CYTOGENETIC STUDIES IN SPERGULARIA:

III. Some Interspecific Hybrids involving S. marina (L.) Griseb.

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THIS paper follows on from the previous and deals with interspecific hybrids of *S. marina* (L.) Griseb. with the tetraploid ($2n=36$) species *S. rupicola* Lebel. ex Le Jolis, *S. purpurea* (Pers.) G. Don (4x), *S. rubra* (L.) J. & C. Presl, *S. nicaeënsis* (Sarato) Burnat and *S. bocconii* (Scheele) Aschers. & Graebn. Brief notes on habit, distribution etc. for *S. marina* and *S. rubra* are given below; similar notes have already been given for the other species in the previous paper. Full descriptions of all species appear in Flora Europaea Vol. I.

S. marina (L.) Griseb. is an annual to short-lived perennial herb which inhabits salt marshes, waste places near the sea and inland salt areas. It is widely distributed in the Northern Hemisphere. The species is tetraploid ($2n=4x=36$).

S. rubra (L.) J. & C. Presl is also an annual to perennial herb which inhabits dry sandy places, ruderal habitats, etc. Like *S. marina* it is widespread in the Northern Hemisphere. The race which was used in hybridisations with *S. marina* is tetraploid but a hexaploid race ($2n=54$) is also known.

Attempts to cross *S. marina* with *S. fimbriata* Boiss., *S. media* (L.) C. Presl, and *S. heldreichii* (Foucaud) Simon & Monnier have been completely blocked by seed incompatibility.

MATERIALS AND METHODS

The source of all material used in the investigation is given in Table 1. Most provenances are of known wild origin.

The various provenances of *S. marina* used in the hybridisations are all completely interfertile and the same is the case for each of the two provenances of *S. rubra*, *S. nicaeënsis* and *S. bocconii*.

Culture of the plants and all other techniques were as described in the previous paper. The two species not previously dealt with, *S. marina* and *S. rubra*, are both homogamous and therefore emasculation of their flowers was necessary prior to anthesis.

RESULTS

F_1 hybrids

Table 1 p. 232 summarises information of parentage, meiotic pairing, pollen fertility and seed fertility in the F_1 hybrids.

These hybrids, like those reported in the previous paper, are morphologically intermediate between their parents and show the characteristic long straggling inflorescences and abnormally small stamens.

The usual pattern occurs in the pollen mother cells with bivalents, trivalents and quadrivalents arranged on the equatorial plate at 1st meiotic metaphase and univalents, when present, scattered through the cell (Plate 25 A, D–F). Lagging of univalents occurs at anaphase and they usually undergo equational division before travelling to the poles. In *S. marina x purpurea* (4x) an octoploid cell containing 36_{II} was seen, and occasional binucleate pollen mother cells occur in *S. marina x nicaeënsis* and *S. marina x rubra*. In *S. marina x rupicola* where all other figures are fairly close to a median of 9_{II} 18_{I}, a single cell was observed which could not be accurately analysed but apparently showed complete pairing.

The pollen is completely abortive in all hybrids except for *S. marina x purpurea* (4x), where the anthers of occasional flowers contain a high percentage of good grains, and *S. bocconii x marina* (and reciprocal) where about 0·5% fertility occurred in all anthers examined. The only F_1 hybrid which has produced seed is *S. marina x purpurea* (4x) where fertility is much lower than 0·5% of that found in the female parent.

The majority of seedlings of *S. marina x rubra* (and reciprocal) are killed by a lethal effect which occurs in most interspecific hybrids involving *S. rubra*. Affected seedlings germinate well but at the stage when the second to fourth pairs of foliage leaves are opening they develop a chlorosis which soon becomes fatal. Thousands of hybrid seedlings involving various provenances of *S. rubra* and including at least seven hundred *S. marina x rubra* (and reciprocal) have succumbed to this chlorosis, and the seventeen plants reported in this paper are the only survivors. These viable *S. marina x rubra* (and reciprocal) hybrids are vigorous, show no tendency to chlorosis, and exhibit the usual characters of the F_1 hybrids already mentioned.

F_2 hybrids

F_2 hybrids have been produced only in *S. marina x purpurea* (4x) where 48 plants have been grown to flowering. They are very variable and show various combinations of the characters of the parental species. Some are morphologically close to *S. marina* and others to *S. purpurea*, whilst the majority are in the intermediate range. An abnormal tussock-like growth habit occurs in some of the F_2 plants due to production of extremely short internodes. Some of the plants have stamens similar in size to the parental species whilst in others the minute hybrid type occurs. Pollen fertility ranges from about 40% to nil and is sometimes very variable from flower to flower of the same plant. Seed fertility ranges from approximately 5% of that normal for *S. marina* to nil.

Cytological examination of the F_2 generation (Table 2) has so far been limited to five plants. One of these was *marina*-like (apart from petal colour) and another was *purpurea*-like, whilst the other three were more or less intermediate. Four of these plants were normal tetraploids but the *purpurea*-like plant possessed one extra chromosome ($2n=37$). Bivalent formation in all plants is high with at least a fair number of cells showing complete or almost complete pairing (see Table 2). The timing of meiosis appears to be disturbed in some of the plants so that precocious separation of bivalents is very common and this undoubtedly accounts for a high proportion of the univalents observed. In plant C (Table 2) secondary associations of bivalents are quite obvious.

DISCUSSION

The discussion is mainly devoted to consideration of meiotic pairing in the hybrids but before proceeding to this topic mention should be made of some other aspects.

As in the other hybrids described in the previous paper the F_1 hybrids involving *S. marina* are morphologically intermediate between their parents, show the characteristic long straggling inflorescences associated with high sterility, and usually have abnormally small stamens. One of these F_1 hybrids occurs in nature. This is *S. marina x rupicola* which has been recorded from two English localities where both parents were growing together (Pugsley, 1911, and Ratter, 1963). In this case the wild and synthetic hybrids correspond exactly.

In the hybrid between *S. marina* and *S. rubra* (4x) a lethal chlorosis normally occurs in the young seedling but when viable plants do occur they exhibit complete sterility associated with meiotic irregularities. The interspecific lethal effect is, therefore, additional to another barrier preventing gene exchange between the species.

Cytology

a) *Cytology of the parental species**. The cytology of *S. rupicola*, *S. purpurea* (4x), *S. nicaeënsis* and *S. bocconii* has already been discussed in the previous paper (p. 207). *S. marina* and *S. rubra* (4x) resemble these species in chromosome size and chiasma frequency. In *S. rubra* (4x) there is regular formation of bivalents at meiosis and this is also the case for the provenances of *S. marina* examined by the writer†. Rodriguez (1953), however, records the frequent occurrence of quadrivalents in Portuguese material of *S. marina*, although he states that in the majority of pollen mother cells only bivalents are formed. It seems, therefore, that although all the provenances of *S. marina* used in the investigation exhibit cytologically diploid behaviour other populations do occur in which quadrivalent formation is common.

b) *Cytology of the hybrids*. The meiotic configurations occurring in the hybrids are summarised in Figure 1 p. 227.

It is immediately apparent that, apart from *S. marina x purpurea* (4x), the hybrids involving *S. marina* contrast with the other tetraploid hybrids discussed in the previous paper in the number of univalents occurring at first meiotic metaphase. The number of univalents in the hybrids of *S. marina* with *S. rupicola*, *S. nicaeënsis* and *S. bocconii* usually approximates to eighteen, i.e. is equivalent to two genomic sets of nine, whilst in *S. marina x rubra* (and reciprocal) the number is usually higher.

S. marina x rubra (and reciprocal) is a suitable starting point for consideration of the significance of meiotic pairing amongst these hybrids. It is characterised by a degree of pairing significantly lower than any of the other hybrids. Often only two or three bivalents per pollen mother cell occur, although in the Denia x Coimbra provenance hybrids some of the cells show up to nine. It is impossible to distinguish whether pairing is due to

*A more detailed account of the cytology of all these species is given in Ratter, 1964.

†A quadrivalent has actually been observed in a single P.M.C. of one provenance of *S. marina* (see Ratter, 1964).

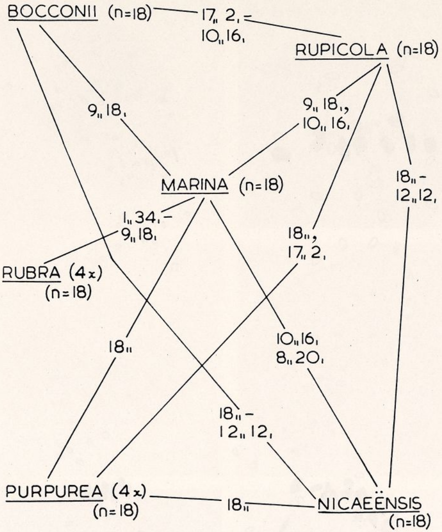

FIG. 1. Pattern of hybridisation involving *S. marina* and also showing hybrid interrelationships of the other species involved. The most frequent configurations at 1st meiotic metaphase are given.

auto- or allosyndesis but in either case the existence of a considerable proportion of pollen mother cells with few bivalents indicates that it is rather weak. We can conclude, therefore, that, at least in this hybrid, neither the capacity for allosyndesis between *rubra* and *marina* chromosomes nor for autosyndesis amongst those of either species is strong enough to bring about regular pairing of complete genomes.

In the F_1 of *S. marina x purpurea* (4x) meiosis is regular with usually complete pairing, but fertility is very low. The F_2 generation of this hybrid contains *marina*-like and *purpurea*-like plants as well as those which are more

FIG. 2. Explanatory diagrams of Plate 25 A, D, E and F.

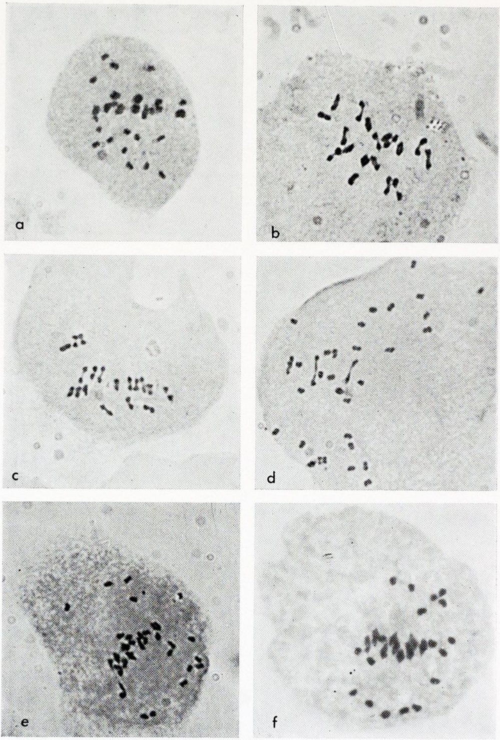

PLATE 25. Pollen mother cells. 1st meiotic metaphase. A–E × 1,350, F × 1,700. a) *S. marina x rupicola* 9_{II} 18_{I}; b) *S. marina x purpurea* (4x) F_1 18_{II}; c) *S. marina x purpurea* (4x) F_1 Plant c 18_{II}; d) *S. marina x rubra* (4x) 3_{II} 30_{I}; e) *S. marina x nicaeënsis* 11_{II} 15_{I} (aneuploid cell); f) *S. bocconii x marina* 9_{II} 18_{I}.

facing p. 228

or less intermediate. The five F_2 plants which have been examined cytologically are tetraploids (or in one case tetraploid plus one chromosome) and show fairly regular pairing. The occurrence of F_2 plants of normal, or nearly normal, tetraploid number approaching the morphology of both parental species suggests that the pairing in the F_1 is allosyndetic, since it appears that only primary segregation following allosyndesis could account for their origin (see also discussion of *S. bocconii x nicaeënsis* p. 209). The conclusion reached in the previous paragraph that any capacity for autosyndesis amongst *S. marina* chromosomes must be weak also tends to support the view that allosyndesis is taking place in this hybrid, since it seems unlikely that the regularly high pairing observed would occur if it depended even in part on autosyndesis of *marina* genomes. The provenances of *S. marina* involved in the hybrids with *S. rubra* and *S. purpurea* (4x) are, however, different so that we cannot be sure that their capacities for pairing are the same (nor can we be sure of similar chromosomal behaviour in different hybrids). The occurrence of 36 bivalents in the single octoploid pollen mother cell observed is presumably the result of preferential pairing between exact homologues suppressing any multivalent formation.

The F_1 hybrids of *S. marina* with *S. rupicola*, *S. nicaeënsis* and *S. bocconii* are characterised by similar meiotic configurations approximating to 9_{II} 18_I. Once again it is impossible to distinguish between auto- and allosyndesis. If autosyndesis is occurring it seems improbable that it is amongst *S. marina* chromosomes since, judging from *S. marina x rubra*, it is unlikely that *marina* genomes would show such regularly high autosyndetic pairing. If allosyndesis is occurring then it indicates that *S. marina* shares one common genome with the other three tetraploid species. It is not surprising that the pairing of *S. nicaeënsis x marina** and *S. bocconii x marina** are similar since, as discussed in the previous paper, the complements of *S. nicaeënsis* and *S. bocconii* appear to show considerable homology. Furthermore *S. nicaeënsis x rupicola* and *S. bocconii x rupicola* both show quite high pairing so that one is tempted to consider that the chromosome complement of *S. rupicola* may be fairly similar to that of *S. nicaeënsis* and *S. bocconii*. If this is correct the similarity of configurations in the hybrids of all three species with *S. marina* would be explained.

It is interesting that *S. marina*, *S. rupicola* and *S. nicaeënsis* all produce hybrids with *S. purpurea* (4x) which show 18 bivalents fairly regularly at 1st meiotic metaphase. This contrasts with the pairing approximating to 9_{II} 18_I occurring in *S. marina x rupicola** and *S. marina x nicaeënsis*.* As previously discussed there is some evidence that allosyndesis accounts for the pairing in *S. marina x purpurea* (4x) and *S. rupicola x purpurea* (4x)†. If this is correct the situation exists where both *S. marina* and *S. rupicola* will show complete allosyndesis with *S. purpurea* (4x) but not with each other. This is, of course, quite possible; an example of a similar case has been reported by Gajewski, (1953) in *Geum* hybrids. There is no evidence available as to whether auto- or allosyndesis is occurring in *S. nicaeënsis x purpurea* (4x), but it seems possible that the behaviour of the *S. nicaeënsis* complement is similar to that of *S. rupicola* in equivalent hybrids.

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*And reciprocal hybrid.

†For discussion of *S. rupicola x purpurea* (4*x*) see previous paper (page 210).

The occurrence of a pollen mother cell in *S. marina x rupicola* showing apparently complete bivalent formation deserves some comment. Since meiotic configurations in this hybrid are otherwise always close to 9_{II} 18_{I} the occurrence of this abnormal figure could suggest that asynapsis (or premature desynapsis) of two of the four genomes present may be normally genically controlled and have broken down here.

CONCLUSIONS

As in the other hybrids discussed in the previous paper little can be deduced of specific relationships from a study of meiotic pairing since it is impossible to distinguish in most cases between auto- and allosyndesis. In *S. marina x purpurea* (4x) evidence from segregation in the F_2 indicates that allosyndesis of the parental complements is probably occurring in the F_1. If this is correct *S. marina* and *S. purpurea* (4x) are at an ecospecific level of differentiation with a very slight capacity for gene exchange. It is very doubtful if this minute capacity for gene exchange is of the slightest importance except under experimental conditions.

The study has demonstrated that hybrid sterility is a barrier to gene exchange amongst the species studied. The only hybrid to show any seed production whatsoever is *S. marina x purpurea* (4x). In the hybrids of *S. marina* with *S. rubra*, *S. rupicola*, *S. nicaeënsis* and *S. bocconii* the occurrence at meiosis of a high proportion of univalents might suggest that sterility is of the chromosomal type but, on the other hand, occurrence of an abnormal figure with 18 bivalents in *S. marina x rupicola* seems to show that genic control of asynapsis cannot be discounted.

SUMMARY

The paper deals with the hybrids of *S. marina* with *S. rupicola*, *S. nicaeënsis*, *S. purpurea* (4x), *S. rubra* (4x) and *S. bocconii*. Details of the F_1 hybrids are given in Table 1 and the pattern of hybridisation and most frequent meiotic configurations are summarised in Figure 1. F_2 hybrids have been produced in *S. marina x purpurea* (4x) only and data concerning them is given in Table 2 (p. 236).

The F_1 hybrids are completely sterile apart from *S. marina x purpurea* (4x) where seed production is less than 0·5% of the normal for the female parent. Irregular meiosis with large numbers of univalents at first metaphase and consequent lagging of chromosomes at anaphase is characteristic of these hybrids except for *S. marina x purpurea* (4x) where bivalent formation is usually complete. In the hybrid between *S. marina* and *S. rubra* (4x) a lethal chlorosis usually occurs in the young seedlings and viable plants are exceptional.

The F_2 hybrids of *S. marina x purpurea* (4x) include plants showing segregation towards both parental species. Most of these F_2 plants are slightly fertile. Initial cytological work on five plants has shown that four were tetraploid and the other tetraploid plus one extra chromosome.

Since it is impossible to distinguish between auto- and allosyndesis in most of the hybrids evidence of specific interrelationships is not available from meiotic pairing. In *S. marina x purpurea* (4x), however, evidence from segregation in the F_2 seems to indicate that allosyndesis occurs in the F_1, and it seems that *S. marina* and *S. purpurea* (4x) are at an ecospecific level of differentiation with a very slight potentiality for gene exchange across a strong partial sterility barrier (a practically identical case to *S. bocconii x nicaeënsis* which was discussed in the previous paper).

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TABLE I

F_1 Interspecific Hybrids

Hybrid combination and source of parents	*No. of plants*	*2n*	*Pairing at M1*				*Notes*
			Configuration III	II	I	*No. of PMC*	
S. rupicola x marina							
♀ Hilbre Is., Cheshire ♂ Glasson, N. Lancs.	6	36		10	16	Not recorded but 9_{II} 18_{I} & 8_{II} 20_{I} the commonest configurations	Large robust perennial. Intermediate in morphology between parents. Pollen fertility 0%. Seed fertility 0%.
				9	18		
				8	20		
S. marina x rupicola							
♀ Glasson, N. Lancs. ♂ Hilbre, Cheshire	20	36		11	14	2	Identical in morphology to the reciprocal hybrid. Pollen fertility 0%. Seed fertility 0%.
				10	16	15	
				9	18	53	
				8	20	8	
				7	22	1	
			Aneuploid cell				
				10	14	1	
						Total 80	
			A PMC was also present with a sideview of M1 not good enough for accurate analysis but apparently showing complete pairing.				
♀ Sacavém, Portugal ♂ Anglesey Wales	5	36		10	16	Not recorded but 9_{II} 18_{I} & 8_{II} 20_{I} the commonest configurations	As above.
				9	18		
				8	20		
S. marina x purpurea (4x)							
♀ Glasson, N. Lancs. ♂ Coimbra, Portugal	3	36		18		43	Slender annual to short-lived perennial. Intermediate in morphology between parents. Pollen fertility varying amongst flowers from 80% to nil. Seed fertility c.0·5%.
				17	2	5	
				15	6	1	
			1	15	3	2	
			Aneuploid cell				
				15	3	1	
			Octoploid cell				
				36		1	
						Total 53	

TABLE I—*contd.*

Hybrid combination and source of parents	*No. of plants*	2*n*	*Pairing at M*I				*Notes*
			Configuration			*No. of PMC*	
			III	II	I		
S. marina x rubra							
♀ Denia, Spain ♂ Coimbra, Portugal	6	36		9	18	4	Slender short-lived perennial. Intermediate in morphology between parents. Pollen fertility 0%. Seed fertility 0%.
				8	20	3	
				7	22	4	
				6	24	10	
				5	26	11	
				4	28	9	
				3	30	10	
				2	32	5	
						Total 56	
S. rubra x marina							
♀ Turku, Finland ♂ Llobregat, Spain	3	36		7	22	2	As above. Eight plants of the reciprocal of this hybrid were also grown. They have not been cytologically examined.
				6	24	5	
				5	26	28	
				4	28	15	
				3	30	18	
				2	32	19	
				1	34	5	
					36	1	
			1	6	21	1	
			1	4	25	1	
						Total 95	
S. marina x nicaeënsis							
♀ Glasson, N. Lancs. ♂ Hèrault, France	20	36		12	12	2	Fairly slender perennial. Intermediate in morphology between parents. Pollen fertility 0%. Seed fertility 0%.
				11	14	4	
				10	16	27	
				9	18	45	
				8	20	12	
			1	12	9	1	
			1	10	13	1	
			1	9	15	1	
			1	8	17	1	
			1	7	19	1	
			2	9	12	1	
			3	10	7	1	
			Aneuploid cells				
				11	15	1	
				10	18	1	
				8	22	2	
				8	18	2	
						Total 103	

TABLE I—*contd.*

Hybrid combination and source of parents	*No. of plants*	2*n*	*Pairing at M*I				*Notes*
			Configuration			*No. of PMC*	
			III	II	I		
S. marina x nicaeënsis (continued)—							
♀ Denia, Spain ♂ Hèrault, France	22	36		13	10	1	As above.
				12	12	1	
				10	16	17	
				9	18	25	
				8	20	11	
				6	24	2	
			1	10	13	1	
			1	9	15	1	
			1	8	17	2	
			1	7	19	1	
			1	6	21	1	
			2	9	12	1	
			2	7	16	1	
				8	$16+1_{IV}$	1	
			2	5	$16+1_{IV}$	1	
					Total	68	
♀ Llobregat, Spain ♂ Barcelona, Spain	30	36		11	14	1	As in *S. nicaeënsis* x *fimbriata* and *S. nicaeënsis* x *purpurea* (2x), reported in the previous paper, the difference in the petal colour of the Barcelona and Hèrault provenances of *S. nicaeënsis* is reflected in the hybrids. Apart from petal colour this hybrid is almost identical to the above.
				10	16	17	
				9	18	15	
				8	20	14	
				7	22	4	
				6	24	1	
				5	26	1	
			1	9	15	5	
			1	8	17	9	
			1	6	21	1	
			2	7	16	1	
			2	6	18	4	
				7	$18+1_{IV}$	1	
			Aneuploid cell				
			1	9	16	1	
					Total	75	
♀ Llobregat, Spain ♂ Hèrault, France	13	36		10	16	4	As above. Petal colour as in Glasson x Hèrault and Denia x Hèrault provenances.
				9	18	6	
				8	20	6	
			1	10	13	1	
			1	8	17	2	
			1	7	19	1	
					Total	20	

TABLE I—*contd.*

Hybrid combination and source of parents		*No. of plants*	2*n*	*Pairing at M*I				*Notes*
				configuration			*No. of PMC*	
				III	II	I		
S. nicaeënsis x marina								
♀	♂							
Hèrault, France	Llobregat, Spain	9	36		10	16	3	Identical to reciprocal hybrid.
					9	18	18	
					8	20	3	
				1	6	21	1	
						Total	25	
S. marina x bocconii								
♀	♂							
Frodsham, Cheshire	Sacavém, Portugal	2	36		9	18	Not recorded	Slender annual to short-lived perennial. Intermediate between parents. Pollen fertility c. 0·5%. Seed fertility 0%.
					8	20		
S. bocconii x marina								
♀	♂							
Par, Cornwall	Glasson, N. Lancs.	9	36		10	16	6	As above.
					9	18	52	
					8	20	7	
					7	22	1	
				1	8	17	1	
						Total	67	

TABLE 2

Details of F_2 plants of S. marina x purpurea (4x)

Plant	*2n*	*Pairing at M* I			*Pollen fertility*	*Seed fertility*	*Notes*
		Configuration		*No. of PMC*			
		II	I				
A	36	18		4	0–c.30%	c.5%	A *marina*-like plant. Pollen fertility varies from flower to flower.
		17	2	3			
		16	4	1			
		15	6	1			
		Total		9			
B	37	18	1	5	c.40%	c.0·5%	A *purpurea*-like plant.
		17	3	3			
		16	5	3			
		15	7	5			
		14	9	4			
		13	11	2			
		11	15	1			
		Total		23			
C	36	18		18	0%	0%	Morphologically intermediate between *S. marina* and *S. purpurea*. Precocious separation of bivalents undoubtedly accounts for many of the univalents. Secondary associations of bivalents occur.
		17	2	16			
		16	4	29			
		15	6	11			
		14	8	7			
		13	10	1			
		12	12	1			
		Aneuploid cell					
		21	3	1			
		Total		84			
D	36	18		16	0–c.5%	0·1%	Morphologically intermediate. Pollen fertility varies from flower to flower.
		17	2	7			
		16	4	2			
		15	6	3			
		14	8	1			
		Total		29			
E	36	18		20	0–c.30%	c.5%	Morphologically intermediate. Pollen fertility varies from flower to flower.
		17	2	5			
		16	1 + 1_{III}	1			
		Aneuploid cell					
		20		1			
		Total		27			