

CYTOGENETIC STUDIES IN SPERGULARIA VI:

The Evolution of True Breeding, Fertile Tetraploids from a Triploid Interspecific Hybrid

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ABSTRACT. The paper reports the derivation of fertile, true-breeding, tetraploid ($2n = 36$) lines from the highly sterile triploid F_1 hybrid of *S. nicaeensis* and *S. purpurea* ($2x$). They were grown until the F_4 generation and belong to two branches, each descended from a near tetraploid F_2 plant. The lines were morphologically very distinct from the two parental species and can be considered as synthetic species. Their evolution was not, however, accompanied by the formation of internal isolating barriers since fertile hybrids were produced both in interline crosses and with *S. nicaeensis*.

The F_1 hybrid of *Spergularia nicaeensis* and *S. purpurea* ($2x$) was reported briefly in a previous publication (Ratter, 1965) which dealt with a number of synthetic interspecific hybrids of *Spergularia*. The purpose of the present paper is to examine the later generations of the hybrid in some detail.

The parental species are both of Mediterranean distribution and probably sometimes come into contact in the field; descriptions are given in Flora Europaea I, but it will be appropriate to give some notes here.

S. nicaeensis Sarato ex Burnat is a tetraploid ($2n = 36$), biennial or short-lived perennial species with a stout woody rootstock, robust decumbent aerial shoots, and short, broadly triangular stipules. The petals and sepals are of more or less equal length (3-4 mm), and the former are pale pink with a whitish base. Pollen fertility is usually over 90%. In nature it is probable that both out- and inbreeding normally occur. The species is nitrophilous and found on saline soils and waste places in the Western Mediterranean region. The stock used in this study originated from seed collected by Dr P. Monnier, University of Montpellier, in Hérault, France.

S. purpurea (Pers.) G. Don fil. (*S. longipes* Rouy) is an annual or biennial species of much more slender form than *S. nicaeensis*. It has a slender tap-root and delicate ascending shoots with silvery, lanceolate acuminate stipules. The petals are uniform rose-purple, 3-4.5 mm long and much exceed the sepals. Pollen fertility is usually over 90%, and it seems probable that the species is predominantly outbreeding (see Ratter, 1969). Under glasshouse conditions, where insect visitors are absent, many of the flowers fail to set seed, due to failure of the stigmas to touch the anthers of the same flower, but if flowers are artificially pollinated seed is always set. The species occurs in sandy waste places in Spain, Portugal and North Africa. There are two chromosome races, a diploid ($2n = 18$) and a tetraploid ($2n = 36$), which are not morphologically distinguishable; the stock used as a parent of the hybrid was diploid and originated from seed collected at Marrakech, Morocco by Dr P. Monnier.

MATERIALS AND METHODS

Plants were grown in a cool glasshouse and cross-pollinations were made using the technique described in Ratter, 1965. The F_1 was produced using *S. nicaeensis* as female parent. It had also been intended to make the reciprocal

cross but loss of *S. purpurea* (2x) from cultivation prevented both this and its use in any backcrosses.

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.

Terminology. In the following account the term line is used to indicate a direct lineal relationship, e.g. an F_4 and the plants of earlier generations from which it is descended, whilst all lines descended from a common F_2 individual are referred to as a branch. Sister plants derived from a common parental individual are referred to as a progeny group.

RESULTS

F_1 generation

Twenty-two plants of the F_1 hybrid were grown. In general, they were intermediate in morphology between the parental species, but in a number of characters were much closer to one parent than the other. The petals, for instance, were rose-purple in colour as in *S. purpurea* and, also as in that species, considerably exceeded the calyx; on the other hand, the plants were perennial with a stout woody rootstock, as in *S. nicaeensis*. As in so many interspecific hybrids of *Spergularia*, the stamens were extremely small, lying in a ring around the base of the ovary and usually far below the level of the stigmas.

At first meiotic metaphase the commonest configurations were $9_{11}9_1$, $1_{111}8_{11}8_1$, $2_{111}7_{11}7_1$, and $3_{111}6_{11}6_1$, but configurations with four and five trivalents were also present. The occurrence of a considerable number of trivalents (nearly 50% of the PMC had two or more) indicates fairly strong homology between all three genomes present, particularly since chiasma frequencies in the parental species are only about 1.4-1.5 per bivalent. The later stages of meiosis were characterized by meiotic irregularities such as very unequal disjunction and lagging of chromosomes at first anaphase, and the pollen was less than 1% fertile.

About 2.5% of the flowers produced small capsules which contained one or two seeds, most of which had aborted embryos. This would represent a seed output approximately 0.0003-0.0007% of that normal for the parental species, assuming that the hybrid and its parents produced the same number of flowers. This assumption, however, is false, since the hybrid, lacking the check to flowering of heavy fruit production, was far more floriferous than its parents. For this reason an accurate estimate of fertility cannot be made but seed output was certainly much less than 0.1% of that normal for the parents and furthermore less than half of the seeds were viable. The low level of fertility can be attributed to two causes: the very low percentage of good pollen, consequent on meiotic irregularities, and the abnormally small stamens which impede the normal selfing mechanism, since pollen cannot easily be deposited on the stigmas of the same flower.

As in other *Spergularia* hybrids of high sterility, continuous flowering resulted in the production of long inflorescences which gave the plant a very straggling appearance.

F₂ generation

Four capsules containing a total of nine seeds were produced by the controlled self-pollination of one of the F₁ plants. Three of these seeds germinated to produce vigorous F₂ plants which are described below.

Plant 1 was a large, robust perennial which resembled *S. nicaeensis* in vegetative characters, apart from having long lanceolate stipules. The flowers were much larger than those of the F₁ or either of the parental species, having long (5.5 mm), narrow petals of *S. purpurea* colour which much exceeded the 3.5 mm sepals. The stamens were of about the same size as *S. nicaeensis* and during the first two years of the plant's life showed no trace of any of the staminal malformations common in *Spergularia* hybrids; in the third year, however, when the plant was in need of re-potting and was lacking in vigour, many of the flowers had androecia of the small hybrid type.

The chromosome number was $2n = 35$ and the chromosomes were usually associated at first meiotic metaphase as $17_{11}1_1$. A number of first meiotic anaphases were observed with seventeen chromosomes going to one pole and eighteen to the other. Pollen fertility varied somewhat from flower to flower and values from 18-40% were recorded, with an overall average of 28%. Approximately 95% of flowers developed into capsules which contained an average of 50 seeds; comparable figures for *S. nicaeensis* were a 100% development of capsules containing an average of 70 seeds. Assuming that this plant and *S. nicaeensis* produced an equal number of flowers, its seed output as a percentage of that of *S. nicaeensis* would be just over 60%. 10% of the seeds were seen to have abortive embryos when examined under the stereomicroscope.

Plant 2 was vegetatively very similar to *S. nicaeensis* and *plant 1*, but differed from the latter in having broadly triangular stipules. The flowers were of similar size to those of *S. nicaeensis*, with petals of *purpurea* colour and rounded outline which very slightly exceeded the sepals. The stamens were well developed in most flowers and of similar size to those of the parental species, but in a few flowers they were somewhat smaller and showed traces of hybrid unbalance.

The chromosome number was $2n = 34$ and the chromosomes were usually associated at first meiotic metaphase as 17_{11} or $16_{11}2_1$. Pollen fertility in the flowers examined varied from 13-16% and averaged 15%. Approximately 75% of the flowers developed into capsules which contained an average of 14.5 seeds and rather less than 10% of these had abortive embryos. Assuming that this plant and *S. nicaeensis* produced equal numbers of flowers, its seed output expressed as a percentage of that of *S. nicaeensis* would be 15%.

Plant 3 was also vegetatively very similar to *S. nicaeensis*. The flowers, however, had petals of *S. purpurea* colour much exceeding the sepals. The stamens were of small hybrid type grouped around the base of the ovary. Pollen fertility was nil and there was no seed set. Unfortunately the chromosome number was not determined.

The year after the three F₂ hybrids described above were grown, a collection of seeds was made from the F₁ hybrids and was sown in the following year to see if other F₂ plants would show a recovery of fertility similar to plants 1 and 2. Seven plants grew from these seeds and were designated *a-g*; details of their androecial development, cytology and fertility are given in Table 1, which also includes data for plants 1, 2 and 3. This group was rather variable,

morphologically: *b*, *e* and *f* were very similar to the F_1 hybrid; *c* was almost identical to *S. purpurea* and would undoubtedly have been identified as this species had its origin been unknown; *g* was also close to *S. purpurea*, whilst *a* resembled that species more closely than it did *S. nicaeensis*; *d* was a very large robust plant morphologically rather similar to plant 1, particularly in floral characters.

As shown by the figures in Table 1 only one of this second batch of F_2 plants (*f*) exceeded the fertility of the F_1 . This was a hexaploid derived by chromosome doubling of the triploid parent, and therefore in a very different category from the part-fertile near-tetraploid plants 1 and 2.

TABLE 1
Chromosome numbers and fertility of *S. nicaeensis* x *purpurea* (2x) F_2

Plant	Androecial development	2n	Assoc. at M1	Pollen fertility %	% Flowers developing into capsules	Average seeds per capsule
1	Normal	35	17 ₁₁ 1 ₁	28	95	50
2	Normal	34	17 ₁₁ , 16 ₁₁ 2 ₁	15	75	14.5
3	Hybrid type	—	—	0	0	0
<i>a</i>	Normal	33	16 ₁₁ 1 ₁	14	0	0
<i>b</i>	Hybrid type	32	16 ₁₁	>1	c.2	2
<i>c</i>	Normal	24	5 ₁₁ 14 ₁	0	0	0
<i>d</i>	Normal	44	21 ₁₁ 2 ₁	5	40	3
<i>e</i>	Hybrid type	32	15 ₁₁ 2 ₁	0	0	0
<i>f</i>	Normal	54	—	20	100	31
<i>g</i>	Normal	30	15 ₁₁ , 3 ₁₁₁ 10 ₁₁ 1 ₁ , 4 ₁₁₁ 7 ₁₁ 4 ₁	>1	0	0

F_3 generation

Small F_3 generations were grown from selfed seed of the F_2 plants 1 and 2. Examination of the F_3 progeny showed that both of the F_2 plants bred fairly true: a remarkable and totally unexpected result. Chromosome data and fertility details for F_3 plants grown in the glasshouse are given in Table 2.

The F_3 generation derived from F_2 1 consisted of four plants grown in the glasshouse (designated *a-d*) and ten grown in an outdoor plot. Three of the indoor and eight of the outdoor plants were more or less identical to the F_2 parent, apart from slight variation in tint of petals, etc. The fourth indoor plant (1*d*) was very similar to the others but had shorter petals, 4.5 mm in length as opposed to 5-6 mm. The remaining two outdoor plants differed from the rest in having minute hybrid type stamens and were completely sterile.

Five F_3 plants derived from F_2 2 were grown in the glasshouse and nine in the experimental plot. They were all very similar to each other and to their F_2 parent but did vary somewhat in petal colour and overall size. One of the outdoor plants showed minute hybrid stamens and was completely sterile, whilst an indoor plant (2*b*) had stamens of somewhat reduced size which only reached the level of the top of the ovary.

TABLE 2
Chromosome numbers and fertility of glasshouse plants of
S. nicaeensis × *purpurea* (2x) F₃

Plant	Androecial development	2n	Assoc. at M1	Pollen fertility %	% flowers developing into capsules	average seeds per capsule	% seeds with abort. embryo
1a	Normal	36	18 ₁₁	27	100	53	30
1b	Normal	—	—	—	100	40	21
1c	Normal	35	17 ₁₁ 1 ₁	64 > 1	72	33	24
1d	Normal	36	18 ₁₁	90	100	74	4
2a	Normal	36	18 ₁₁	—	100	32	13
			2 ₁₁₁ 14 ₁₁ 2 ₁				
2b	Slightly smaller than normal	34	17 ₁₁	c.1	24	3.4	30
			1 ₁₁₁ 13 ₁₁ 5 ₁				
2c	Normal	36	multivalents common	15-6	11	9	11
2d	Normal	36	18 ₁₁	—	100	42	6
2e	Normal	36	18 ₁₁	60	100	80	> 1

F₄ generation

F₄ progenies, each of ten individuals, were grown from the selfed seed of the nine glasshouse F₃ plants. Three of the latter (1a, 1d and 2e) produced very uniform offspring almost identical in morphology to themselves, whilst the others bred fairly true, although a certain amount of variation occurred in the progenies, particularly in the characters of petal length and colour.

In most of the F₄ progenies there were a few individuals of low fertility or even complete sterility. The majority, however, were of as great or greater fertility than their F₃ parents, whilst every group apart from 2c contained some plants with a seed set approximately as high as *S. nicaeensis*. The percentage of plants per progeny group of approximately *S. nicaeensis* level of fertility was as follows (designating each group by the number of its F₃ parent): 1a, 80%; 1b, 70%; 1c, 80%; 1d, 90%; 2a, 40%; 2b, 30%; 2c, 0%; 2d, 70%; 2e, 40%.

Chromosome numbers were determined for at least two representatives of each F₄ progeny group and all plants examined had 2n = 36 with regular meiosis. Pollen fertility was very variable, ranging from 0% in some of the sterile plants to as high as 90%, and sometimes showing a considerable variation from flower to flower.

Backcrosses of the F₄ to *S. nicaeensis*

Highly fertile F₄ plants from the 1a, 1b, 2b and 2d progeny groups were backcrossed to *S. nicaeensis*, using the hybrid as female parent.

As might be expected, the offspring of these crosses were morphologically close to *S. nicaeensis* but also showed characters of their hybrid parents. Fertility was very high, as in both parents, and all had 2n = 36 and regular meiosis.

Hybridization of different F₄ progeny groups

Highly fertile F₄ plants of the 1b, 1d, 2b and 2d progeny groups were crossed in the combinations 1b × 2b and 1d × 2d. In each combination reciprocal

hybridizations were carried out, i.e. each plant was used both as male and female parent; thus four progeny groups were produced and ten plants of each were grown.

$1d \times 2d$ and its reciprocal were groups of morphologically similar, uniformly fertile plants, similar to their parents (and *S. nicaeensis*) in level of seed set. $1b \times 2b$ and its reciprocal, on the other hand, were morphologically somewhat variable and showed great variation in fertility, ranging from three plants showing similar fertility to their parents to a number of others with small hybrid type stamens and no seed set. The contrast in variability between the two combinations was most striking and must have reflected different levels of heterozygosity in the parents.

All plants examined cytologically had $2n = 36$ and meiosis was regular, showing 18_{11} at first meiotic metaphase, except in one plant of $1b \times 2b$ where there was variation between $15_{11}6_1$ and $7_{11}22_1$.

Backcrosses of the F_1 to *S. nicaeensis*

One of the F_1 plants was backcrossed to *S. nicaeensis*, using the latter as pollen parent. Most of the flowers pollinated developed into small capsules containing 1-4 seeds. The six backcross progeny which were grown to maturity were very variable but fell more or less within the range of variation pattern of the F_2 produced by selfing. Fertility was variable: one plant was of comparable fertility to F_{21} , two others approached the fertility of F_{22} , another was similar in fertility to the F_1 hybrid, whilst no seeds at all were produced by the remaining two plants. Some of the more sterile plants showed a variable percentage of flowers with small hybrid type androecia.

Only two of the plants were examined cytologically. The plant of comparable fertility to F_{21} had $2n = 36$ and regular meiosis, whilst one of the completely sterile plants had $2n = 31$ and showed very irregular disjunction at first meiotic anaphase.

DISCUSSION

The evolution of the fertile tetraploid lines. The most interesting feature of the results presented in this paper is the derivation in only two generations of fertile true-breeding tetraploid plants, such as $1a$, $1d$ and $2e$ of the F_3 , from a triploid hybrid of high sterility. The tetraploids combine characters of both parental species with others of some novelty (e.g. the very large flowers of branch 1 and the rounded petals of branch 2) and the two branches, as represented by their fertile members, would certainly be recognized as distinct species if they had been discovered in the field. Since all the morphological characters of the two branches and a great deal of fertility were already present in the F_2 generation, the production of these fairly true-breeding near-tetraploid F_2 plants from the triploid F_1 hybrid comes close to the evolution of new species in a single step.

The tetraploid condition arose via a near tetraploid F_2 ($2n = 35$ in plant 1 and 34 in plant 2) and by the F_3 six of the eight plants for which chromosome numbers were determined were tetraploid, whilst in the F_4 all counted were of this level. The most likely explanation for the establishment of near tetraploidy in the F_2 is the fusion of gametes of approximately diploid level; origin involving unreduced triploid gametes seems unlikely since to produce

the chromosome numbers of the F_2 they would have to fuse with hypohaploid gametes.

The morphology of the F_2 and succeeding generations indicates that their complements contain both *nicaeensis* and *purpurea* chromosomes, and it seems possible, judging from the high intergenomic homology shown in the F_1 (see p. 118), that a fair degree of interchangeability of chromosomes of the two species may occur within genomes without causing serious genetic unbalance. Following this argument, the complements of the true-breeding lines were probably made up of 'shuffled' genomes, but there must have been sufficient similarity between pairs of these genomes for each chromosome to find an exact homologue at meiosis and thus allow true breeding to occur. Presumably preferential pairing must have played a large part in bringing about such accuracy in association amongst the four genomes present. An alternative explanation to account for true-breeding is that it was the result of apomixis, but this seems unlikely because of the change in chromosome number between F_2 and F_3 . In any case, if apomixis did occur it must have been of the pseudogamous type, since flowers never set seed if they had small stamens which could not bring about self-pollination by touching the stigmas.

Highly fertile offspring were produced by crossing representatives of both branches with *S. nicaeensis*, showing that their evolution had not been accompanied by the establishment of any reproductive barrier separating them from this species. Fertility in hybrids between the branches varied: $1d \times 2d$ hybrids and some $1b \times 2b$ hybrids were fully fertile, whilst other $1b \times 2b$ plants had small 'hybrid type' stamens and were completely sterile. The occurrence of sterile plants with malformed androecia was, however, no higher in the hybrid than in the $1b$ and $2b$ lines themselves and there seems therefore to be no evidence indicating that establishment of reproductive barriers separating the two branches has occurred. Absence of barriers to gene exchange, however, does not vitiate the claim that the branches as represented by their fertile true breeding lines are comparable to species, since there are a number of recognized wild species of *Spergularia* which produce F_1 hybrids of high or fair fertility, e.g. *S. bocconii* and *S. nicaeensis*, *S. heldreichii* and *S. purpurea* (4x).

At this stage it should be pointed out that not all the F_2 plants were fertile near tetraploids (see Table 1). Apart from the two progenitors of the F_3 and F_4 generations, only one other plant (*f*) was highly fertile and this was a hexaploid, presumably produced by the union of two unreduced gametes. The others consisted of a near pentaploid ($2n = 44$), four plants intermediate between triploid and tetraploid levels ($2n = 30, 32$ (two plants), and 33), and one intermediate between diploid and triploid levels ($2n = 24$). The occurrence of healthy, well-developed plants exhibiting such intermediacy in chromosome number indicates an insensitivity to genetic unbalance caused by aneuploidy.

Comparison with previously recorded cases. A number of cases have been recorded where interspecific F_1 hybrids of varying fertility have given rise without doubling of chromosome number to stable fertile derivatives of intermediate morphology (see Grant, 1966a, for list). The best documented case is that of the fertile, stable lines derived from the highly sterile *Gilia*

malior \times *modocensis* (Grant, 1966a, b & c). In these a stepwise increase in fertility was obtained generation by generation and highly fertile lines were established by the F_3 , some stabilized at euploid and others at aneuploid level.

The lines derived from *S. nicaeensis* \times *purpurea* (2x) differ from the other examples cited by Grant in that their F_1 progenitor is a triploid and the recovery of fertility has been accompanied by the restitution of the tetraploid condition of the original female parent. Other noteworthy differences occur between the *Spergularia* and *Gilia* lines. For instance, in *Spergularia* the bottleneck of hybrid sterility was largely passed in one generation, associated with the change in ploidy, whilst in *Gilia* achievement of fertility was a more or less gradual stepwise process*. A similar situation occurred with regard to segregation for morphological characters: the F_2 *Spergularia* hybrids produced fairly uniform progeny, whereas in *Gilia* a fair degree of segregation occurred during the earlier selfed generations. The fertile derivatives of the *Gilia* hybrid also differ in that those tested by Grant were separated by sterility barriers, at least in part of chromosomal nature, from each other and from their parental species, whereas those of *Spergularia* are in both cases interfertile. The last difference is of course related to the degree of genomic differentiation occurring: *Gilia malior* and *modocensis* genomes show considerable structural differences whilst in *Spergularia* there is evidence to indicate that interspecific genomic homologies are very wide (Ratter, 1969).

Androecial abnormalities. In common with so many interspecific *Spergularia* hybrids, dysfunction in androecial development occurred in many of the plants reported in this paper resulting in stamens which were too small to function efficiently. Susceptibility to such dysfunction is apparently a character of great sensitivity as it can vary not only between morphologically identical sister individuals, but also with environmental fluctuations; for instance, the same plant in different years may produce either normal or abnormal androecia (e.g. F_{21}), or indeed such a change may occur during a single growing season.

Abnormalities producing undersized androecia are probably far more potent barriers to interspecific gene exchange in *Spergularia* than chromosomal sterility.

CONCLUSIONS

S. nicaeensis \times *purpurea* (2x) adds another example to the few known cases where recovery of fertility of sterile interspecific hybrids has occurred without a doubling of chromosome number. The evolution of fertile forms has, however, involved a readjustment of ploidy level from triploid to tetraploid. The two branches, each of which include true breeding lines, are no less distinct in morphology and breeding characteristics than a number of recognized natural species of *Spergularia* and therefore could be considered to rank as synthetic species.

* The level of 100% fertility had not been obtained in the *Spergularia* hybrids when the study was ended at the F_4 , in that even in the most fertile lines, e.g. 1c, 1d, some plants of subnormal fertility occurred. There seems little doubt, however, that selection could rapidly have achieved lines with 100% fertility.

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