CYTOGENETIC STUDIES IN SPERGULARIA: V

Some Interspecific Hybrids involving S. media (L.) C. Presl

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This paper follows on from the previous one (Notes R.B.G. Edinb. 29:213–223, 1969) and deals with interspecific hybrids of *S. media* (L.) C. Presl with *S. rupicola* Lebel ex Le Jolis, *S. purpurea* (Pers.) G. Don fil. (tetraploid race), *S. capillacea* (Kindb. & Lange) Willk., and the new world species *S. macrotheca* (Hornem.) Heynh. Brief notes on habit, distribution etc. for *S. media* and *S. macrotheca* are given below; similar notes have already been given in the previous paper for the other species. Full descriptions of all species, with the exception of *S. macrotheca*, appear in Flora Europaea Vol. 1. A full description of *S. macrotheca* is given in Rossbach's monograph of *Spergularia* in North and South America (1940).

S. media (L.) C. Presl is a perennial herb with woody rootstock which inhabits salt marshes, waste places near the sea, saline deserts and similar habitats; it is more or less cosmopolitan. The breeding system, at least in north temperate regions, probably involves a balance between out- and inbreeding. Normally $2n = 2x = 18$, although tetraploid races are known.

S. macrotheca (Hornem.) Heynh. is a very robust new world perennial. It is distributed along the Pacific coast of north America from northern Baja California to Vancouver Island, and on Guadalupe Island, Mexico. The plant which was used in the successful synthetic hybridization was one grown from seed collected from a Guadalupe herbarium specimen (*Copp* 174) by Dr. P. H. Raven of Stanford University, California. These Guadalupe plants differ considerably in morphology from typical mainland *S. macrotheca* and originally were described as a separate species, *Tissa talinum* Greene (*Tissa* Adans. is a generic synonym of *Spergularia* (Pers.) Presl); Rossbach (1940) considers, however, that this form does not merit specific separation. The Guadalupe stock grown at Edinburgh is probably normally outbreeding, since self-pollination usually fails due to the receptive surface of the stigmas failing to come in contact with the anthers during anthesis.

The chromosome number of the plants grown from the Guadalupe seed was $2n = 72$, the octoploid number for the genus. Meiosis was rather difficult to observe accurately at first meiotic metaphase but appeared to show normal bivalent pairing. Seeds from two mainland collections of typical *S. macrotheca* morphology (*Moran* 6836 and *Moran* 8279) have also been sent to me by Dr. P. H. Raven and the plants grown from these showed $2n = 36$. Attempts to cross these mainland stocks with *S. media* have been blocked by seed incompatibility.

MATERIALS AND METHODS

The source of all material used in the investigation is given in Table I. All stocks apart from *S. capillacea* are of known wild origin.

Culture of the plants and all other techniques were as described in the previous paper and in Ratter 1965a.

Results

Table I summarises information of parentage, meiotic pairing, and fertility of the F_1 interspecific hybrids. Table 2 gives the same information for the F_2 generation of *S. media* × *purpurea*. Notes on each of the hybrids are given below.

S. media × **rupicola.** Repeated attempts to synthesize a hybrid between these species, using a number of stocks of each parent, failed due to seed incompatibility. Eventually in the combination *S. media* ♀ from Glasson × *S. rupicola* ♂ from Gt. Orme two capsules were produced which each contained, in addition to numerous aborted seeds, two seeds with almost full-sized embryos and fairly well-filled perisperm. Three of these seeds germinated and produced very robust perennial plants intermediate in morphology between the parental species. The intermediacy, however, was not shown in the indumentum since the plants were densely glandular throughout as in *S. rupicola* whereas the Glasson stock of *S. media* was completely glabrous. The stamens were of the small hybrid type so common in interspecific hybrids of *Spergularia* and the plants were completely sterile and consequently produced characteristic long straggling fruitless cymes (for discussion of these characters see Ratter 1965a). All three plants were tetraploid (2n = 36) and not triploid (2n = 27) as would be expected from the chromosome numbers of the parents (*S. media* 2n = 18, *S. rupicola* 2n = 36).

Table I gives the results of the meiotic analyses and Plate 8a shows a typical MI configuration.

S. media × **purpurea** (4x). Three plants of the F_1 hybrid have been grown. The cotyledons of all three seedlings were malformed and twisted and the growth of the seedlings was arrested for about 12 days between the opening of the cotyledons and the opening of the first pair of foliage leaves; a long delay in development at this stage has never been observed in other seedlings growing under similar conditions. Following the opening of the first pair of foliage leaves growth was rapid and vigorous hybrid plants were produced.

The hybrids were intermediate in morphology between the parents but certainly tended more towards *S. purpurea*, the male parent, than to *S. media*. They were of annual habit with slender tap-root as in *S. purpurea*, whereas *S. media* is perennial with thick, woody rootstock. Floral characters also were closer to *S. purpurea* than to *S. media*. The androecia of many flowers were of small hybrid type but in others more or less normally developed stamens were present. The pollen contained approximately 5% of well-formed grains with dense contents which might possibly have been viable. Production of seeds was less than 0·2% of that of the female parent, and only about half of the seeds were viable; they were similar in size and tuberculation to those of *S. purpurea* but also had vestiges of the wings which characterize *S. media* seeds. The hybrid was triploid (2n = 27) and details of the meiotic analyses are given in Table I and illustrated in Plate 8b.

An F_2 generation of twenty-one plants was raised. Two of the plants showed severe abnormalities in the development of leaves and failed to flower, another showed minor abnormalities but flowered sparsely; the remainder flowered more or less freely and details of chromosome number

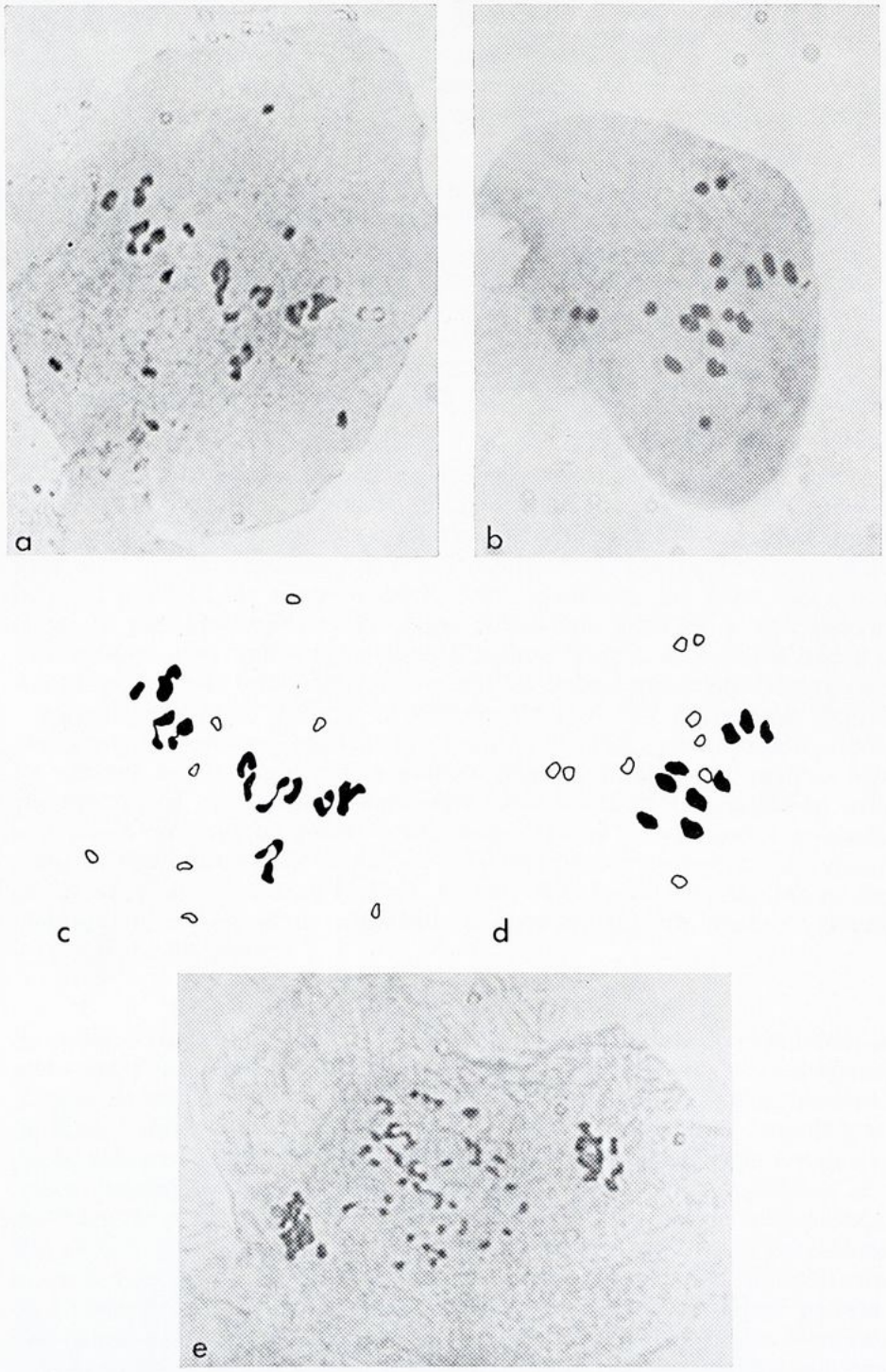

PLATE 8. Pollen mother cells × 1,500: a, *S. media* × *rupicola* M1, 6_{iii} 5_{ii} 8_{i}; b, *S. media* × *purpurea* (4x) F_1, M1, 9_{ii} 9_{i}; c and d, explanatory diagrams of a and b; e, *S. media* × *macrotheca*, T1, showing laggards.

and meiotic analyses, where they are available, are given in Table 2. A fair amount of morphological variation occurred in the F_2 and most of the plants resembled *S. purpurea* more closely than did the F_1 parent. The percentage of pollen grains which appeared to be fertile seemed remarkably high in most of the F_2 hybrids, but no plant showed higher seed fertility than occurred in the F_1.

S. media × capillacea. Although meiotic observations were not obtained this hybrid has been included here because it grew to maturity and flowered freely. It was more or less intermediate between its parents, but with a more compact growth form and brighter green foliage than in either parent. The stamens did not develop beyond small club-shaped primordia in which meiosis did not take place. This early abortion of the androecium was presumably a more drastic manifestation of the same disharmony which produces exceptionally small stamens in so many interspecific *Spergularia* hybrids.

S. media × macrotheca. As previously mentioned the stock of *S. macrotheca* used in this hybridization was from Guadalupe and had $2n = 72$. Only a single plant of this hybrid has been obtained although numerous attempted hybridizations have been carried out. The flowers were perfectly intermediate between those of the parental species but vegetatively the plant was much closer to the subshrubby habit of the Guadalupe stock of *S. macrotheca*. The stamens were well developed with anthers lying level with the stigmas, but the pollen was totally aborted so that the hybrid was completely sterile.

Meiosis was more difficult to observe than in any *Spergularia* hybrid previously encountered, partially as a result of the high chromosome number. In addition to the eight pollen mother cells where accurate analysis was possible, many others which were unfit for detailed analysis could be seen to contain numbers of quadri- and tri-valents and in one case a 5-valent; some of these latter cells contained only one or two univalents and many of the anaphases observed had no, or very few, laggards, although in the majority there were many univalents and many laggards. Plate 8c shows a 1st meiotic telophase.

Discussion

S. media × rupicola. The tetraploidy of the hybrids of this combination is presumably the result of fusion of normal 2x pollen grains of the tetraploid *S. rupicola* with unreduced egg cells of the diploid *S. media*. The complement of these hybrids should therefore consist of two completely homologous *media* genomes, which will pair normally, and two genomes from *S. rupicola* which, judging from the evidence afforded by *S. rupicola* × *capillacea* discussed in the previous paper, should be capable of considerable autosyndesis. We should expect therefore a high level of bivalent pairing to take place in the hybrid but the actual observations reveal a complication: the existence of a considerable degree of homology between all four genomes present. The actual extent of this homology as shown by the occurrence of multivalents in many of the cells is surprisingly high—considering that parental chiasma frequencies are unlikely to exceed 1·5 per bivalent a configuration of $3_{iv}\ 4_{iii}\ 4_{ii}\ 4_{i}$ probably represents somewhere near the maximum association which could be expected even of four completely homologous genomes. The majority of pollen mother cells, however, show much lower association

TABLE I

F_1 Interspecific Hybrids

Hybrid combination and source of parents	*2n*	*No. of plants*	*Pairing at M1*				*Notes*
			Configuration			*No. of PMC*	
			IV	III	II	I	
S. media × *rupicola* Glasson, Gt. Orme, N Lancs., Wales. England.	36	3	1	6	1	7+1v	1	Vigorous robust perennial with thick woody root-stock, intermediate in morphology between parents. Stamens very small. Pollen fertility nil. Seed fertility nil.
			3	4	4	4	1	
			2	5	4	5	1	
			2	4	4	8	1	
			2	3	5	9	1	
			1	6	4	6	1	
			1	6	2	10	1	
			1	5	4	9	1	
			1	5	3	11	1	
			1	4	8	4	1	
			1	4	5	10	1	
			1	3	11	1	1	
			1	2	10	6	1	
				6	5	8	1	
				6	4	10	1	
				5	8	5	2	
				5	6	9	2	
				5	5	11	1	
				5	4	13	2	
				4	8	8	3	
				4	7	10	2	
				4	6	12	2	
				3	11	5	1	
				3	9	9	5	
				3	8	11	1	
				3	7	13	1	
				2	11	8	1	
				2	8	14	2	
				1	9	15	1	
				1	8	17	1	
					14	8	2	
					13	10	3	
					12	12	6	
					11	14	5	
					10	16	1	
					9	18	3	
			Apparent aneuploid cells					
			2	5	3	9	1	
				6	5	7	1	
				5	7	6	1	
				3	6	13	1	
				2	10	9	1	
					12	14	1	
						Total	68	

TABLE I (*Contd.*)

Hybrid combination and source of parents	2*n*	*No. of plants*	*Pairing at M1*				*Notes*
			Configuration IV	III	II	*No. of PMC* I	
S. media × *purpurea* (4x) Parkgate, Cheshire, England. Cordoba, Spain.	27	3	1		7	9 1	Annual with slender taproot (as in S. purpurea). Morphology tending more towards *S. purpurea* than *S. media*. The androecium of many flowers is of the small hybrid type but in others ± normally developed stamens present. Pollen fertility c. 5%. Seed fertility less than 0·2%
				1	9	6 1	
				1	8	8 7	
					11	5 3	
					10	7 11	
					9	9 40	
					8	11 7	
					7	13 1	
			Apparent aneuploid cells				
					11	6 1	
					9	10 1	
					9	8 1	
					Total	74	
S. media × *macrotheca* South of Tunis, Tunisia. (ssp. *tunetana*) Guadalupe Island, Mexico (Pacific).	45	1	1	4	14	1 1	In vegetative form close to the subshrubby habit of the male parent. Otherwise more or less intermediate. Pollen fertility nil. Seed fertility nil.
				4	11	11 1	
				3	14	8 1	
				1	12	18 1	
					20	5 1	
					16	13 1	
					11	23 1	
					10	25 1	
					Total	8	
			Many figures which were not good enough for accurate analysis showed many trivalents and in one case a 5-valent. Figures were also present which had only 1 or 2 univalents.				
S. media × *capillacea* Parkgate, Cheshire, England. Coimbra, Portugal.	18	12					More or less intermediate in morphology between parents but with a rather compact growth form and bright green foliage. Stamens failing to develop past a primordial stage and hence no meiosis in them. The hybrid is completely sterile.

TABLE 2

S. media × *purpurea* F_2

Plant No.	*2n*	*Most Common Meiotic Associations*	*Apparent Pollen Fertility*
601	44	19_{ii} 6_{i}	c. 90%
602/L	43	20_{ii} 3_{i} 1_{iii} 18_{ii} 4_{i} 2_{iii} 17_{ii} 3_{i} 19_{ii} 5_{i}	c. 30%
603/1	32 or 33	3_{iii} 9_{ii} 5_{i} 13_{ii} 7_{i}	c. 90%
604/1	±44	1_{iii} 17_{ii} 5_{i} 19_{ii} 6_{i} 20_{ii} 4_{i}	c. 90%
604/2	42	20_{ii} 2_{i}	c. 50%
605/1	±45	1_{iii} 19_{ii} 7_{i} 20_{ii} 5_{i} 21_{ii} 3_{i} 19_{ii} 7_{i}	c. 40%
606/1	42		c. 80%
607/1	47 or 48	23_{ii} 1_{i}	c. 70%
608/1	47	19_{ii} 9_{i} 18_{ii} 11_{i}	less than 1%
608/2	43 or 44		c. 60%
609/2	41	17_{ii} 7_{i} 16_{ii} 9_{i} 15_{ii} 11_{i}	c. 70%
610/L	33	$1_{,ii}$ 14_{ii} 2_{i} 2_{iii} 12_{ii} 3_{i} 2_{iii} 11_{ii} 5_{i} 1_{iii} 14_{ii} 2_{i}	c. 70%
611/1	48	1_{iii} 19_{ii} 7_{i} 21_{ii} 6_{i} 20_{ii} 8_{i}	c. 30%
611/2	46		c. 15%

than this, perhaps because of some or all of the following factors: (a), competition in pairing; (b), only partial homology of the *media* genomes with those of *rupicola*; (c), only partial homology of the *rupicola* genomes with each other.

The existence of considerable homology between the *media* genome and both genomes of *S. rupicola* is interesting, since in the previous paper (Notes R.B.G. Edinb. 29:217) high homology was also shown to occur between the *rupicola* genomes and that of *S. capillacea*. We therefore must conclude that the very dissimilar diploid species *S. media* and *S. capillacea* have genomes which possess a fair degree of homology. Unfortunately no direct confirmation of this is possible from the hybrid *S. media* × *capillacea* since its androecium fails to develop to the stage where meiosis occurs.

S. media × **purpurea** (4x). The closer resemblance of this hybrid to *S. purpurea* than to *S. media* can possibly be attributed to the presence of two *purpurea* genomes and only one from *media*. In other *Spergularia* hybrids between perennial and annual species the perennial condition with thick rootstock (as in *media*) has normally been dominant to annual condition with slender taproot (as in *purpurea*), but here the situation is reversed.

The majority of pollen mother cells show configurations of $9_{ii}\ 9_{i}$. From the evidence discussed in the previous paper and in Ratter 1965a it was concluded that *S. purpurea* (4x) is an autopolyploid or segmental allopolyploid, and the most likely explanation of the $9_{ii}\ 9_{i}$ configuration therefore is that the two *purpurea* genomes are exhibiting autosyndesis and the *media* chromosomes remain unpaired. Presumably the occurrence of trivalents reveals some homology between the *media* and *purpurea* genomes; there is, however, never more than one trivalent per cell, possibly indicating that this homology is rather low, or that its expression is obscured by low chiasma frequency or preferential pairing of *purpurea* genomes.

Most of the F_2 hybrids for which chromosomal information is available approximate to the pentaploid level ($2n = 45$). They have probably originated by the fusion of an unreduced triploid and a near diploid gamete. The spread in number around the euploid $2n = 45$ is ± 3, apart from one $2n = 41$ plant which carries the deviation to -4. The two plants which do not fall into the foregoing category are intermediate between triploid and tetraploid levels, one with $2n = 32$ or 33 and the other with $2n = 33$; these plants are not morphologically separable from the rest of the F_2 generation. The lack of any increase in fertility of this generation over the F_1 is not surprising considering the prevalence of aneuploid numbers usually around an uneven (5x) ploidy level.

S. media × **macrotheca.** In this hybrid the *S. media* chromosomes find partners amongst those of *S. macrotheca* in at least some pollen mother cells, for example in the cell showing $1_{iv}\ 4_{iii}\ 14_{ii}\ 1_{i}$ and in those where although the figures are not fit for detailed analysis there are clearly only one or two univalents present. The occurrence of quadrivalents and trivalents also indicates that the *S. macrotheca* chromosomes have some capacity for autosyndesis, if the possibility that these associations are the result of segmental interchange is discounted. This capacity for autosyndesis reveals that the octoploidy of the Guadalupe stock of *S. macrotheca* is at least in part autopolyploid or segmental allopolyploid.

Summary

Four synthetic interspecific F_1 hybrids are reported in this paper: *S. media* × *rupicola*, *S. media* × *purpurea* (4x), *S. media* × *capillacea* and *S. media* × *macrotheca*. An F_2 generation of *S. media* × *purpurea* (4x) has also been raised and studied. Details of meiosis and fertility of the hybrids are given in Tables 1 and 2. In addition chromosome numbers for *S. macrotheca* are reported for the first time: the Guadalupe Island race of this species is octoploid ($2n = 72$), whilst two Californian stocks are both tetraploid ($2n = 36$).

All three F_1 plants of *S. media* × *rupicola* were tetraploid, not triploid as would be expected from the chromosome numbers of the parents. They have presumably arisen by fusion of normal pollen of the tetraploid species *S. rupicola* with unreduced egg-cells of the diploid *S. media*. The meiotic configurations of this hybrid revealed considerable homology amongst all four genomes present.

S. media × *purpurea* (4x) exhibited meiotic configurations which can be explained as the result of autosyndesis of the two genomes contributed by *S. purpurea* (4x), with the *media* genomes remaining unpaired. It is the only one of the F_1 hybrids reported in this communication which showed any fertility; most of the F_2 generation approximated to the pentaploid level ($2n = 45$) and none showed higher fertility than the F_1.

Twelve plants of *S. media* × *capillacea* were raised but no study of meiosis in pollen mother cells was possible since the stamens in all flowers failed to develop beyond tiny club-shaped primordia.

Meiosis in the pentaploid hybrid *S. media* × *macrotheca* is characterized by complicated configurations which are rather difficult to analyse. It is interesting, however, that in some of the pollen mother cells almost all the chromosomes managed to find partners.

Acknowledgments

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References

Ratter, J. A. (1965a). Cytogenetic studies in Spergularia: II, An attempt to discover cytogenetic relationships of some species. *Notes R.B.G. Edinb.* 26:203–223.

Rossbach, R. P. (1940). Spergularia in North and South America (1st part). *Rhodora* 42:57–83.