂			18	1		Very vigorous robust per-
Hérault,           Hilbre,			17	2	5	ennial. Intermediate be-
France           Cheshire			16	4	9	tween parents. In the
			15	6	12	octoploid pollen mother
			14	8	7	cell the unpaired chromo-
			13	10	17	somes are not true uni-
			12	12	10	valents but are the result of
			11	14	2	precocious separation of
			10	16	1	bivalents (they are ar-
			1	2	9	ranged regularly in opposite
			29	14	1	pairs). The fact that quad-
					—	rivalent formation does not
				Total	66	appear to have occurred in
						this cell indicates that there
						is preferential pairing.
						Pollen fertility c. 0.1%.
						Seed fertility 0%.

TABLE I—contd.

Hybrid combination and source of parents	2n	No. of plants	Pairing at M <sub>1</sub>			Notes	
			Configura- tion		No. of PMC		
			111	11			
S. bocconii x rupicola ♀ ♂ Par, Cornwall	36	2	17	2	3	Fairly robust perennial. Intermediate between parents.	
Hilbre, Cheshire			16	4	4	Pollen fertility 0%.	
			15	6	9	Seed fertility 0%.	
			14	8	10		
			13	10	6		
			12	12	6		
			11	14	4		
			10	16	3		
			9	18	1		
			I	14	5		
			I	13	7		
			I	12	9		
			I	11	11		
			I	10	13		
			I	8	17		
			2	10	10		
			Aneuploid cell				
			14	9	1		
			Total		59		
S. nicaeensis x purpurea (4x) ♀ ♂ Hérault, France	36	3	18		7	Short-lived perennial. Intermediate between parents.	
			17	2	1	Pollen fertility c. 50%.	
			16	4	1	Seed fertility c. 5%.	
			2	15	1		
			16	+ I <sub>IV</sub> I			
			Total		11		
S. nicaeensis x bocconii ♀ ♂ Barcelona, Spain	36	15	18		1	Vigorous annual to short-lived perennial. Intermediate between its parents. As in <i>S. nicaeensis</i> x <i>fimbriata</i> the white petal colour of <i>S. nicaeensis</i> Barcelona provenance combined with the pink of <i>S. bocconii</i> gives a characteristic petal pattern which is pink in the centre and white at the margins.	
			17	2	3	Pollen fertility 1-2%.	
			16	4	11	Seed fertility 0%.	
			15	6	16		
			14	8	8		
			13	10	7		
			12	12	3		
			I	14	5		
			I	11	11		
			Total		51		

TABLE I—contd.

Hybrid combination and source of parents	2n	No. of plants	Pairing at M1			Notes	
			Configura- tion		No. of PMC		
			111	11			
S. <i>bocconii</i> x <i>nicaeensis</i>	36	26	16	4	5	Petal colour as in the reciprocal hybrid.	
♀ Denia, Spain	♂ Barcelona, Spain		15	6	11	Pollen fertility 1-2%.	
			14	8	19	Seed fertility 0% (but see footnote p. 206).	
			13	10	11		
			12	12	6		
			10	16	2		
			I	15	3		
			I	14	5		
			I	13	6+IV I		
			Total			57	
♀ Denia, Spain	♂ Hérault, France	36	28	18	7	Differs from the other hybrids of this combination (where <i>S. nicaeensis</i> Barcelona provenance is the female parent) in its petal colour which is a normal pink.	
			17	2	7		
			16	4	7		
			15	6	18		
			14	8	8		
			13	10	3		
			12	12	3		
			I	14	5	Pollen fertility c. 5%.	
			I	12	9	Seed fertility >1% (but see footnote p. 205).	
			I	9	14+IV I		
			Total			56	
S. <i>nicaeensis</i> x <i>heldreichii</i>	36	37	18	2	2	Annual to short-lived perennial. Intermediate between parents.	
♀ Hérault, France	♂ Tangier		17	2	6		
			16	4	9		
			15	6	13	Pollen fertility c. 1%.	
			14	8	6	Seed fertility 0%.	
			13	10	12		
			12	12	5		
			I	12	9		
			2	11	8		
			Aneuploid cells				
			I	5	I		
			12	11	I		
			Total			57	

TABLE I—*contd.*

Hybrid combination and source of parents	2n	No. of plants	Pairing at M1			Notes
			Configura- tion	No. of PMC		
	111	11	1			
S. heldreichii x nicaeensis Tangier Hérault, France	36	10	17 16 15 14 13 12 11 I I	2 4 6 8 10 12 14 7 5	3 8 5 14 10 7 3 1 1	As above.
			<i>Aneuploid cell</i>			
			13	9	1	
			Total			53
S. bocconii x heldreichii ♀ ♂ Denia, Tangier Spain	36	44	18 17 16 15 14 13	20 9 10 6 5 2	— — — 52	Vigorous annual. Intermediate between parents. Pollen fertility 0%. Seed fertility 0%.
			High polyploid cells were observed but were uncountable.			
S. heldreichii x bocconii ♀ ♂ Tangier Denia, Spain	36	19	18 17 16 15 I	15 12 4 2 1	— — — — —	As above.
			Total			34

TABLE 2  
Preliminary Cytological data from  $F_2$  hybrids

Hybrid combination	No. of plants	2n	Pairing at $M_1$			Pollen fertility	Seed fertility	Notes
			configura-	No. of				
			111	11	1			
S. <i>bocconii</i> x <i>nicaeensis</i> ( <i>Denia</i> x <i>Hérault</i> )	1	36	18	25		c.30%	c.30%	A <i>bocconii</i> -like segregate showing comparatively high fertility.
			17	2	1			
			15	2 + I <sub>IV</sub> I				
	1	36	18	14		c.30%	c.30%	A <i>nicaeensis</i> -like plant also showing high fertility.
			17	2	3			
	1	36	18		7	c.7%	c.7%	An intermediate plant. There are unusually few PMC per anther.
			15	6	1			
			1 16	1	1			
	1	40	19	2	6	c.10%	1-2%	A <i>nicaeensis</i> -like plant of aneuploid chromosome number.
			18	4	5			
			17	6	1			
			1 17	3	1			
								Only four plants of the $F_2$ generation of this hybrid have, as yet, been examined cytologically.
S. <i>rupicola</i> x <i>purpurea</i> (4x)	1	38	18	2	9	c.50%	c.3%	The morphology of this plant tends towards <i>S. rupicola</i> but a number of <i>S. purpurea</i> characters are also present.
	1	39	18	3	4	c.2%	c.1%	Tending towards <i>S. purpurea</i> morphology.
			1 14	8	1			
			2 15	3	2			
	1	36	18		11	c.1%	c.1%	More or less intermediate between the parental species.
			17	2	2			
			16	4	2			
			1 16	1	1			
								Only three plants of this $F_2$ hybrid have been examined cytologically.