CYTOGENETIC STUDIES IN SPERGULARIA: IV

Some Further Interspecific Hybrids

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Two previous communications (Ratter 1965a, 1965b) have dealt with synthetic interspecific hybrids of Old World *Spergularia* species, concentrating on attempts to obtain information of relationship and evolution by means of meiotic analysis. The present paper follows similar lines and reports hybrids amongst the species listed below.

	2n	Distribution	Notes
S. rupicola Lebel ex Le Jolis	36	Atlantic Coast of Europe to c. 58° N in Scotland.	Robust perennial with large conspicuous fls. Probably there is considerable out-breeding in this sp., but nevertheless vigorous offspring are produced by selfing.
S. purpurea (Pers.) G. Don	18, 36	Mediterranean region.	Slender annual with very conspicuous flowers. Probably predominantly outbreeding. Both diploid (2n = 18) and tetraploid (2n = 36) races were used in the hybridizations reported here.
S. rubra (L.) J. & C. Presl	36, 54	Widespread in northern hemisphere.	Annual to perennial. Probably both out- and inbreeding occur in nature. Both the tetraploid (2n = 36) and the hexaploid (2n = 54) races were used in the hybridizations reported here.
S. capillacea (Kindb. & Lange) Willk.	18	Portugal and NW Spain.	Habit ± similar to *S. rubra*. Breeding system probably also similar.
S. bocconii (Scheele) Aschers & Graebn.	36	SW Europe and Mediterranean region.	Slender annual or short-lived perennial. Probably predominantly inbreeding.
S. heldreichii (Foucaud) Simon & Monnier	36	Mediterranean region extending to the atlantic coast of France.	Slender annual. Probably predominantly inbreeding.

Descriptions of all species are given in Flora Europaea Vol. I. The notes on breeding system are in the main based on the size and conspicuousness of flowers and their pollen output, rather than on direct observations of pollination in the field. Flower size varies from about 9–10 mm diameter in *S. rupicola* to 4–6 mm diameter in *S. bocconii* and *S. heldreichii.* The latter two species also generally show a reduced number of stamens (usually 2–5 in *S. bocconii* and 6–8 in *S. heldreichii*), whereas in *S. rupicola* and *S. purpurea* 10 stamens nearly always occur. The number of pollen grains per anther

FIG. 1. Explanatory diagrams of Plate 7 a–d.

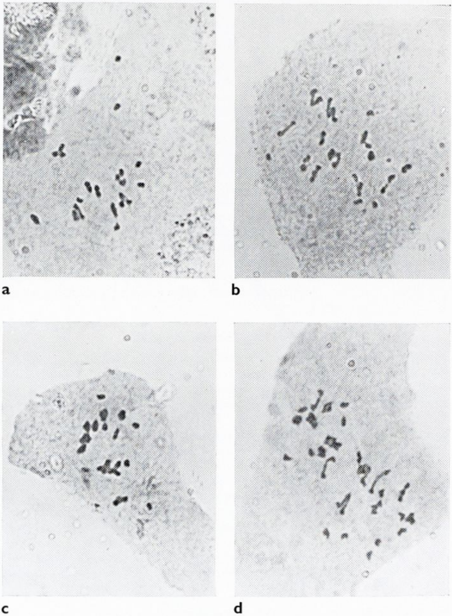

Plate 7. Pollen mother cells. 1st meiotic metaphase × 1,500. a, *S. rupicola* × *capillacea*, 4_{iii} 5_{ii} 5_{i}; b, *S. purpurea* (4x) × *heldreichii*, 1_{iv} 1_{iii} 11_{ii} 6_{i}—the figure appears to be lacking one chromosome (2n = 35), two of the univalents have obviously been produced by dissociation of a bivalent; c, *S. rubra* (4x) × *capillacea*, 4_{iii} 5_{ii} 5_{i}; d, *S. rubra* (6x) × *bocconii*, 3_{iii} 12_{ii} 12_{i}.

is also spectacularly different: c. 5000 grains in *S. rupicola* and *S. purpurea* but only c. 1000 in *S. heldreichii* and c. 100–380 in *S. bocconii*. This information suggests that *S. rupicola* and *S. purpurea* are adapted towards outbreeding and *S. bocconii* and *S. heldreichii* towards inbreeding. For *S. purpurea*, further information from two sources reinforces this conclusion: 1, the stigmas seldom touch the anthers of the same flower, as they do during anthesis in most other species of *Spergularia*, so that in the absence of insect pollination fruit-set normally fails; 2, seeds collected from wild populations of this species produce plants very variable in morphology, indicating that considerable heterozygosity must exist in nature. *S. rubra* and *S. capiliacea* appear to fall somewhere between the other species, having intermediate flower size and pollen output, and rarely a reduction in stamen number.

Most interspecific crosses attempted amongst these species are blocked by a variety of barriers, the most important of which is seed incompatibility. The present communication is, however, devoted only to hybrids which successfully reached flowering and consideration of barriers operating before this stage is being reserved for a future publication. The existence of such barriers, however, accounts for the absence of most of the possible hybrid combinations apart from *S. rupicola* × *purpurea* (4x), *S. rupicola* × *bocconii* and *S. bocconii* × *heldreichii* which are omitted here since they have already been reported in a previous paper (Ratter 1965a).

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 I and has been given in more detail previously (Ratter, 1964).

The techniques of culturing the plants and of hybridization were the same as described previously (Ratter, 1965a).

Meiosis was studied in pollen mother-cells 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 7 years storage showed no deterioration (in fact these long-stored buds appeared to show an actual improvement in staining quality).

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

RESULTS

Table I summarizes parentage, meiotic pairing, pollen fertility and seed fertility of the five F_1 interspecific hybrids which have been grown to flowering. Plate 7 shows meiosis in pollen mother cells of the hybrids.

The F_1 hybrids were intermediate in most characters between their parental species. They were vigorous and showed no signs of malformations except in the stamens of *S. rupicola* × *capillacea*, *S. rubra* (4x) × *capillacea*, and *S. bocconii* × *rubra* (6x) which were abnormally small. Such abnormally small stamens are a common feature of interspecific hybrids in *Spergularia* and in many other genera (see Ratter, 1965a). The long straggling inflorescences previously commented upon (Ratter 1965a) were present in all hybrids except *S. purpurea* (4x) × *heldreichii*, and its reciprocal, where fertility was too high to allow the superabundant flowering which produces them.

In pollen mother cells the usual pattern occurred with bivalents, trivalents and quadrivalents arranged on the equatorial plate and univalents, when present, scattered through the cell. At first meiotic anaphase and telophase the univalents could be seen as laggards which usually divided equationally and then travelled to the poles. Exclusion of lagging chromosomes from the daughter nuclei appeared to be rare in all hybrids and polyspory was not observed.

Pollen fertility varied from nil in *S. rupicola* × *capillacea* to c. 50% in *S. purpurea* (4x) × *heldreichii*, and seed fertility from nil in *S. rupicola* × *capillacea*, *S. rubra* (4x) × *capillacea* and *S. bocconii* × *rubra* (6x) to 20–25% of that normal for the female parent in *S. heldreichii* × *purpurea* (4x).

When F_1 hybrids showed any fertility, F_2 generations were raised. Eight F_2 plants of *S. purpurea* (4x) × *heldreichii* were grown. They were of fairly uniform morphology but showed some slight segregation towards the parental species, especially in petal colour. Pollen fertility varied from plant to plant, ranging from nil to c. 30%, whilst seed fertility, expressed as a percentage of that of *S. heldreichii*, was from nil to c. 5%. Meiosis was examined in four plants, all of which had $2n = 36$ and showed regular formation of bivalents. Eight plants of the reciprocal hybrid were also raised; their range of morphology and fertility was similar to *S. purpurea* (4x) × *heldreichii* and apart from the fact that one of the four examined cytologically had $2n = 37$ they also corresponded in cytology.

Nine F_2 hybrids of *S. rubra* (6x) × *S. purpurea* (2x) were raised and all were examined cytologically. Five plants were tetraploid ($2n = 36$) as in the F_1, one had $2n = 37$, and the remaining three were octoploid ($2n = 72$). Meiosis of the tetraploid plants was more or less regular with normal bivalent formation, and the same was the case in the $2n = 37$ plant whose extra chromosome either remained unpaired or was associated in a trivalent. The octoploid plants, however, showed rather irregular meiosis with many quadrivalents, trivalents and univalents at M I and many lagging chromosomes at the anaphase separation. The five tetraploid plants included one with high fertility (c. 30% of parental *S. rubra* seed output), two with c. 5% fertility, another with less than 1% fertility and another completely sterile. The $2n = 37$ plant had c. 10% seed fertility whilst one of the octoploids had c. 7%, another c. 3% and the third was completely sterile, exhibiting a teratological bushy form with distorted flowers which never opened. Apart from this one malformed plant the generation was fairly uniform; slight segregation towards the parental species is detectable amongst the plants but it was not possible to distinguish the ploidy levels morphologically.

DISCUSSION

For convenience in discussion the individual hybrid combinations are first treated separately and then the more general significance of the results is dealt with in the conclusions subsection.

1. S. rupicola × capillacea

The meiotic analyses show that occurrence of trivalents is common in this hybrid and that they may reach as many as four per P.M.C. (see Table 1). Formation of trivalents presumably indicates homology of all three genomes present in this triploid hybrid, and the fact that their number can be as high as four per cell seems to suggest a high degree of homology. Unfortunately critical determinations of chiasma frequency are not available for the parental species, but from rather casual observations it seems unlikely that the frequencies exceed 1·5 per bivalent, so that, even with complete homology between all three genomes present in the hybrid, configurations probably could not be expected showing more trivalents than have been observed. It is worth pointing out here that configurations showing the highest degree of association are more significant in revealing genomic homologies than those showing less association, since the latter may be the result of preferential pairing of complete or nearly complete homologues causing the exclusion of chromosomes with a lower degree of homology, or may merely reflect low chiasma frequency. In this hybrid, therefore, the pattern of meiotic association reveals that all three genomes (two derived from *S. rupicola* and one from *S. capillacea*) show considerable homology. This carries with it the corollary that *S. rupicola* is evidently a segmental allotetraploid or an autotetraploid since its two genomes (or "subgenomes") are obviously at least partially homologous.

2. S. purpurea (4x) × heldreichii and reciprocal.

With the exception of *S. bocconii* × *nicaeensis* (Ratter 1965a, see footnote p. 205) this interspecific hybrid, with a seed set of c. 20–25% of that normal for the *S. heldreichii* parent, is the most fertile which has yet been synthesized in *Spergularia*. The degree of fertility is surprising when the high frequency of multivalent formation observed in the meiotic analysis of *S. heldreichii* × *purpurea* (4x) is taken into account. Possibly these analyses, which were derived from five flowers coming from two plants (three from one, two from the other), are atypical and the normal situation is more accurately represented by the analyses in the reciprocal hybrid where configurations of eighteen bivalents predominate.

The occurrence of cells in *S. purpurea* (4x) × *heldreichii* containing as many as five quadrivalents reveals that a high degree of homology must exist between all four genomes present, particularly since it is unlikely that the chiasma frequency of either parent is above 1·5 per bivalent. This indicates not only a homology between the *S. purpurea* (4x) and the *S. heldreichii* complements but also shows that both species have a high capacity for autosyndesis (since both auto- and allosyndesis occur in the quadrivalents). Such a capacity for autosyndesis shows that both *S. purpurea* (4x) and *S. heldreichii* are autotetraploids or segmental allotetraploids.

It is notable that none of the F_2 generation are more fertile than their F_1 parents, and in fact some of the plants show a reduced fertility or are completely sterile. In this respect they differ from *S. bocconii* × *nicaeensis* where the F_2 showed an increase in fertility over the F_1 (Ratter 1965a).

3. S. rubra (4x) × capillacea

The discussion for *S. rupicola* × *capillacea* is equally applicable to this hybrid. The number of trivalents per cell reaches five, indicating a high degree of homology between all three genomes present, and cells with three or four trivalents are quite common. Considering once more that the chiasma frequencies of the parents are unlikely to be more than 1·5 per bivalent, the association observed in cells with five trivalents is probably near the maximum to be expected even from three completely homologous genomes.

The two species *S. rubra* (4x) and *S. capillacea* are certainly morphologically very similar so that the close relationship of their genomes is not surprising. It seems probable therefore, on grounds of morphology and of chromosome behaviour at meiosis, that *S. rubra* (4x) represents an autopolyploid derivative of *S. capillacea*, or alternatively a segmental allopolyploid derived from the hybridization of *S. capillacea* and a closely related species.

4. S. rubra (6x) × purpurea (2x)

The extreme paucity of meiotic analyses in this hybrid makes any hypothesizing as to their significance rather unsatisfactory. Nevertheless the degree of fertility would seem to indicate that regular eighteen bivalent associations must be relatively common (four out of the six figures analysed were of this type). The occurrence of eighteen bivalent associations reveals homology of the genome of *S. purpurea* (2x) with at least one of those of *S. rubra* (6x).

The most interesting feature of the F_2 generation was the occurrence of three octoploid plants which were presumably produced by the union of unreduced gametes. Variation in fertility in this generation is considerable but cannot be correlated with ploidy level (see p. 216); in the octoploids sterility can be related to the observed meiotic abnormalities, but in the tetraploids it is associated with apparently normal meiosis.

5. S. bocconii × rubra (6x)

Meiotic configurations in this hybrid unfortunately cast no light on genomic relationships since it is impossible to determine the nature of the observed pairing. Two pieces of information from other hybrids do give some clue as to what might be happening: i, the occurrence of pollen mother cells with eighteen bivalents in *S. rubra* (6x) × *purpurea* (2x) has shown that at least two of the genomes of hexaploid *S. rubra* must be capable of autosyndesis (admittedly the stocks of hexaploid *S. rubra* used in *S. bocconii* × *rubra* (6x) and *S. rubra* (6x) × *purpurea* (2x) are different, but they are very similar morphologically and it seems safe to assume that they are cytologically similar); ii, evidence from other hybrids involving *S. bocconii* (Ratter, 1965a) indicates that a capacity for autosyndesis also exists amongst the genomes of this species. We can conclude therefore that a considerable capacity for autosyndesis exists amongst the *rubra* (6x) and *bocconii* genomes of this hybrid, but there is no evidence to show to what extent the actual pairing which occurs is auto- or allosyndetic.

Conclusions

The most significant findings which emerge from the cytological results are: (a), the capacity for autosyndesis which the genomes of the tetraploids *S. rupicola*, *S. rubra* (4x), *S. purpurea* (4x) and *S. heldreichii* exhibit in hybrids; (b), the high degree of homology existing between the genome of

S. capillacea and both genomes of *S. rupicola* and of *S. rubra* (4x); and (c), the equally high homology occurring between the genomes of *S. purpurea* (4x) and *S. heldreichii*.

The capacity for autosyndesis indicates that the four species, *S. rupicola*, *S. rubra* (4x), *S. purpurea* (4x) and *S. heldreichii* are autopolyploids or segmental allopolyploids. They are all, however, cytologically diploid in behaviour regularly showing meiotic configurations of eighteen bivalents.* A possible explanation of their cytological behaviour is that preferential pairing of completely homologous genomes controls regular bivalent formation in the parental species, but in the hybrids where exact homologues are not present the system breaks down with resultant multivalent formation—such an explanation of course presupposes that these species are segmental allopolyploids and not autopolyploids. The meiotic configurations exhibited in other hybrids involving *S. rupicola*, *S. purpurea* (4x) and *S. heldreichii* reported in previous communications (Ratter 1965 a & b) are consistent with auto- or segmental allopolyploidy in these species.

The capacity for syndesis between genomes of different species indicates the occurrence of rather widespread homologies in the genus. For instance evidence from meiotic association in *S. purpurea* (4x) × *heldreichii* indicates a high degree of similarity of the chromosomal complements of the rather dissimilar tetraploid parents, whilst the genome of *S. capillacea* not only shows high homology with both of those present in *S. rubra* (4x) but also with both of *S. rupicola*.

SUMMARY

Five synthetic interspecific F_1 hybrids are reported in this paper. These are *S. rupicola* × *capillacea*, *S. purpurea* (4x) × *heldreichii* (and reciprocal), *S. rubra* (4x) × *capillacea*, *S. rubra* (6x) × *purpurea* (2x) and *S. bocconii* × *rubra* (6x). In addition F_2 generations of *S. purpurea* (4x) × *heldreichii* (and reciprocal) and *S. rubra* (6x) × *purpurea* (2x) have been raised. Details of meiosis and fertility of the F_1 hybrids are given in Table 1.

The F_1 hybrids are intermediate in morphology between their parents and are vigorous. *S. rupicola* × *capillacea*, *S. rubra* (4x) × *capillacea* and *S. bocconii* × *rubra* (6x) are totally sterile, whilst *S. rubra* (6x) × *purpurea* (2x) has some fertility and in *S. purpurea* (4x) × *heldreichii* (and reciprocal) fertility reaches 20–25% of the normal seed-set for *S. heldreichii*. Characteristic features of the sterile hybrids are the long straggling inflorescences and, in most, the abnormally small stamens.

Analyses of meiotic pairing in the F_1 hybrids *S. rupicola* × *capillacea*, *S. purpurea* (4x) × *heldreichii* (and reciprocal) and *S. rubra* (4x) × *capillacea* show that the tetraploid species *S. rupicola*, *S. purpurea* (4x), *S. heldreichii* and *S. rubra* (6x), are autopolyploids or segmental allopolyploids; they also reveal the occurrence of rather widespread genomic homologies amongst species in the genus.

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*A very few exceptional cells have been seen in *S. rupicola* and *S. purpurea* (4x) which contained single quadrivalents (Ratter 1964).

References

Ratter, J. A. (1964) Cytogenetic studies in Spergularia: I, Cytology of the old world species. *Notes R.B.G. Edinb.* 25:293–303.

——(1965a) Cytogenetic studies in Spergularia: II, An attempt to discover cytogenetic relationships of some species. *Ibid.* 26:203–223.

——(1965b) Cytogenetic studies in Spergularia: III, Some interspecific hybrids involving S. marina (L.) Griseb. *Ibid.* 26:224–236.

TABLE I

F_1 Interspecific Hybrids

Hybrid combination and source of parents	$2n$	*No. of plants*	*Pairing at M*I *Configuration* IV	III	II	I	*No. of PMC*	*Notes*
S. rupicola × *capillacea* Hilbre Is., Cheshire. Coimbra, Portugal.	27	4		4	6	3	2	Vigorous robust perennial with thick woody rootstock. Morphology intermediate between parents Stamens minute. Pollen fertility 0%. Seed fertility 0%. (Seed fertility is expressed as a percentage of that normal for the female parent.)
				4	5	5	2	
				4	4	7	3	
				3	7	4	1	
				3	6	6	2	
				3	5	8	1	
				2	8	5	1	
				2	7	7	1	
				2	6	9	3	
				2	5	11	1	
				2	4	13	1	
				1	9	6	1	
				1	8	8	3	
				1	7	10	3	
				1	6	12	1	
				1	5	14	3	
					11	5	1	
					9	9	1	
					8	11	9	
					7	13	9	
					6	15	3	
					5	17	1	
					Total		53	
S. purpurea × *heldreichii* (4x) Cordoba, Spain. Tangier, Morocco.	36	3	5	1	6	1	1	Slender plant intermediate in morphology between parents. Stamens similar in size to *heldreichii* parent, 10 in number. Pollen fertility c.50%. Seed fertility approximately 20–25% that of parent.
			3	4	3	6	1	
			3	3	5	5	1	
			3	2	9		1	
			3	2	7	4	1	
			3	1	8	5	1	
			2	3	8	3	1	
			2	2	7	8	1	
			1	2	12	2	1	
			1	2	7	12	1	
			1		16		1	
				3	10	7	1	
				2	14	2	1	
				2	13	4	2	
				2	12	6	1	
				1	16	1	1	
				1	15	3	1	
					18		6	
					17	2	1	
			Apparent aneuploid cells					
			1	3	9	4	1	
				3	12	4	1	
				3	8	10	1	
						Total	28	

TABLE I (*Contd.*)

Hybrid combination and source of parents	2*n*	*No. of plants*	*Pairing at M*I					*Notes*
			Configuration IV	III	II	I	*No. of PMC*	
S. heldreichii × *purpurea* (4x) Tangier, Morocco. Cordoba, Spain.	36	5	1		16		1	Identical in morphology to reciprocal hybrid. Pollen fertility c.2–50%. Seed fertility c.20% of ♀ parent (80–90% of flowers develop into small capsules).
			1	1	13	3	1	
				3	13	1	1	
				2	15		2	
				1	15	3	1	
					18		20	
					17	2	1	
			Apparent aneuploid cells					
				2	14	4	1	
				1	16	3	1	
						Total	29	
S. rubra (4x) × *capillacea* Ness, Cheshire. Coimbra, Portugal.	27	10	1		8	7	1	Intermediate in morphology between the very similar parents. Stamens minute. Pollen fertility 0–1%. Seed fertility nil.
				5	5	2	1	
				5	4	4	1	
				4	6	3	3	
				4	5	5	4	
				4	4	7	1	
				3	8	2	1	
				3	7	4	5	
				3	6	6	8	
				3	5	8	1	
				2	9	3	4	
				2	8	5	5	
				2	7	7	6	
				2	6	9	1	
				1	12		1	
				1	10	4	1	
				1	9	6	4	
				1	8	8	2	
					11	5	1	
					9	9	1	
			Apparent aneuploid cells					
			2	3	4	4	1	
				5	6	1	1	
				4	6	4	1	
				3	8	3	1	
				3	7	7	1	
				3	7	6	2	
				3	7	5	1	
				2	9	7	1	
				2	6	8	1	
					11	6	1	
					11	4	1	
					9	8	1	
					7	11	1	
						Total	66	

TABLE I (*Contd.*)

Hybrid combination and source of parents	2*n*	*No. of plants*	*Pairing at M* I					*Notes*
			Configuration				*No. of PMC*	
			IV	III	II	I		
S. rubra (6x) × *purpurea* (2x)	36	11	$\mathrm{I_{v}}$+ $\mathrm{I_{iv}}$	4	5	5	1	Intermediate in morphology between parents.
Hèrault, France. — Marrakesh, Morocco.					18		4	Stamens slightly smaller than normal for *S. rubra*.
				4	10	4	1	Pollen fertility 1–3%.
						Total	6	Considerable seed fertility.
S. bocconii × *rubra* (6x)	45	39		4	10	13	1	Intermediate in morphology between parents.
Par, Cornwall. — Zagreb, Yugoslavia.				3	12	12	1	Stamens small with short filaments.
				3	10	16	1	Pollen fertility 1%.
				2	15	9	1	Seed fertility nil.
				1	13	16	1	
					15	15	1	
					14	17	1	
					13	19	1	
					11	23	1	
					10	25	1	
					8	29	1	
			Apparent aneuploid cell					
				1	10	23	1	
						Total	12	