HYBRIDS AND CHROMOSOMES IN THE GENUS SONNERATIA (SONNERATIACEAE)

J. MULLER and S. Y. HOU-LIU

INTRODUCTION

In the Flora Malesiana Backer and van Steenis (1951) recorded five species of the genus *Sonneratia*, three of which occur in Malesia, *viz.* *S. caseolaris* (L.) Engler, *S. alba* J. E. Smith, and *S. ovata* Backer. In the course of a palynological study of recent and fossil *Sonneratia* pollen (Muller, 1964), it was discovered that in Brunei, NW. Borneo, hybridization occurs between these species.

It is the purpose of this note A. to describe the morphology of the hybrids, and B. to report on a preliminary cytological examination of the species and the hybrids for determining the chromosome numbers and for detecting irregularities in chromosome behaviour at meiosis in the hybrids.

Voucher specimens and permanent slides of trees marked in the field have been deposited in the Rijksherbarium, Leiden (Holland).

ACKNOWLEDGMENTS

The authors are grateful to Prof. Dr. C. G. G. J. van Steenis, Director of the Rijksherbarium, Leiden, for his advice and encouragement and free access to the facilities of his institute, to the Netherlands Foundation for the Advancement of Tropical Research (WOTRO) for a grant which made the cytological study possible, to Mr. J. P. van Niel, Brunei Shell Petroleum Co., Seria, Brunei, for continued collecting of flower bud material, to the staff members of the Rijksherbarium for their kind assistance and especially to Mr. J. van Brummelen for taking the photomicrographs and to Prof. Dr. H. J. Gloor, Director of the Genetics Laboratory, Leiden, for advice.

Part A — OCCURRENCE OF NATURAL HYBRIDS ON THE BRUNEI COAST

(by J. Muller)

Description of hybrids

The *Sonneratia* trees growing in the estuaries of the Brunei and Tutong Rivers (State of Brunei, NW. Borneo) can be divided into six groups as shown on table I. The morphological characters of groups I, IV, and VI agree completely with the descriptions of *S. ovata* (fig. 1A, 3A), *S. alba* (fig. 1C, 3C), and *S. caseolaris* (fig. 1E, 3E) respectively, as given in the Flora Malesiana. *S. ovata* is very rare and has only been found twice: a single tree in the island of Labuan and a second one upstream from Brunei town, both on the landward side of the mangrove belt. *S. alba* is common on the coast of Labuan, Klias, and Brunei Bay and is locally abundant on the banks of the Brunei River as far as 20 km from the sea. It also occurs in a few scattered specimens in the estuary of the Tutong

COLLECTION NO.		GROUP I Muller 5, 7	GROUP II Muller 12,14,18	GROUP III Muller 1, 6, 8, 9, 15,17,21,22	GROUP IV Muller 4,16,19	GROUP V Muller 11	GROUP VI Muller 2, 20
DETERMINATION		Sonneratia ovata Backer	Sonneratia cf. ovata	Sonneratia alba × ovata	Sonneratia alba J. Smith	Sonneratia alba × caseolaris	Sonneratia caseolaris (L.) Engl.
Leaf	shape apex base petiole colour	ovate, small, fairly uniform round subcordate long dark green	ovate, small fairly uniform round subcordate long dark green	broadly oval, large, variable round / emarginate flat / rounded long dark green	oval uniform round/emarginate cuneate long grey green	oval/elliptical uniform round cuneate short grey green	elliptical uniform acute cuneate-sessile very short light green
Bud	size shape apex base	small oval,constricted in the middle flat contracted	small oval, constricted in the middle flat contracted	medium oval, slightly constricted in the middle slightly acute contracted	medium ellipsoid acute conical	large conical, constricted in the middle acute slightly contracted	medium ellipsoid-barrel shaped, variable acute / apiculate flat / conical
Calyx	surface lobes base	granulate long, appressed cupshaped	granulate long,appressed cupshaped	smooth long, appressed cupshaped	smooth short, reflexed funnel shaped	smooth long, slightly reflexed bell shaped	smooth long, horizontal flat
Fruit	shape wall	flattened oval thin	flattened oval thin	oval thin	rounded oval thick, woody	flattened oval thin	oval very thin
Style	base	thin, depressed	thin, depressed	thin, flat	thin, flat	conical, depressed	conical, flat
Petals		absent	vestigial, white	conspicuous, white	white/tinged with red	red	red
Stamens		white	white	white	white	red	red
Pollen	type size fertility	ovata 40 μ 100%	ovata or aberrant 42 μ 50-90%	ovata 47 μ 1-50%	alba 60 μ 100%	caseolaris 49 μ 1%	caseolaris 50 μ 100%
Flowering		good	good	good	good	poor	good
Fruiting		good	good	good	good	very poor	good

Table 1. Morphological characters.

River near Danau. *S. caseolaris* [1] has a more restricted distribution, but is locally abundant on the banks of the Brunei River, upstream from Brunei town. It is thus restricted to the more brackish part of the Brunei River estuary, but its ecological range overlaps with that of *S. alba*.

The pollen fertility of these three species proved to be always near to 100 %.

Three other groups of *Sonneratia* trees were found which showed a mixture of characters of these three species and often reduced pollen fertility.

Group II is very similar to *S. ovata* except that vestigial white petals are present and that in one tree reduced pollen fertility and abnormal pollen occurred. It is designated as *Sonneratia cf. ovata* and has only been found in the Tutong River estuary where at least six trees occur.

The trees of group III (fig. 1B, 2A, 3B) are intermediate between *S. ovata* and *S. alba* in leaf shape and apex of bud. They resemble *S. ovata* in the colour and shape of the leaf, the contracted base and middle of the bud, the long calyx lobes which are appressed to the fruit, the thin wall of the fruit, and in pollen type. They resemble *S. alba* in the rather smooth surface of the bud and in the presence of fairly conspicuous white petals. Shape of buds, fruits, and leaves are pictured in fig. 1, 2A, and 3B. The trees are in the field at once distinguishable from *S. alba* by their large, dark green leaves. A peculiarity noted in several trees of this group was the occasional occurrence of abnormally developed branches with elliptical yellowish green leaves and grooved flower buds. At anthesis the anthers of these flowers had difficulty in releasing their pollen, which was only for 1 % fertile. Of the normal branches pollen fertility was generally around 50 %, although some trees showed a lower fertility.

The trees of group III occur mainly in a rather restricted area along the banks of the Brunei River, upstream from Brunei town and are especially frequent along the left bank of Sungei Damuan, close to the locality of *S. ovata*. Two specimens were also found near Danau in the Tutong River estuary.

Group III is thought to represent the first hybrid generation of a cross between *S. ovata* and *S. alba*.

Group II might then represent either F 2 segregates of this cross or back crosses with *S. ovata*.

The trees of group V (fig. 1D, 2B, 3D) show a combination of characters of *S. alba* and *S. caseolaris*. They are very rare and to date only three specimens have been found in a very restricted locality close to Brunei town. They resemble *S. alba* in the grey-green colour of the leaves, and they resemble *S. caseolaris* in the thin wall of the fruit, the conical, depressed base of the style, the red petals and stamens, and in pollen type; they are intermediate in length of petiole and shape of leaf between those two species. The most interesting characteristics are, however, to be found in the obviously disturbed flowering and fruiting. The buds grow to an excessive size and have apparently difficulty in opening because very few flowers were observed. The ovary meanwhile starts premature expanding, causing the bud to develop a peculiar conical shape with a constriction in the middle, quite unlike the buds of either *S. alba* or *S. caseolaris*. This phenomenon is clearly visible in fig. 1D. The few fruits which could be collected had developed into a flat disc-like oval shape and were half sunken into the bell-shaped calyx, thus being intermediate in character between the fruits of *S. alba* and *S. caseolaris*. In fig. 2B one of the

[1]) In passing I may point attention to the fact that in the sterile state *S. alba* and *S. caseolaris* are rather similar. I found, however, that *S. caseolaris* can always be distinguished from its congeners by a small knob-like thickening at the tip of the leaf, a difference hitherto unobserved.

fruits is almost wholly enclosed by the calyx, while the other has emerged halfway. The pollen fertility was found to be very low.

This group is thought to represent the first hybrid generation of a cross between *S. alba* and *S. caseolaris*. In the area where they were found, both putative parents grow mixed together.

Discussion

Hybrids between species of the genus *Sonneratia* have not been described before. The evidence presented above, which is confirmed by the cytological observations, strongly suggests that they do occur under special conditions. The main factor responsible is probably the rather unusual situation that the three parent species grow side by side in the Brunei River estuary. Usually they are ecologically separated, *S. alba* being the more marine species, *S. ovata* the least marine, growing on the landward side of the mangrove swamps and thus normally having little chances of contact with *S. alba*, while *S. caseolaris* is intermediate in ecological requirements.

The very narrow mangrove belt alongside the rather steep banks of the Brunei River and its tributaries have telescoped the various ecological zones together and thus increased the chances of contact, especially between *S. alba* and *S. ovata*. Another factor possibly promoting hybridization may have been the scarcity of *S. ovata* which stands thus a large chance of being cross-fertilized with *S. alba* pollen. This is also suggested by the obvious concentration of the *alba* × *ovata* hybrids around the single observed locality of *S. ovata*.

In the Tutong River estuary conditions are less clear, mainly because of human interference along the river banks. However, also here the mangrove zones are rather narrow due to steep banks.

The reduced fertility of the hybrids indicates that *S. alba*, *S. ovata*, and *S. caseolaris* are 'good' species, but the sterility barrier between *S. ovata* and *S. alba* appears lower than between *S. alba* and *S. caseolaris*. [1]

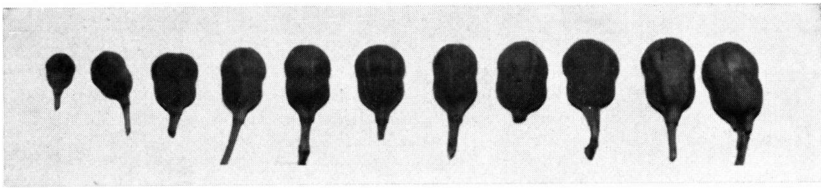
Part B — CHROMOSOME NUMBERS (by S. Y. Hou-Liu)

Materials and methods

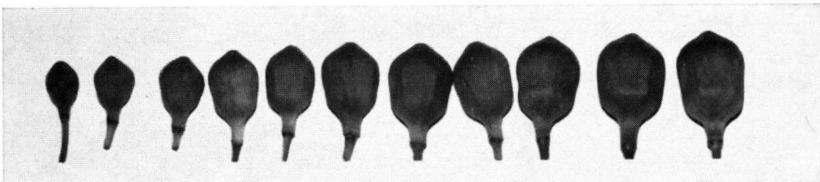
Flower buds at different stages of development were collected in the field at various times of the day and were killed and fixed immediately in Carnoy's solution (3 parts absolute alcohol and 1 part acetic acid by volume). In order to keep the material as short as possible in the fixative, it was air-freighted to Holland and stored in a refrigerator upon reception.

Each bud contains many stamens at various stages of development and good, countable chromosome figures were found in the material fixed between 9 and 11 o'clock in the morning. Observations on the meiotic division were made from iron-acetocarmine smear preparations (Johansen, 1940, and also Smith-White, 1942, 1948). Acetocarmine is preferable to aceto-orcein for this group. Suitable slides were temporarily sealed with balsamum copaivae. Usually the colour of the chromosomes appears deeper after a few days and good figures could then be photographed. Because of technical difficulties and lack of time, only some selected slides were permanently sealed, using 'Euparal' as a mounting medium. Text illustrations were traced from enlargements of the photomicrographs.

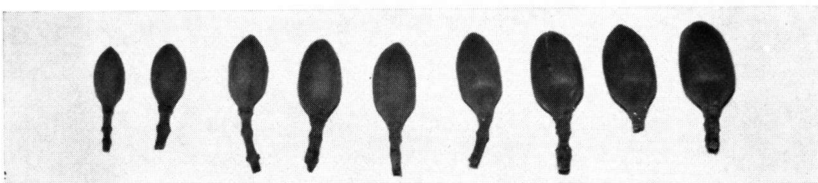
[1]) It is the intention to present detailed pollen-morphological data on the *Sonneratia* species and hybrids described here in a later publication.



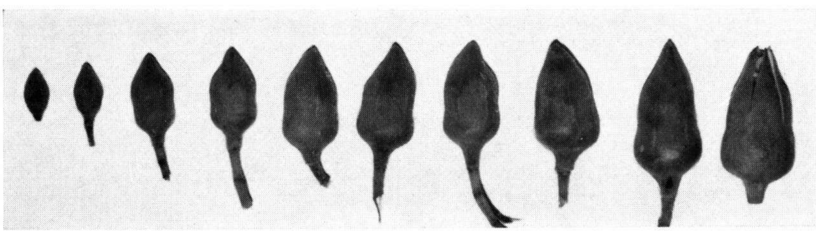
A Sonneratia ovata



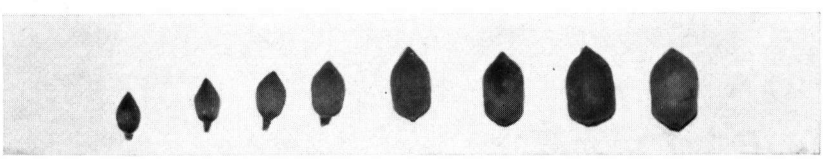
B Sonneratia alba × ovata



C Sonneratia alba



D Sonneratia alba × caseolaris



E Sonneratia caseolaris

Fig. 1. A—E. Bud development in *Sonneratia* species, × $\frac{1}{3}$.

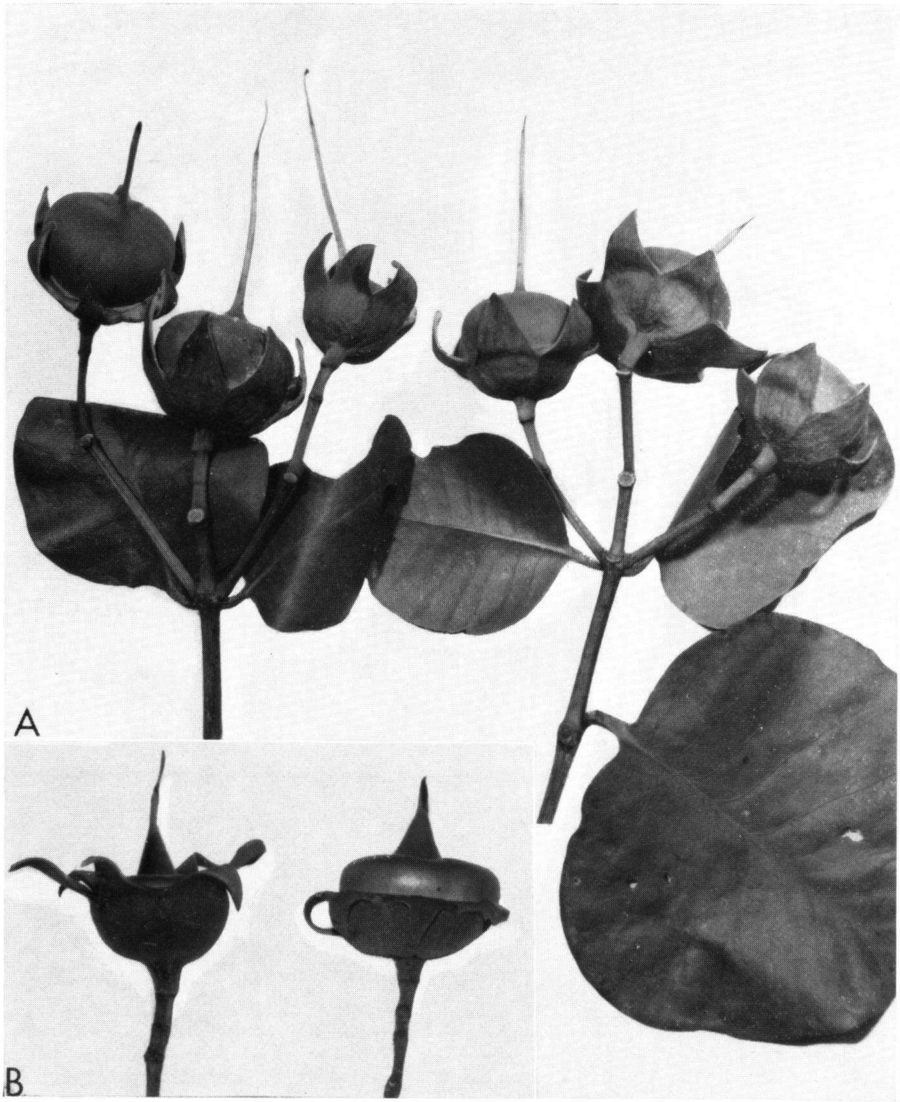


Fig. 2. A. *Sonneratia alba* × *ovata*; B. *S. alba* × *caseolaris*; both × ½.

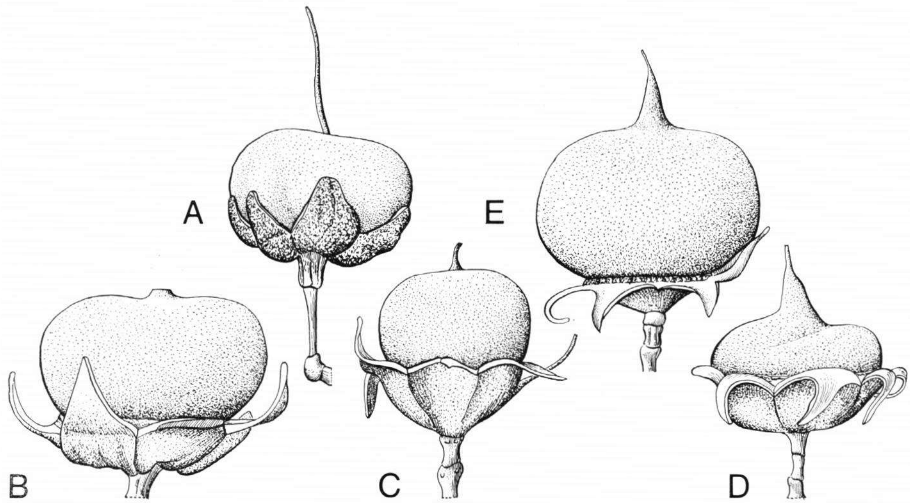


Fig. 3. A. *Sonneratia ovata*; B. *S. alba* × *ovata*; C. *S. alba*; D. *S. alba* × *caseolaris*; E. *S. caseolaris*. — A. Labuan, *Muller 5*, 28-12-63; B. Brunei, *Muller 8*, 9-6-64; C. Labuan, *Muller s.n.*, 28-12-63; D. Brunei, *Muller 11*, 30-5-64; E. Brunei, *Muller s.n.*, 30-5-64; all × ½.

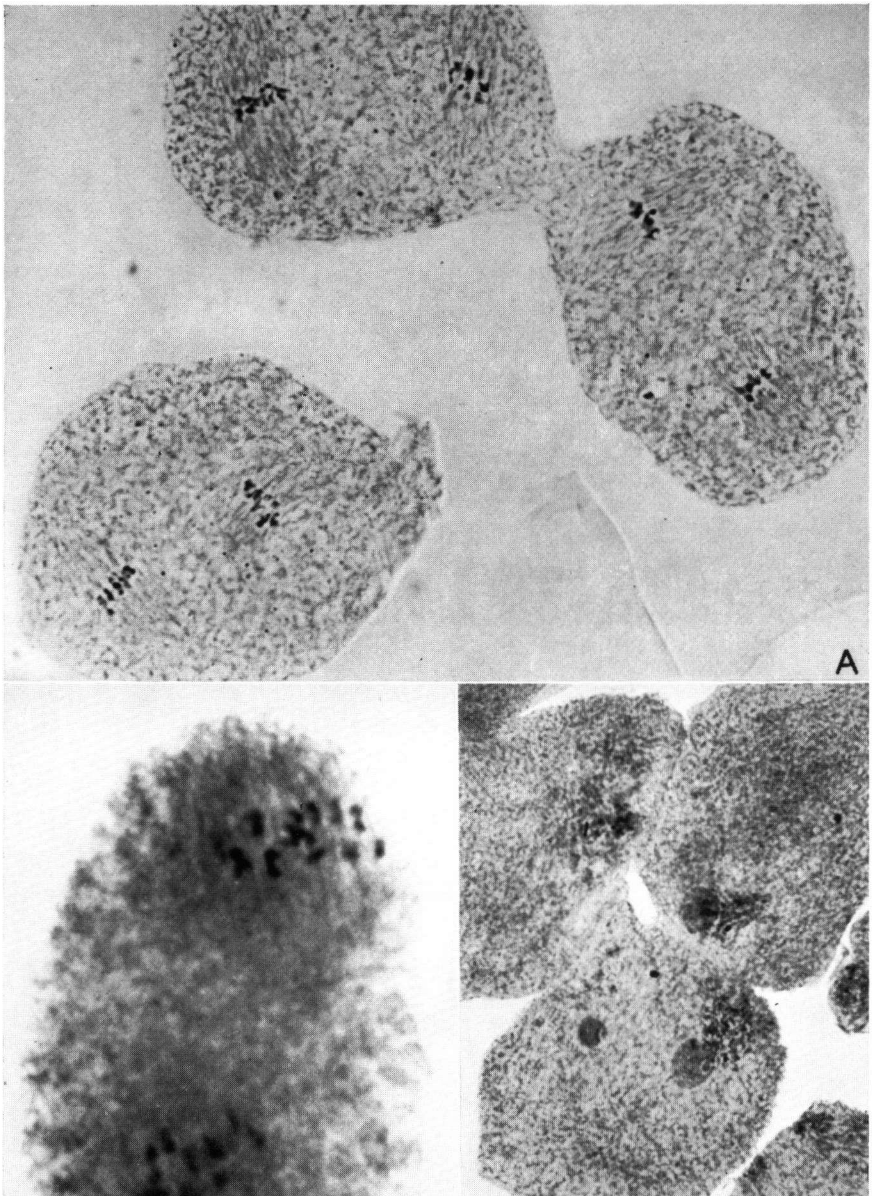


Fig. 4. Photomicrographs of pollen mother cells of *Sonneratia*. — A. *S. alba*, metaphase II, spindles lying in the same plane or perpendicular to each other, × 500; B. *S. ovata*, metaphase II, in oblique view, showing two chromosome complements, × 2000; C. *S. alba* × *caseolaris*, prophase I, showing the synizetic knots, × 750.

Table II. Data of material of *Sonneratia* in which meiosis was found

Name	Locality	Number of the plant	Material collected and fixed	Size of bud (mm) in which meiosis was observed
S. ovata	Brunei	Muller 7	3-III-'65	17 × 12
			9:00 a.m.	
			10:00 a.m.	20 × 15
			2:00 p.m.	17 × 13
S. cf. ovata	Danau Jetty	Muller 14	21-V-'65 10:00 a.m.	19 × 15
S. alba × ovata	Brunei	Muller 9	16-III-'65	24—25 × 13—14
			9:00 a.m.	
			10:00 a.m.	19 × 12—13
S. alba	Brunei	Muller 19	16-III-'65	28—30 × 13—15
			9:00 a.m.	
			10:00 a.m.	23—24 × 13
			2:00 p.m.	28—30 × 13—14
S. alba × caseolaris	Brunei	Muller 11	26-V-'65	31 × 14—16
			9:00 a.m.	
			10:00 a.m.	35 × 15
			11:00 a.m.	35 × 16
S. caseolaris	Brunei	Muller 20	26-V-'65	22—24 × 14—15
			9:00 a.m.	
			11:00 a.m.	24 × 15

The smear preparations are always full of artifacts as small granules, which sometimes obscured the observation of the chromosomes (compare fig. 4). These precipitates may be caused by storing the material too long in the fixative and/or by the reaction of tannin or other chemical substances contained in the cells with the iron ions as described by Li (1954).

Upon learning of these difficulties, Dr. H. L. Li, Morris Arboretum, Philadelphia, kindly told me his experience and sent me his paper just quoted. In order to obtain satisfactory preparations, future workers may try, if possible, to keep the material not too long in the fixative, to prepare the stain free from iron, and to avoid using metal instruments in handling the material.

Observations

(1) Sonneratia ovata, S. alba, and S. caseolaris

The chromosome behaviour at meiosis is normal and appears similar in all three species mentioned. The developmental stage within one anther is rather uniform. Owing to technical difficulties a detailed study of the meiotic process has not been made, but some remarks on the successive stages observed during the chromosome counts follow.

The pollen mother cells are subglobose, broadly ellipsoid or ellipsoid; at the resting stage each possesses a rather big nucleus, containing a distinct, excentric nucleolus. At first metaphase the chromosomes become arranged on the equatorial plane and the spindle fibers appear clearly visible. Because of the cell shape, it is very difficult to obtain a good polar view. The chromosomes are rather small and crowded together. The

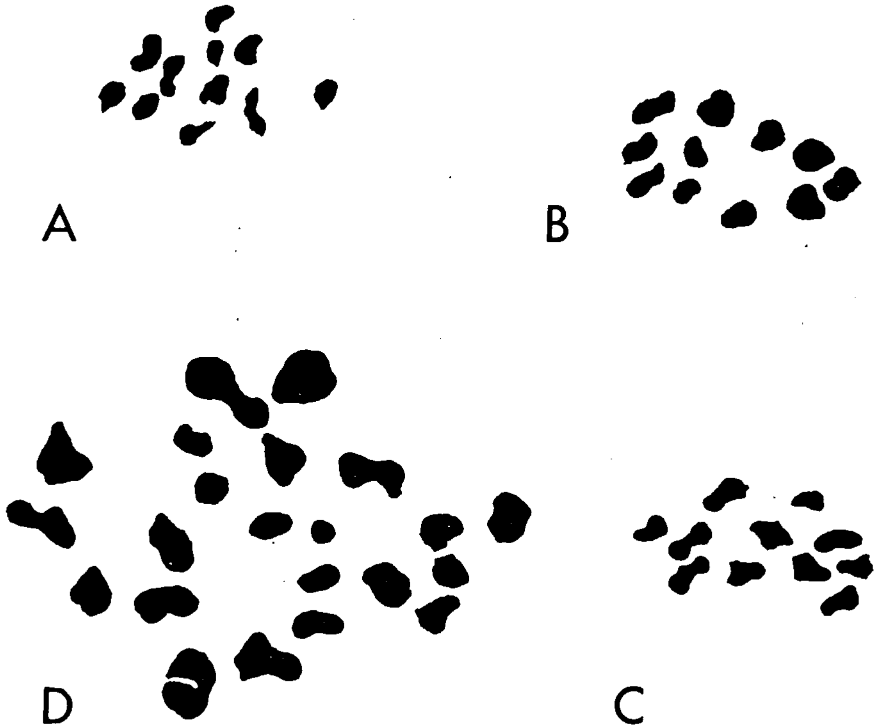


Fig. 5. Chromosome complements of *Sonneratia* in polar view (in A—C, only one chromosome complement of a dividing pollen mother cell at secondary metaphase is shown). — A. *S. alba*, at metaphase II, n = 11; B. *S. caseolaris*, at metaphase II, n = 11; C. *S. ovata*, at metaphase II, n = 11; D. *S. alba* × *caseolaris*, tapetal cell, at metaphase, 2n = 22; all × 3000.

chromosome complement of all three species is n = 11. During the early first anaphase the bivalents are held together by one or occasionally two terminal chiasmata. At second metaphase and anaphase the two sets of spindle fibers usually lie in the same plane or sometimes at right angles to each other (fig. 4A). In this latter case, therefore, one may have a good chance to obtain a polar view, favourable for chromosome counting. From second telophase up to tetrad and pollen grain formation, development appears regular and can easily be observed. As already stated in the first part of this study, pollen grain fertility in all three species is always nearly 100 %.

(2) Sonneratia alba × *caseolaris*

The material examined from this suspected hybrid frequently showed that the pollen mother cell was either in the resting stage containing a distinct nucleus with one (occasionally two?) excentric nucleolus (i) or in prophase. In the latter case the chromosome threads form a compact tangling mass (fig. 4C), with an appearance similar to the synizetic knot shown by Smith-White (1950, cf. his fig. 5) as occurring in *Darwinia (Myrtaceae)*. It is not certain whether such a knot, found in the pollen mother cell of the present material, is due to cytological difficulty or just represents the stage with long duration as described by Smith-White (1948, p. 31, cf. also fig. 81 & 82) in *Eucalyptus paniculata (Myrtaceae)*.

In some preparations, each made from one anther, a small group (or sometimes groups) of cells in first metaphase and/or anaphase were observed among the numerous pollen mother cells, either in resting stage or in prophase.

The vegetative cells, possibly tapetal cells, are very active. Regular mitotic divisions were observed, similar to those found in certain Angiosperms by Cooper (1933). The diploid number of these cells is $2n = 22$ (fig. 5D), which may serve to confirm the meiotic count ($n = 11$). Some cells contain rather higher chromosome numbers, but unfortunately none of them has a good figure favourable for counting. Tetraploids may possibly also occur here, similar to those mentioned by Cooper (1933) and Smith-White (1948).

The conspicuous synizetic knot, localized cell activity, together with the intermediate gross morphology and low pollen fertility could be attributed to interspecific hybridization (Sax, 1933).

(3) Sonneratia alba × ovata

In preparations of this suspected hybrid, meiotic activity is rather localized. One may find here and there a group of cells in certain stages of development. In first prophase sometimes only a few cells with the synizetic knot were observed. Cells in regular first metaphase are present, but it is very difficult to obtain a good figure for counting. Sometimes the chromosomes appear irregularly distributed along the spindle at first metaphase, almost from one end to the other. Also the number found is usually less than 11, occasionally more. These phenomena may be due to the presence of univalent, unpaired chromosomes and/or secondary pairing between bivalents (Smith-White, 1942, 1948). A further cytological examination is desirable for elucidation of these irregularities.

Conclusions

(1) The appropriate size of flower buds in which good meiotic stages were observed has been indicated in table II.
(2) Good chromosome figures were obtained from the material fixed between 9 and 11 AM.
(3) The chromosome number of all three species is the same: $n = 11$.
(4) Tapetal cells with regular mitotic division and the somatic chromosome number $2n = 22$ were observed.
(5) Irregular chromosome behaviour at meiosis was observed in the material of the suspected hybrids, *S. alba × caseolaris* and *S. alba × ovata*.
(6) The cytological findings agree with the observations on the morphology and on the fertility of the pollen grains.

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