

CYTOGENETIC STUDIES IN SPERGULARIA: VII

Cryptic speciation in *S. media* (L.) C. Presl and *S. marina* (L.) Griseb.

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ABSTRACT. Results of intraspecific crosses between geographically disjunct stocks of *S. media* and *S. marina* are reported, and data on their fertility are summarized. In some interstock hybrids in both species the type of androecial abnormalities characteristic of many interspecific hybrids of *Spergularia* occurred and fertility was very low. Meiosis in these interstock hybrids was regular but pollen fertility was usually much reduced. From the data obtained it is concluded that *S. media* ssp. *tunetana* is as strongly isolated from the other *S. media* stocks as many recognised *Spergularia* species are from each other. The other *S. media* stocks all showed reduction of fertility in hybrids and this may indicate incipient speciation. *S. marina* hybrids involving the Falkland Islands stock showed greatly reduced fertility.

During the course of a cytotaxonomic study in *Spergularia* a number of diverse stocks of *S. media* (L.) C. Presl and *S. marina* (L.) Griseb. were accumulated for use in hybridizations. Morphologically they showed considerable variation but nevertheless clearly fell within the limits of the two species. In addition to the programme of interspecific hybridizations for which the stocks had been assembled, intraspecific crosses between stocks were also carried out and it is the results of the latter which form the subject of this paper.

Plants were grown in a cool glasshouse and cross-pollinations were made using the technique described in Ratter, 1965a. Meiosis was studied in propionocarmine squashes of anthers from flower-buds which had been fixed in 3:1 ethanol:acetic acid and stored in a 'deep-freeze' at -12°C . Pollen fertility was estimated by observing the percentage of grains which were well-formed and stained deeply in acetocarmine.

The results of crossing within the two species are considered separately below and then are discussed together.

S. media (L.) C. Presl (*S. marginata* (DC.) Kittel)

S. media is a robust, principally outbreeding, perennial which occurs in maritime habitats throughout the world, and also in inland saline areas. The species is diploid ($2n=18$) apart from a few isolated tetraploid pockets (Monnier, 1962). For a description the reader is referred to Monnier and Ratter's account in the *Flora Europaea* vol I, whilst Monnier (1953, 62) deals with intraspecific diversification in North Africa.

The stocks used in the crosses were grown from seed which originated from the following sources, but had in most cases passed through a number of generations in cultivation: (1) British plants from Clevedon (Somerset, v.c. 6), Anglesey (N Wales, v.c. 52) and Glasson (N Lancs. v.c. 60); (2) SW European plants from Montpellier, France (Monnier 145), and Aragon, Spain; (3) a Turkish collection from Istanbul (Demiriz 1184); and (4) a collection made at Tunis (Monnier 1270).

TABLE 1. Fertility of *S. media* intraspecific hybrids and parental stocks; n.d.=not determined.

Parentage	No. of plants	Pollen fertility %	% of flowers developing into capsules	Seeds per capsule	
				Average	Extreme
Glasson × Istanbul	8	0-23	64	28.9	3-80
Clevedon × Montpellier	5	0-14	34	34.0	3-67
Istanbul × Aragon	5	0-60	8	50.1	14-98
Anglesey × Tunis	3	0-4	0-1	6.25	2-12
Montpellier × Tunis	8	0-8	0-3 (-50)	5.7	1-24
Glasson	10	30	82	54.6	5-100
Anglesey	10	37	100	81.9	58-105
Clevedon	10	n.d.	100	87.5	70-104
Montpellier	10	70	100	163.8	145-181
Aragon	10	n.d.	100	183.6	116-222
Istanbul	10	90	99	61.5	35-97
Tunis	10	43	30	42.5	2-85

TABLE 2. Fertility of *S. marina* intraspecific hybrids and parental stocks; n.d.=not determined.

Parentage	No. of plants	Pollen fertility %	% of flowers developing into capsules	Seeds per capsule	
				Average	Extreme
S Atlantic × Sahara	8	70-80	100	67.5	32-91
N Mediterranean × S Atlantic	6	16-25	100	41.5	17-75
Falkland Islands × S Atlantic	4	1.2-5.7	32	1.8	1-4
Falkland Islands × Istanbul	6	0-5	10	2.0	1-6
S Atlantic	10	0-57	c 100	92	68-112
N Mediterranean	10	n.d.	c 100	64	44-87
Sahara	10	12-30	c 100	78.5	29-110
Istanbul	10	60	c 100	n.d.	n.d.
Falkland Islands	10	35-52	c 100	35	6-78

The Clevedon and Glasson stocks belonged to the form of the N European race with unwinged seeds, sometimes recognized as var. *aptera* or var. *angustata*, whilst the Anglesey stock belonged to the same race but had broadly winged seeds. The Montpellier, Aragon and Istanbul stocks were all of the Mediterranean race, whilst the Tunis stock belonged to the form which Dr P. Monnier has named ssp. *tunetana* (Monnier, 1962). All stocks were diploid.

The inter-stock cross pollinations yielded 100% of capsules well-filled with normal seed which germinated to produce vigorous F_1 plants intermediate in morphology between their parents. Table 1 gives data on fertility of both the intraspecific F_1 hybrids and the parental stocks.

The abnormalities of the androecium already described in interspecific *Spergularia* hybrids (Ratter, 1965 a, b) occurred to a varying degree in the intraspecific hybrids. Thus in the Glasson \times Istanbul, Clevedon \times Montpellier and Istanbul \times Aragon hybrids some of the flowers had all the stamens very small, forming a cluster around the base of the ovary, whilst in others from one to nine of the ten stamens were of this type with the remainder normally developed. Flowers with these abnormally small stamens are incapable of setting seed by selfing, since the dehiscent anthers are not long enough to touch the stigmas of the same flower, and therefore they remain sterile under glasshouse conditions where insect visitors are virtually absent. In the hybrids involving the Tunis stock (ssp. *tunetana*) androecial abnormality was particularly pronounced. In the Montpellier \times Tunis hybrid two plants had all the flowers with well developed stamens of normal size (reaching somewhat above the top of the ovary and level with the stigmas) whilst another two had stamens only of the minute 'hybrid' type; the other four plants lay between these extremes with stamens either reaching about halfway up the ovary or having a mixture of both normal and minute stamens. The three plants of the Anglesey \times Tunis hybrid showed a similar gradation in androecial development: one with nearly all stamens reaching part way up the styles, another with them usually reaching the top of the ovary and the third with all stamens of the minute 'hybrid' type.

Meiosis in all hybrids was perfectly regular with the occurrence of nine bivalents and chiasma frequencies similar to their parents. Pollen fertility, however, was usually much reduced and was notably variable from flower to flower: in all hybrids a considerable number of flowers produced only abortive pollen, whilst in only one case was pollen fertility above 50% observed. There seemed to be no correlation between normality of staminal development and pollen fertility. As shown in Table 1, the pollen fertility of three of the parental stocks grown under the same conditions as the hybrids, and in the same year, was less than 50%. Examination of specimens of the same stocks collected from the field, however, showed fertility of over 90%. The reduced fertility of the parental plants in culture is perhaps the result of inbreeding depression, since a number of generations of this predominantly outbreeding species were grown from greenhouse-harvested seed, no doubt of selfed origin, and at least one of the stocks (that from Glasson) showed some morphological abnormalities.

The number of flowers developing into capsules and the number of seeds per capsule was much lower in the hybrids than in the parents (Table 1). In the case of the two hybrids involving ssp. *tunetana* the number of flowers

developing into capsules was particularly low and rather variable from plant to plant, ranging from 3% to nil, apart from one aberrant plant which showed 50%. Failure of fruit development can be attributed to two causes: low pollen fertility and the failure of selfing due to the presence of small 'hybrid-type' stamens (see above). It is noteworthy that *ssp. tunetana*, under the conditions of culture, had only a 30% development of capsules and this seemed to be due to a partial failure of selfing; at anthesis this stock has its stigmas standing upright well-removed from the dehiscing anthers and it is only later that they spread into the receptive position, and even then frequently fail to come into contact with the anthers. The other stocks, in contrast, are nearly homogamous, with stigmas already spreading at anthesis and only requiring a minor curving to bring them into contact with the anthers. The hybrids of low fertility (Anglesey \times Tunis, Montpellier \times Tunis, and Istanbul \times Aragon) showed the long, straggling inflorescences, so characteristic in interspecific hybrids of *Spergularia*, resulting from the continual production of flowers in the absence of the normal check due to fruiting.

***S. marina* (L.) Griseb.** (*S. salina* J. & C. Presl)

S. marina is a slender, predominantly inbreeding, annual to short-lived perennial of tetraploid chromosome number ($2n=36$) which, like *S. media*, is widespread in maritime habitats and inland saline areas.

The stocks used in the crosses included three grown from seed collected by Dr P. Monnier, University of Montpellier; these belonged to the south Atlantic and the north Mediterranean races (both French collections) and to the north African Sahara race. The other two stocks came from Istanbul and from the Falkland Islands, the latter collected by Dr D. Moore of the University of Reading. All these stocks clearly fell within the circumscription of *S. marina* (L.) Griseb. although there was a fair degree of variation amongst them. The Falklands stock, for instance, closely resembled the typical light-green, glabrous populations so common in the British Isles, whilst the Mediterranean and north African plants were darker green and much hairier.

As in *S. media*, the inter-stock cross pollinations yielded 100% of capsules well-filled with normal seed which germinated to produce vigorous F_1 plants morphologically intermediate between the parents. Table 2 gives data on fertility of the F_1 and the parental stocks.

The S Atlantic \times Sahara and N Mediterranean \times S Atlantic hybrids showed normal androecial development similar to their parents, but the hybrids involving the Falkland Islands stock exhibited the reduction of staminal size so often associated with hybridity in *Spergularia*. The stamens of the latter seldom exceeded the top of the ovary and were often much shorter, so that the efficiency of the self-pollinated mechanism was much reduced, and this, combined with low pollen fertility in most flowers, resulted in a much reduced seed output with consequent production of long straggling inflorescences. In contrast the S Atlantic \times Sahara and N Mediterranean \times S Atlantic hybrids had levels of seed output more or less similar to their parents.

Meiosis in all hybrids was perfectly regular, showing 18 bivalents at M1 and chiasma frequencies of 1.4-1.5 per bivalent, but despite this much

abortion of pollen occurred. There seemed, however, to be little significant difference between the pollen fertility of the N Mediterranean \times S Atlantic and S Atlantic \times Sahara hybrids and their parents, but pollen fertility of the hybrids involving the Falkland Islands stock was obviously much reduced. The low pollen fertility of the parental stocks (see Table 2) is surprising and since the species is normally autogamous inbreeding depression cannot be invoked as a cause. Low pollen fertility of this degree will have no effect on seed output, since 1,000–1,500 pollen grains are produced per anther, and with an average of three to four anthers per flower there would be plenty of viable grains to bring about self-fertilization of the hundred or so ovules in the ovary, even with a fertility of only a few per cent.

In the capsules of the hybrids involving the Falkland Islands stock, where there were usually only one, two or three seeds, these were of remarkably large size (av. diam. 0.9 mm, excluding wing) and were almost always winged. This furnishes some supporting evidence for the claim of Salisbury (1958) that winged seeds are produced as a result of higher availability of nutrients, since in capsules with so few developing seeds there would presumably be much less competition for available nutrients.

DISCUSSION

Some interesting conclusions can be drawn from the hybridization of the very disjunct stocks of *S. media* and *S. marina* just described.

In *S. media* most of the interstock hybrids showed a much reduced fertility and, in their abnormal androecia, evidence of the type of unbalance which characterizes interspecific hybrids in the genus. In the case of the hybrids involving ssp. *tunetana*, fertility was considerably lower than in some hybrids between morphologically dissimilar species (e.g. *S. bocconii* \times *nicaeensis*, *S. nicaeensis* \times *purpurea* (4x) and *S. marina* \times *purpurea* (4x), Ratter 1965 a, b): clearly ssp. *tunetana* is as strongly isolated from the other *S. media* stocks as these species are from each other. The hybrids of the other stocks showed levels of fertility which indicate the occurrence of some degree of reproductive isolation (see fig. 1) and their parents perhaps might be considered as incipient species.

There is no evidence of any reduction in fertility in hybrids of the S Atlantic (French), N Mediterranean (French) and Sahara stocks of *S. marina*. Hybrids of the Falkland Islands stock with the S Atlantic and Istanbul stocks, on the other hand, showed much reduced fertility and evidence of unbalance in the androecial characters. Here we appear to be dealing with a case of cryptic speciation in that the Falkland Islands stock is at least partially isolated from the other two stocks by high sterility of the F_1 .

Observations of meiosis in both the *S. media* and *S. marina* hybrids indicate that chromosomal sterility, at least at the microscopically visible level, is probably not one of the factors operative in reducing fertility and hence bringing about speciation. This finding is in accordance with results obtained from interspecific hybrids of *Spergularia* where genomic homologies seem to be remarkably wide (Ratter 1965 a, b, 1969 a, b). Such maintenance of homology is interesting in a group where one of the major evolutionary trends seems to have been towards inbreeding and where polyploidy is frequent: under such circumstances it might have been expected that

chromosomal repatterning and resultant erosion of homology would have played an important part in speciation (see Grant, 1958 p 355). On the other hand, irregularities in the development of the androecium, resulting in the production of small 'hybrid-type' stamens, prevent the operation of the normal pollination mechanism and must represent an important barrier to gene flow.

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