CYTOGENETICS AND TAXONOMY OF THE GENUS GLOBBA L. (ZINGIBERACEAE) IN MALAYA II: CYTOGENETICS*

LIM SIEW-NGO**

ABSTRACT. A detailed cytological investigation was conducted on all but one of the twelve species, five subspecies and eight varieties of Malayan *Globba* (Zingiberaceae). Somatic chromosome numbers recorded for the genus so far are 22, 24, 32, 44, 48, 64 and 80; those of the Malayan species being confined to 32, 48 and 80. Chromosome association in taxa with somatic numbers of 32 and 48 was strongly suggestive of their being diploid and triploid respectively. In some taxa, however, an unusual subgrouping of chromosomes, predominantly into associations of eight, indicated that the original basic number might be 8. Taxa with 32 and 48 chromosomes would thus be allotetraploid (AABB) and hexaploid (AAABBB) respectively. A general correlation of increasing sterility with decreasing chromosome association and increasing meiotic irregularity has been observed both in the generally highly fertile taxa with $2n = 32$ and in the relatively sterile $2n = 48$. On the basis of cytology, morphology and distribution, the evolution of the genus in Malaya is discussed and a scheme of specific inter-relationships proposed.

INTRODUCTION

This paper presents the results of a detailed cytological investigation of the genus *Globba* in Malaya. Somatic chromosome numbers of all but one of the twelve species, five subspecies, eight varieties and a natural hybrid which occur in Malaya have been determined. Live material of the remaining species, *G. fasciata* Ridl., was not available. In addition, meiosis and pollen viability were analysed in as many Malayan taxa as possible. Due to limited material, a full cytological investigation of *G. fragilis*, *G. pendula* ssp. *montana*, *G. unifolia* var. *unifolia* and *G. leucantha* var. *peninsularis* was not possible; however, an analysis of chromosome association in metaphase I was made in *G. fragilis* and counts were made of the three latter taxa. These cytological studies were conducted with the dual purpose of determining the basic number of the genus and of gaining a deeper perception of specific inter-relationships within it.

MATERIALS AND METHODS

Details of locality, altitude, collector, herbarium number and chromosome number of each individual collection of living material are recorded in Lim (1969; Table IV).

Chromosome numbers were determined mainly in pollen mother cells but mitotic counts were made also from root tip squashes and anther wall cells. Young, actively dividing root tips were excised at about 12 noon, pre-fixed with distilled water for one hour and then with a mixture of saturated para-dichlorobenzene and alpha-monobromonaphthalene (1: 1) for one hour and twenty-five minutes. Fixation for about 18 hours was carried out in three parts absolute ethanol to one part glacial acetic acid. Roots were then hydrolysed in normal hydrochloric acid at 60°C for 10 to 15 minutes and stained in feulgen reagent for three hours.

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** Dr (Mrs) Chong Siew-Ngo née Lim, University of Nanyang, Singapore.

TABLE 1

Chromosome numbers of the Malayan species of *Globba*

	Species	Code	Herbarium accession number	Somatic count 2n	PMC count n
1	*G. marantina*	M	KLU 4831	80	—
2	*G. cernua* ssp. *cernua*	R2a	KLU 4844	32	16
		R2b	KLU 8205	32	16
		R3a	KLU 4843	48	24
		R3b	KLU 4796	48	24
		R3d	KLU 4832	48	24
	G. cernua ssp. *crocea*	Rc2	KLU 4791	32	16
		Rc3	KLU 4817	48	24
	G. cernua ssp. *porphyria*	Rp	KLU 8240	32	16
3	*G. unifolia* var. *unifolia*	—	—	—	—
	G. unifolia var. *sessiliflora*	U	KLU 7367	32	—
4	*G. fragilis*	F	KLU 4847	32	16
5	*G. curtisii*	C	KLU 4793	48	24
6	*G. holttumii* ssp. *holttumii*	H	KLU 4822	48	24
	G. holttumii ssp. *aurea*	Ha	KLU 8206	48	24
7	*G. x intermedia*	RA	KLU 4848	48	24
8	*G. patens* var. *patens*	A2b	KLU 8211	32	16
		A2c	KLU 4849	32	16
		A3	KLU 8232	48	24
	G. patens var. *costulata*	Aa	KLU 8209	32	16
9	*G. variabilis* ssp. *variabilis*	V	KLU 8208	48	24
	G. variabilis ssp. *pusilla*	Vp	KLU 8246	32	16
10	*G. albiflora* var. *albiflora*	B	KLU 8221	32	16
	G. albiflora var. *aurea*	—	—	32	16
11	*G. pendula* ssp. *pendula*	P2a	KLU 4799	32	16
		P2b	KLU 8213	32	16
		P3	KLU 8203	48	24
	G. pendula ssp. *pendula* var. *elegans*	Pe	KLU 4830	32	16
	G. pendula ssp. *montana*	Pa	KLU 8219	32	16
12	*G. leucantha* var. *peninsularis*	L	KLU 8200	32	16

Young flower buds were collected between 9 and 11 a.m. The individual anthers were fixed overnight in Dyer's fixative, consisting of ten parts of absolute ethanol, two parts of glacial acetic acid, two parts of chloroform and one part of formalin. After being treated overnight in a mordanted fixative of three parts of absolute ethanol to one part of glacial acetic acid saturated with ferric acetate, the anthers were washed with three hourly changes of 70% ethanol and stained in alcoholic-hydrochloric acid-carmine (Snow, 1963) for four days at 60°C. The stained anthers were rinsed in 70% ethanol and squashed in 45% acetic acid.

Pollen viability was estimated by aceto-carmine stainability and germination in Brewbaker & Kwack's solution (1963).

RESULTS

i. *Somatic chromosomes.* Chromosome numbers determined in this investigation are listed in Table 1; with the exception of *G. albiflora* var. *aurea*, the counts are new. Somatic chromosome numbers were found to be 32, 48 and 80.

TABLE 2

Chromosome association at first meiotic metaphase of taxa with 2n = 32

Chromosome association						Number of PMC examined in taxa*												
I	II	III	IV	V	VI	B	Vp	A2b	Rp	Aa	Rc	R2a	A2c	F	Pe	P2a	R2b	P2b
—	16					36	49	67	99	18	18	26	29	18	55	38	8	8
2	15					3	2	20	13	5	—	8	9	2	14	7	11	3
4	14						—	2	—	1	—	3	2	—	5	14	24	1
6	13						—		—		—		—	2	—	9	9	—
8	12						—		—		—		—	—	—	5	3	—
10	11						—		—		—		—	—	—	3	2	—
12	10						—		—		—		—	—	—	1	1	—
1	14	1					—		—		—		—	—	2			1
	13	2					—		—		—		—	—	—			5
6	10	2					—		—		—		—	—	—			1
	14	—	1				2		4		3		5	—	8			23
2	13	—	1				—		2					—	4			1
	12	—	2				1		—					2	—			32
4	12	—	1						—						—			1
2	11	—	2						—						—			2
6	11	—	1						—						1			—
	11	2	1						—									3
	10	—	3						—									1
2	9	—	3						—									2
	8	—	4						—									1
	12	1	—	1					—									6
2	11	1	—	1					—									1
	13	—	—	—	1				—									1
	11	—	1	—	1				1									—
	9	—	2	—	1													1
Total number PMC						39	54	89	119	24	21	37	45	24	89	77	58	96
Mean II/PMC						15·91	15·81	15·73	15·73	15·70	15·70	15·62	15·48	15·33	15·31	14·66	14·03	12·80
						±0·27	±0·67	±0·98	±0·73	±0·54	±0·70	±0·63	±0·75	±1·31	±1·02	±1·60	±1·30	±1·64
% II†						99·5	98·8	98·3	98·2	98·2	98·2	97·6	96·8	95·8	95·6	91·6	87·7	66·3
% PMC with multivalents						—	5·6	—	5·9	—	14·3	—	11·1	8·33	16·9	—	—	85·4

* Taxa represented by abbreviations adopted in Table 1

† $\% \text{ II} = \frac{\text{observed no. of II}}{\text{potential no. of II}} \times 100$

Chromosomes in somatic cells are not suitable for karyotype analysis and comparison. The absolute size of the chromosomes varies from 0·7 μm to 2·5 μm, and their small size gives a superficial appearance of uniformity in the karyotype. No clear distinctions could be drawn between the different chromosomes in the complement. Sharma & Bhattacharyya (1959) reported the presence of one to two secondary constrictions in the chromosomes of two of the *Globba* species they studied; but no such features have been observed in the Malayan species examined.

Somatic complements of $2n = 32$, 48 and 80 are shown in plates $4a_1 — d_2$.

Plate 4 (e_1, e_2) shows an endopolyploid cell with 160 chromosomes in the root tip of *G. marantina* ($2n = 80$); endoployploidy has not been observed in the other species. Inconstancy of chromosome numbers, such as was reported in *G. hookeri* Clarke and *G. racemosa* Smith by Sharma & Bhattacharyya (1959), has not been observed in Malayan *Globba*.

ii. *Meiotic analysis in taxa with* $2n = 32$.

(a) Chromosome association

Chromosome association in metaphase I is tabulated in Table 2. The taxa, represented by the code adopted in Table 1, are arranged in order of decreasing mean number of bivalents per pollen mother cell (PMC).

Chromosomes were associated predominantly in bivalents. Full association to form sixteen bivalents occurred in more than 60% of cells in nine of the twelve taxa examined. The mean number of bivalents exceeded 15·3 in all the taxa except *G. pendula* ssp. *pendula* (P2a, P2b) and *G. cernua* ssp. *cernua* (C2b). These showed 14·66 ± 1·60, 14·03 ± 1·30 and 12·80 ± 1·64 mean number of bivalents per PMC respectively. Expressed in terms of percentage of possible bivalent formations, the degree of chromosome association was evidently high, the value exceeding 90% for ten of the twelve taxa examined.

Plate $2(a_1–b_2)$ shows metaphase I plate with 16 bivalents, and 11 bivalents plus 10 univalents, respectively.

Univalents were of generally low occurrence in all taxa except *G. pendula* ssp. *pendula* (P2a) and *G. cernua* ssp. *cernua* (P2b). The number varied from 0 to 4 in most cases.

Multivalents occurred in seven of the thirteen taxa investigated. They were observed in 85·4% PMC in *G. pendula* ssp. *pendula* (P2b), but only in 5·6% to 16·9% PMC in the other taxa. Quadrivalents were of more common occurrence than the other multivalents. Plate $5(c_1, c_2)$ shows metaphase I with a hexavalent.

(b) Chromosome segregation

Segregation was mainly disjunctional (92·3% PMC and above) in the first eight taxa in Table 3. These show a high degree of bivalent association. The remaining four taxa showed a higher frequency of non-disjunction. *Globba pendula* ssp. *pendula* var. *elegans* (Pe) showed 20·8%, *G. pendula* ssp. *pendula* var. *pendula* (P2a) 58·5% and (P2b) 31·7% respectively and *G. cernua* ssp. *cernua* (C2b) 45·6% non-disjunction. These values do not exhibit a definite correlation with the decreasing degree of bivalent association. The high incidence of non-disjunctional segregation may be partly accountable by the occurrence of multivalents and univalents in these taxa. Non-disjunctional segregation usually involved only a single pair of homologous chromosomes.

(c) Meiotic irregularities and pollen fertility

Strong correlations have generally been observed in these taxa between degree of bivalent association, meiotic irregularities and pollen viability.

Noncongression in the first and second metaphases, laggards in the first anaphase, micronuclei and polyspory showed a general increase in frequency of occurrence with decreasing bivalent formation.

Pollen viability, as estimated both by stainability and germination, also decreased with decreasing bivalent formation. This was as expected, as meiotic irregularities also increased with decreased bivalent association.

In cases with roughly equivalent levels of bivalent formation, presence of multivalents was observed to increase meiotic irregularities and pollen inviability of the taxon.

Seed-set probably occurs, even if only rarely, in all the taxa with $2n = 32$; as estimated from field observations, herbarium sheets and previous records of Holttum (1950) and Ridley (1924, 1925), it showed a general correlation with normal meiotic behaviour and high pollen viability.

Detailed information on the above subject is given in Lim (1969).

iii. *Meiotic analysis in taxa with* $2n = 48$

(a) Chromosome association

Chromosomes were observed to associate predominantly as trivalents. The analysis is recorded in Table 3. Taxa are arranged in order of descending degree of association as trivalents.

The maximum number of 16 trivalents occurred in five taxa, with frequency of 25·8% in *G. holttumii* ssp. *holttumii* (H), 8% in *G. variabilis* ssp. *variabilis* (V), 3·8% in *G. cernua* ssp. *crocea* (Rc3), 6% in *G. curtisii* (C) and *G. patens* var. *patens* (A3). The range of trivalent number in the individual taxa varied from 11–16 to 3–13. The lowest number of trivalents was not less than 8 in a majority of the taxa.

The mean values of trivalents, as recorded in Table 3, exceeds 8 in all cases. Plate $6(a_1–c_2)$ show metaphase I plates with 15 trivalents, 1 bivalent, 1 univalent; 14 trivalents, 2 bivalents, 2 univalents and 13 trivalents, 2 bivalents, 5 univalents respectively.

In seven taxa the total number of chromosome associations whether as bivalents, trivalents or multivalents, were found to exceed 16, with a frequency varying from 1·5% in *G. holttumii* ssp. *holttumii* (H) to 22·5% in *G. pendula* ssp. *pendula* (P3). These cells are grouped under (b) in Table 3. Such associations were indicative of chromosome pairing within the functional genome.

Chromosome associations involving multivalents are grouped under (c) in Table 3. The mean values of multivalents are low.

(b) Chromosome segregation

Chromosome segregation varied from 24: 24 to 17: 31. The majority were between 24: 24 and 20: 28. If segregation were at random, chromosome numbers at the poles should correspond approximately to a binomial expansion of $(\frac{1}{2} + \frac{1}{2})^n$ where n is 16, the functional basic number. Chi-squared tests have shown that segregation was random in all the taxa except *G. holttumii* ssp. *holttumii* (H), *G. curtisii* (C) and *G. patens* var. *patens* (A3). Both C and H were near random, but not A3.

TABLE 3

Chromosome association at first meiotic metaphase of taxa with 2n = 48

	Chromosome association						Number of PMC in taxa*									
	I	II	III	IV	V	VI	H	V	Rc3	C	R3a	A3	Ha	R3b	P3	R3d
(a)	—	—	16				17	8	3	3		4				
	1	1	15				13	25	14	7		1		1		
	3	—	15				3	1	3	1		1	1	—		
	2	2	14				12	22	10	12	6	3	1	1		
	4	1	14				6	—	3	—	—	1	3	—		
	6	—	14				1	—	1	—	—	—	—	—		
	3	3	13				4	21	12	7	10	5	2	3	3	1
	5	2	13				2	1	2	—	—	2	3	1	—	—
	7	1	13				—	—	—	—	—	—	1	—	—	—
	9	—	13				—	—	1	—	—	—	—	1	—	—
	4	4	12				1	10	12	4	8	10	4	2	7	1
	6	3	12				3	—	1	—	—	1	—	4	—	—
	8	2	12				—	—	—	—	5	—	3	1	—	—
	10	1	12				—	—	—	—	—	1	—	—	—	—
	12	—	12				—	—	—	—	—	—	—	—	—	—
	5	5	11				1	4	5	2	1	10	1	6	8	1
	7	4	11				—	1	—	1	1	1	1	3	1	2
	13	1	11				—	—	—	—	—	1	1	—	—	—
	15	—	11				—	—	—	—	—	1	—	—	—	—
	6	6	10				—	—	2	1	1	5	2	2	11	7
	8	5	10				—	—	—	1	5	4	—	3	1	2
	10	4	10				—	—	—	—	—	—	3	2	—	—
	7	7	9				—	—	1	1	—	4	6	2	12	5
	9	6	9				—	—	—	—	1	—	2	4	1	2
	11	5	9				—	—	1	—	—	1	1	3	—	1
	8	8	8				—	—	1	—	2	—	2	2	12	12
	10	7	8				—	—	—	—	—	—	—	—	—	6
	12	6	8				—	—	—	—	1	—	4	1	1	2
	14	5	8				—	—	—	—	—	2		2	—	3
	9	9	7				—	—	—	—	1			—	4	3
	11	8	7				—	—	—	—				—	—	3
	10	10	6				—	—	—	—				—	1	1

(b)	—	3	14				—	1	3	—					—	—	—
	1	4	13				—	1	1	1					—	—	—
	2	5	12				—	—	2	1					—	1	—
	3	6	11				—	—	—	2					1	1	—
	2	8	10				—	—	—	—					1	—	—
	4	7	10				—	—	—	—					1	—	—
	5	8	9				1	—	1	—					2	6	3
	3	9	9					—		—					1	3	—
	4	10	8					—		1					1	3	—
	6	9	8					—		—					—	1	—
	7	10	7					—		—					—	—	2
	6	12	6					—		—					—	1	
	7	13	5					—		—					—	1	
	8	14	4					—		—					—	1	
(c)	2	—	14	1				—		1					—		
	1	—	13	2				1		—					—		
	3	1	13	1				—		1					—		
	2	3	12	1				2		—					—		
	3	4	11	1				—		1					—		
	4	5	10	1				—		1					—		
	6	4	10	1				—		—					1		
	2	7	8	2				—		1‡‡					—		
	15	4	7	1				—							1		
	4	11	6	1				—							1‡‡		
	2	2	11	1	1			1									
	2	2	12	—	—	1		1									
PMC Number							66	100	79	50	42	57	41	54	80	57
Mean III/PMC							14·48	13·74	13·15	12·96	11·71	11·71	10·85	10·11	9·35	8·65
							±1·29	±1·35	±1·73	±1·97	±1·82	±1·99	±2·08	±2·13	±1·78	±1·34
% III†							90·5	85·8	82·2	81	73·2	73·2	67·8	63·2	58·4	54·1
% PMC in (b)							1·5	2·0	8·9	12·0	0	0	0	13·0	22·5	8·8
% PMC with IV, V and VI							0	5	0	10	0	0	0	5·6	0	0

* Taxa represented by abbreviations adopted in Table 1

† $\%\ \text{III} = \dfrac{\text{observed no. of III}}{\text{potential no. of III}} \times 100$

‡‡ Associations that also show intragenomic association

In H, C and A3, equal disjunction of 24: 24 was usually in excess of expectation, and nondisjunctional 22: 26, 21: 27 and 20: 28 lower in frequency than expected.

Triads were observed in division II. These were formed as a result of an additional plane of cytokinesis at the end of division I and were closely related to complement fractionation and secondary association of chromosomes.

(c) Meiotic irregularities and pollen fertility

In all the taxa investigated with $2n = 48$, meiosis was found to be irregular and pollen viability greatly reduced relative to those of taxa with $2n = 32$. The degree of meiotic irregularity and pollen sterility showed a general correlation to the degree of trivalent formation in metaphase I. Usually, the relatively fertile taxa showed a high degree of trivalent association in division I whilst the least fertile showed the lowest degree of trivalent association.

Meiotic irregularities common to all the taxa were: noncongression of chromosomes in the first and second metaphases, laggards in the first anaphase, nondisjunctional segregation, micronuclei and polyspory in tetrads. Other abnormalities of less common occurrence are the formation of triads in the second division and marked chromosome subgrouping.

Noncongression of chromosomes onto the equatorial plate occurred in more than 77% of the first metaphases in all the taxa except *G. holttumii* ssp. *holttumii* (H, 60·6%) and *G. cernua* ssp. *cernua* (R3a, 27·8%). The chromosomes involved were mainly univalents.

The frequency of occurrence of laggards varied from 60% of cells in *G. variabilis* ssp. *variabilis* to 100% in *G. pendula* ssp. *pendula*; they occurred less frequently in *G. cernua* ssp. *cernua* (17·6% in R3a, 37·5% in R3d) The mean number of chromosomes involved varied from $1 \cdot 2 \pm 0 \cdot 4$ to $5 \cdot 44 \pm 3 \cdot 89$ per cell, but as many as eight laggards were seen frequently in the first meiotic anaphases of P3 where micronuclei were common in the subsequent interphase and second prophase.

The formation of triads instead of dyads in the second division occurred with the frequency of 27·3% in *G. cernua* ssp. *crocea* (Rc3), 14·1% in *G. curtisii* (C), 9·1% in *G. patens* var. *patens*, 5% and 2·1% in R3b and R3d of *G. cernua* ssp. *cernua* (see plate 7b). This contributed to the unbalanced numbers in the resultant spores. One interesting instance of a segregation of 16: 16: 16 was observed.

Micronuclei and one to six supernumerary microspores have been recorded in tetrads. The combined frequency of occurrence of both micronuclei and polyspory generally showed a parallel to the frequencies of meiotic irregularities in the preceeding stages, particularly in metaphase II.

Pollen fertility in these ten triploid taxa varied between 10 and 50% (estimated on stainability) and is evidently related to meiotic irregularities: as might be expected it decreases with the increase in such irregularities.

Seed-set has not been observed in the field nor in herbarium specimens except in *G. variabilis*; 9·8% of the 121 herbarium sheets of this species in the Singapore Botanic Garden Herbarium had seeds. *G. variabilis* Ridl. s.s. has so far been found to be solely $2n = 48$. Live specimens for cytological investigation were from the Gombak Forest Reserve, Genting Simpah and Sungei Lallang Forest Reserve (Selangor), Fraser's Hill (Pahang) and Bukit

Timah Forest Reserve (Singapore). The fruit-bearing herbarium specimens were from Temerloh, Jerantut and Kuala Tahan (Pahang), Gunong Panti and Kuala Semberang (Johore) and Kinta (Perak). These might be chromosomal races with 2n = 32; or, considering the relatively high fertility of the species, it appears possible that plants with 2n = 48 could be fruit-bearing.

(d) Chromosome subgrouping

Subgrouping of chromosomes has been observed at various meiotic stages in some of the taxa. It is strongly akin to the phenomenon observed in *Rubus* cultivars and termed "complement fractionation" by Thompson (1962), wherein "the chromosome complement is subdivided into independent operating groups within a cell". In *Globba* however, the chromosomal groups do not always operate independently.

Chromosome subgrouping has been observed in the following:

G. patens var. *patens* (in both 2n = 32 and 2n = 48),
G. patens var. *costulata* (2n = 32),
G. pendula var. *elegans* (2n = 32),
G. cernua ssp. *cernua* (only in 2n = 48),
G. curtisii (2n = 48) and
G. variabilis ssp. *variabilis* (2n = 48).

At metaphase I the chromosomes most frequently associated in groups of eight as subgroups of eight bivalents in the 2n = 32 taxa and eight trivalents in the 2n = 48 taxa. The commonest situation observed in the 2n = 32 taxa was two subgroups of eight bivalents, whilst in the 2n = 48 taxa a subgroup of eight and another of sixteen trivalents was commonest. Considerable variation both in the number of chromosomes involved in the subgroups and in the number of subgroups themselves did, however, occur.

Plate 7a shows a PMC exhibiting subgrouping at metaphase I; whilst 7b, c and d show in the later stages of meiosis the types of figures associated with the formation of subgroupings.

Chromosome subgrouping has also been observed in root tip cells of *G. marantina* (2n = 80).

Detailed observations of the phenomenon of chromosomal subgrouping in *Globba* will be presented in a future paper.

Discussion

Nature of the 2n = 32 and 2n = 48 taxa

The high degree of bivalent association and the low incidence of univalents and multivalents in the thirteen Malayan taxa investigated with 2n = 32 indicate that they are functional diploids.

Similarly the noticeably high degree of trivalent association in the species examined with 2n = 48 suggests that these taxa are triploids; a conclusion supported by the high frequency of meiotic irregularities and the low pollen viability.

The degree of trivalent formation generally gives a good indication of the extent of homology in the three genomes of a triploid: a high degree of trivalent formation is usually a manifestation of autotriploidy, whilst allotriploids are characterised by asynapsis and paucity of trivalents, and segmental allotriploids show a pattern of synapsis intermediate between these two extremes. The situation can of course be greatly modified by

variations in chiasma frequency; for instance, occurrence of a low chiasma frequency may result in an autotriploid with three completely homologous genomes producing only bivalents and univalents, thus appearing exactly like an allotriploid.

The degree of trivalent association in triploid *Globba* varied from 54·1% to 90·5% (see Table 3). Taxa higher up in the range are probably mostly autotriploids, e.g. *G. holttumii* ssp. *holttumii* (H), *G. variabilis* ssp. *variabilis* (V), *G. cernua* ssp. *crocea* (Rc 3) and *G. patens* var. *patens* (A3) where the maximum association of 16 trivalents frequently occurred; this conclusion is strongly supported by the close morphological resemblance of Rc3 and A3 to their diploid chromosome races R2A and A2c. On the other hand, the relatively low degree of trivalent association in *G. holttumii* ssp. *aurea* (Ha, 67·8%), *G. cernua* ssp. *cernua* (R3b, 63·2%; R3d, 54·1%) and *G. pendula* ssp. *pendula* (P3, 58·4%) appears indicative of lesser homology between the genomes present, possibly as a result of hybrid origin.

Basic Number

Three different basic numbers (x = 11, 12 & 16) have been proposed for the genus *Globba* by previous workers; the chromosome numbers of the seven species which they determined are listed in Table 4.

On the basis of the somatic chromosome number of 48 of a single species (*G. bulbifera* Roxb.), Raghavan & Venkatasubban (1943) proposed that the basic number of the genus was 12. Sharma & Bhattacharyya (1959) reported 2n = 44 for the same species; and, on the basis of chromosome counts and karyotypes of *G. hookeri* Clarke (2n = 22) and *G. racemosa* Smith (2n = 24), they suggested an alternative basic number of 11 or 12 for the genus. Mahanty (1963) inferred that *x* equals 12 from observations of ten metaphase 1 plates of *G. atrosanguinea* Teysm. & Binn. (2n = 48); the inference hinged

TABLE 4

Somatic chromosome numbers of seven species of *Globba* determined by previous workers.

	Species	Locality	Author	2n
1	*G. bulbifera* Roxb.	India and Malaya	Raghavan & Venkatasubban (1943)	48
	G. bulbifera Roxb.		Chakravorti (1948)	48
	G. bulbifera Roxb.		Sharma & Bhattacharyya (1959)	44
2	*G. hookeri* Clarke	Reg. Himalayas	Sharma & Bhattacharyya (1959)	22
3	*G. racemosa* Smith	Reg. Himalayas	Sharma & Bhattacharyya 1959)	24
4	*G. atrosanguinea* Teysm. & Binn.	Borneo	Mahanty (1963)	48
5	*G. winitii* C. H. Wright	Thailand	Mahanty (1965)	32
6	*G. albiflora* var. *aurea* (Ridl.) Holtt.	Malaya	Mahanty (1965)	32
7	*G. heterobractea* K. Schum.	Philippines	Mahanty (1965)	64

mainly on the strong correlation between trivalent and univalent number in seven of the ten cells (cf. Table 1, p. 54 & fig. 38, p. 74: Mahanty 1963). A similar correlation has not been observed in any of the ten Malayan taxa investigated. Furthermore, the relatively large number of trivalents (3 to 10 per cell, mean = 5·7) and the low occurrence of quadrivalents (0 to 4 per cell, mean = 1·6) pointed to the unlikeliness of *G. atrosanguinea* being a tetraploid. On further analysis of meiosis of *G. winitii* C. H. Wright (2n = 32), *G. albiflora* var. *aurea* (2n = 32) and *G. heterobractea* K. Schum. (2n = 64), Mahanty (1965) viewed 12 as the original basic number and 16 as a secondary basic number of the genus. Chakravorti (1948, 1952) studied the meiotic division of *G. bulbifera* Roxb., 2n = 48, and concluded that it was an autotriploid.

The present investigation has shown that Malayan *Globba* taxa with 2n = 32 behave meiotically as diploids and those with 2n = 48 as triploids, suggesting at first glance that the basic number is 16. However, the occurrence in the 2n = 32 taxa of multivalents (predominantly quadrivalents) and of triads in the second division indicate that these taxa may themselves be polyploid. Evidence from chromosome subgroupings at meiosis in both 2n = 32 and 48 taxa (see page 274–5) also supports this view. Similar chromosome subgroupings have been reported in high polyploid *Rubus* cultivars (Thompson, 1962), hybrids of *Triticum timopheevi x Aegilops bicornis* (Li & Tu, 1947), in *Ribes* and *Rubus* (Vaarama 1949, 1953) and in *Lycopersicon esculentum* (Gottschalk 1958, a, b, 1959). In all these previously recorded examples chromosomes aggregated predominantly in numbers that corresponded to the basic number of the respective genera. The two subgroups of seven bivalents observed in 60% metaphase I plates of the allotetraploid hybrid *Rubus procerus x R. laciniatus* have been interpreted as belonging to different genomes (Bammi, 1965). This suggests that in Malayan *Globba* the prevailing occurrence of subgroups of eight bivalents in 2n = 32 taxa, and eight trivalents in 2n = 48 taxa, indicates an original basic number of x = 8. The fact that subgroupings occur probably means that the putative x = 8 genomes involved are different and therefore that allopolyploidy is involved; the 2n = 32 plants which functioned meiotically as diploids would thus be allotetraploids with a genomic constitution AABB, and taxa with 2n = 48 would be hexaploids (AAABBB).

The proposed basic number of 8 seems to fit well into the somatic numbers of 24, 32, 48, 64 and 80 so far counted in the genus, but not the counts of 2n = 22 for *G. hookeri* and 2n = 44 for *G. bulbifera* made by Sharma & Bhattacharyya (1959). The above hypothesis, if true, would imply that *G. racemosa* with 2n = 24 (Sharma & Bhattacharyya, 1959) must be a true triploid, and *G. marantina* (2n = 80) a decaploid—this high degree of polyploidy perhaps accounts for the complete sterility of the latter species.

Based on the chromosome numbers of 48 species in 11 genera of Zingiberacea, Sato (1960) showed that a wide range of basic numbers occurs in the family, those proposed being 8, 9, 11, 12, 13 and 17. It is interesting to note that x = 8, which the writer proposes as the basic number of Malayan *Globba*, corresponds to the lowest number given by Sato. Unfortunately, none of the other genera in the tribe Globbeae (*Hemiorchis*, *Gagnepainia* and *Mantisia*) have yet been investigated cytologically; so no information bearing on the basic number of *Globba* is available from this source.

Cytological Evolution in the Genus Globba in Malaya.

If we assume, as seems fairly likely for the reason already given, that the basic number in Malayan *Globba* is 8 and the 2n = 32 species represent allotetraploids, then we can state that two extremely important evolutionary trends have been (1), the establishment of allotetraploidy in the first place and (2), the derivation of hexaploids by triploidization of allotetraploids. All cytologically investigated *Globba* populations from Malaya have in fact been polyploid and it seems probable that diploids do not occur in the country—nor in fact have such 2n = 16 taxa been recorded from any part of the range of the genus.

The adaptive value of the permanent hybrid condition of allotetraploids is well known and this, coupled with a combination of both sexual and asexual reproduction, is probably an important factor that has contributed to the evolutionary success of allotetraploid *Globba*.

Hexaploid forms occur in six of the twelve *Globba* species in Malaya, and are geographically more limited in distribution than the tetraploids; they are generally of triploid cytological behaviour and are largely maintained by vegetative reproduction by means of bulbils and vegetative spread. However, although seeds have not been observed, the possibility of sexual reproduction is suggested by morphological evidence of introgressive hybridization of *G. patens* var. *patens* into *G. cernua* ssp. *cernua* (to be dealt with in detail in another paper), the relatively high pollen fertility of some hexaploid taxa, and interclonal variability, particularly in hexaploid *G. cernua* ssp. *cernua*.

The decaploid *G. marantina* (2n = 80), on the other hand, has sacrificed the advantages of sexual reproduction by an almost complete failure of flowering. Its widespread geographic distribution is explained by the remarkable ability of its bulbils and rhizomes to survive adverse conditions and its ability to colonise open habitats where there is relatively little competition.

The absence of higher polyploids other than the decaploid *G. marantina* in Malaya, indicates that hexaploidy may represent the highest level consistent with the selective advantage of chromosome duplication whilst avoiding the excessive imbalance of higher ploidy levels.

Inter-relationship of Malayan Globba species

On the basis of cytology, morphology and distribution, a scheme of inter-relationships among the Malayan species of *Globba* is proposed and presented in figure 1. The two anther appendage species and the four anther appendage species form two distinctly different natural groups. The affinity between *G. pendula* Roxb., *G. leucantha* Miq. and *G. fasciata* Ridl. (see Notes R.B.G. Edinb. 31: 265, 1972) has been suggested by Holttum (1950). *G. albiflora* Ridl. represents an independent line of its own—its uniqueness lies in its distinct pollen grain morphology and anther appendage attachment. Its limited distribution to the north of the Malay Peninsula indicates that it might be related to species in Thailand and the north.

Of the species in the four anther-appendage group, *G. patens*, *G. variabilis* and *G. fragilis* are apparently closely related (see Notes R.B.G. Edinb. 31: 259, 1972). The affinity between *G. patens* and *G. variabilis* has been suggested by Holttum (1950). *Globba holttumii* and *G. cernua* would seem related to these (see *l.c.*: 250, 1972). A close genetical relationship between *G. patens*

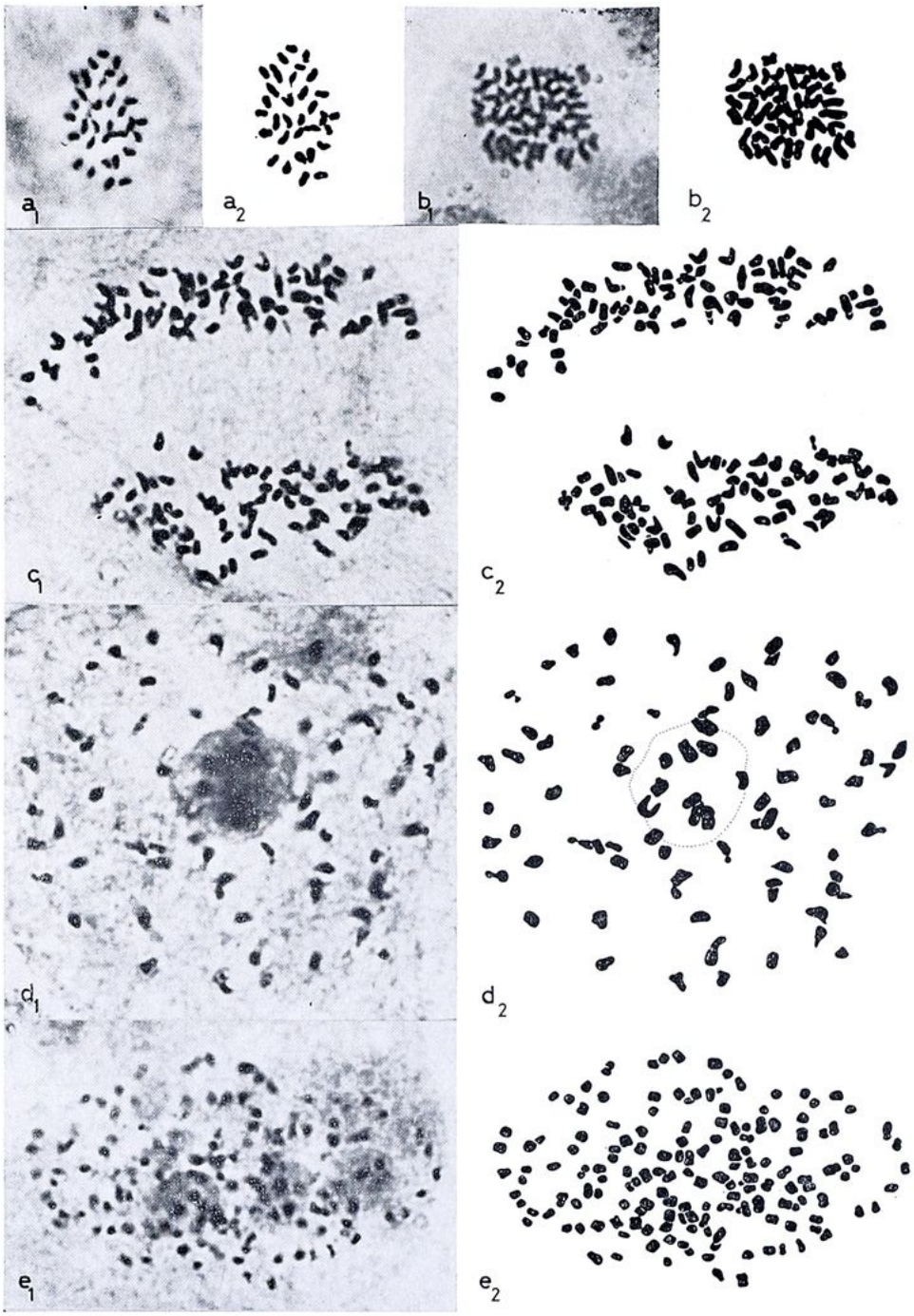

PLATE 4. Somatic chromosomes of some *Globba* species, x 3000: a_1, a_2, c-metaphase in a root cell of *G. pendula* with 2n = 32; b_1, b_2, c-metaphase in a root cell of *G. cernua* ssp. *crocea* with 2n = 48; c_1, c_2, anaphase in a root cell of *G. marantina* with 2n = 80; d_1, d_2, late prophase in a root cell of *G. marantina* with 2n = 80; e_1, e_2, endopolyploid cell in root tip of *G. marantina* showing 160 chromosomes.

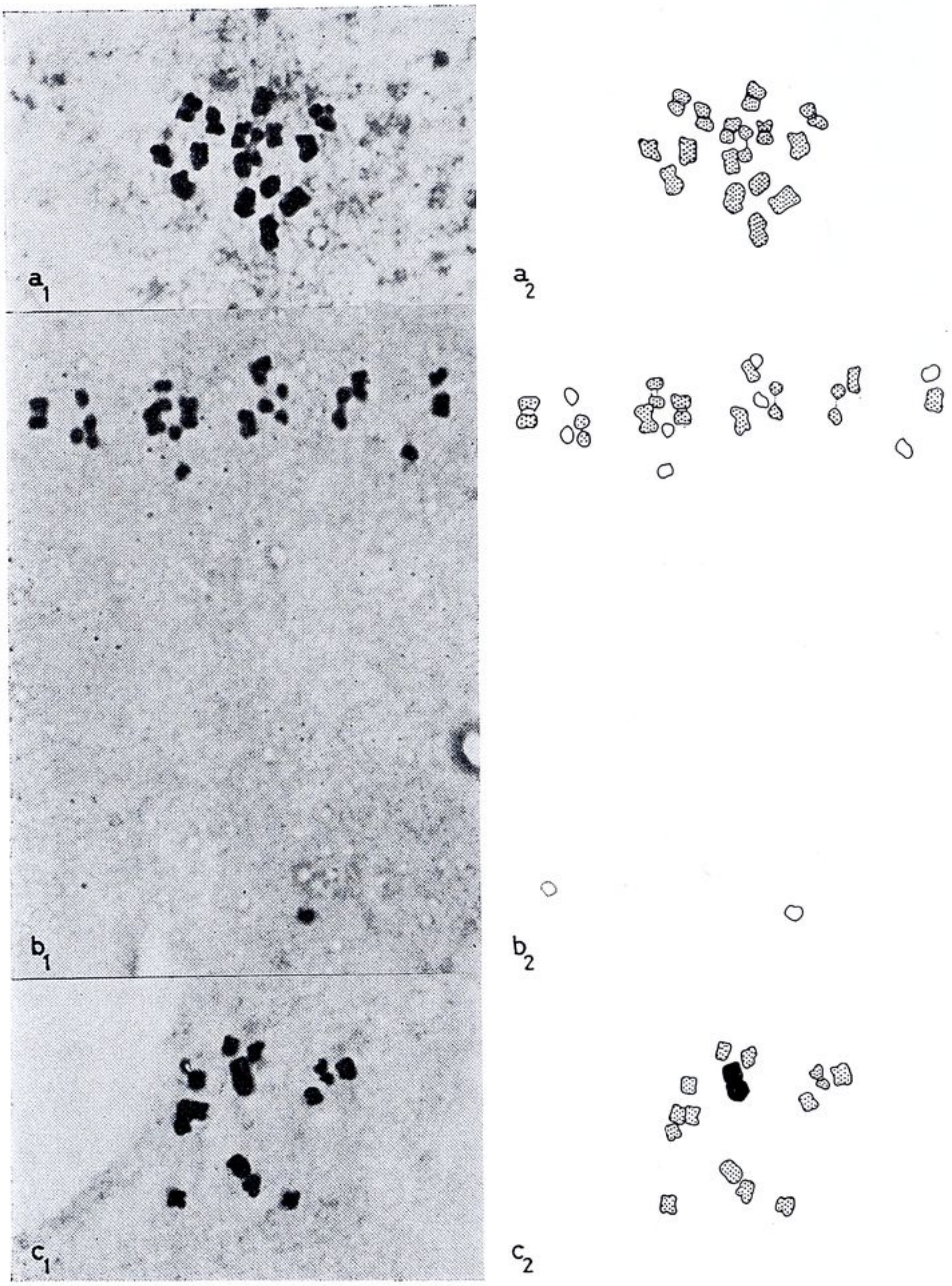

PLATE 5. Metaphase I in pollen mother cells of *Globba* species with 2n = 32, x 2500: a_1 a_2, *G. ceruna* ssp. *porphyria*, 16 II; b_1, b_2, *G. pendula* (P2a), 11 II 10 I; c_1, c_2, *G. pendula*, (P2b), 13 II 1 VI; hexavalent solid and bivalents dotted in drawing c_2.

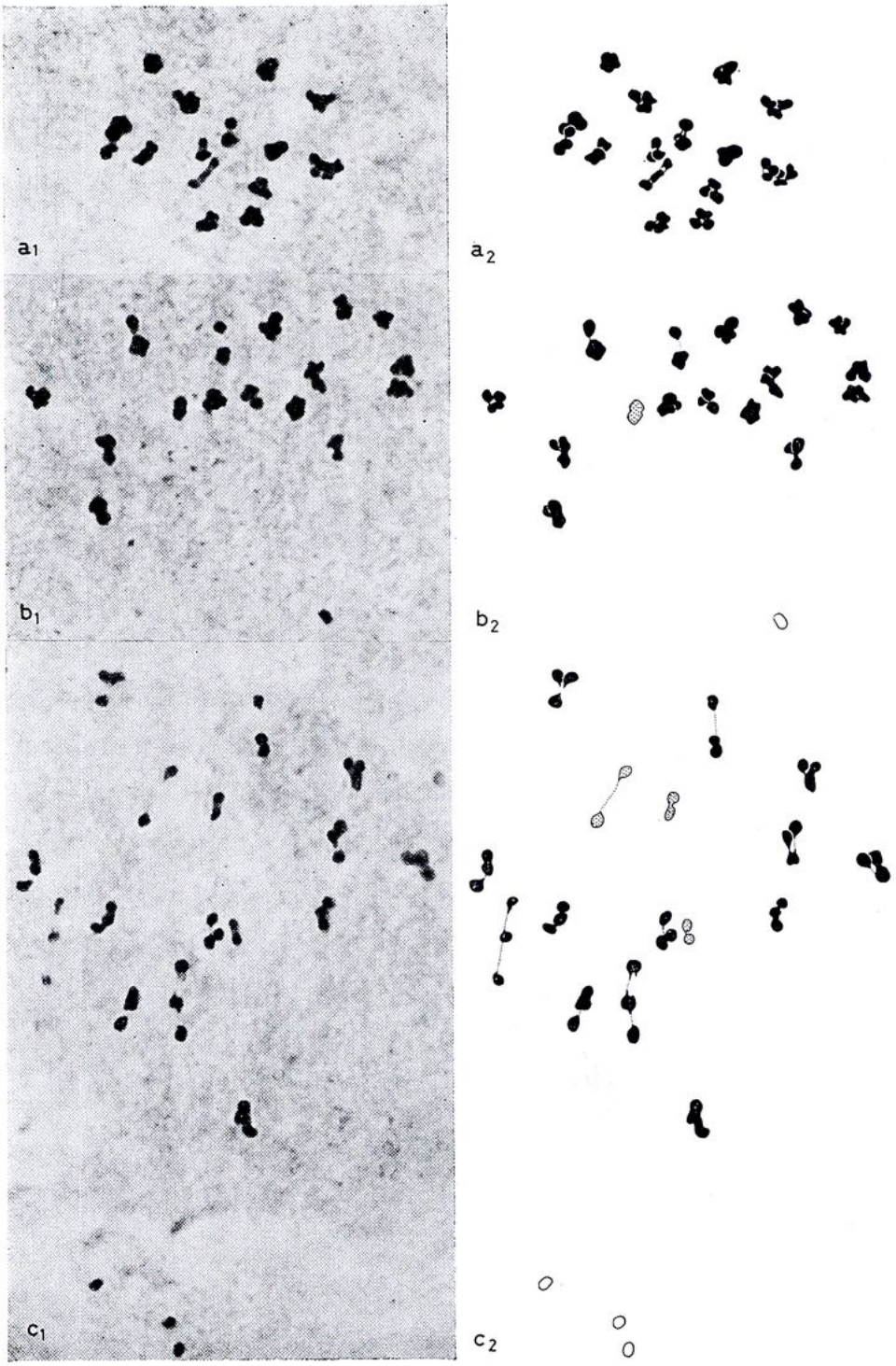

PLATE 6. Metaphase I in pollen mother cells of *Globba* species with 2n = 48, x 2500: a_1, a_2, *G. curtisii*, 16 III; b_1, b_2, *G. holttumii*, 15 III 1 II 1 I—single noncongressing univalent lower right; c_1, c_2, *G. cernua* ssp. *crocea*, 13 III 3 II 3 I—three noncongressing univalents lower left.

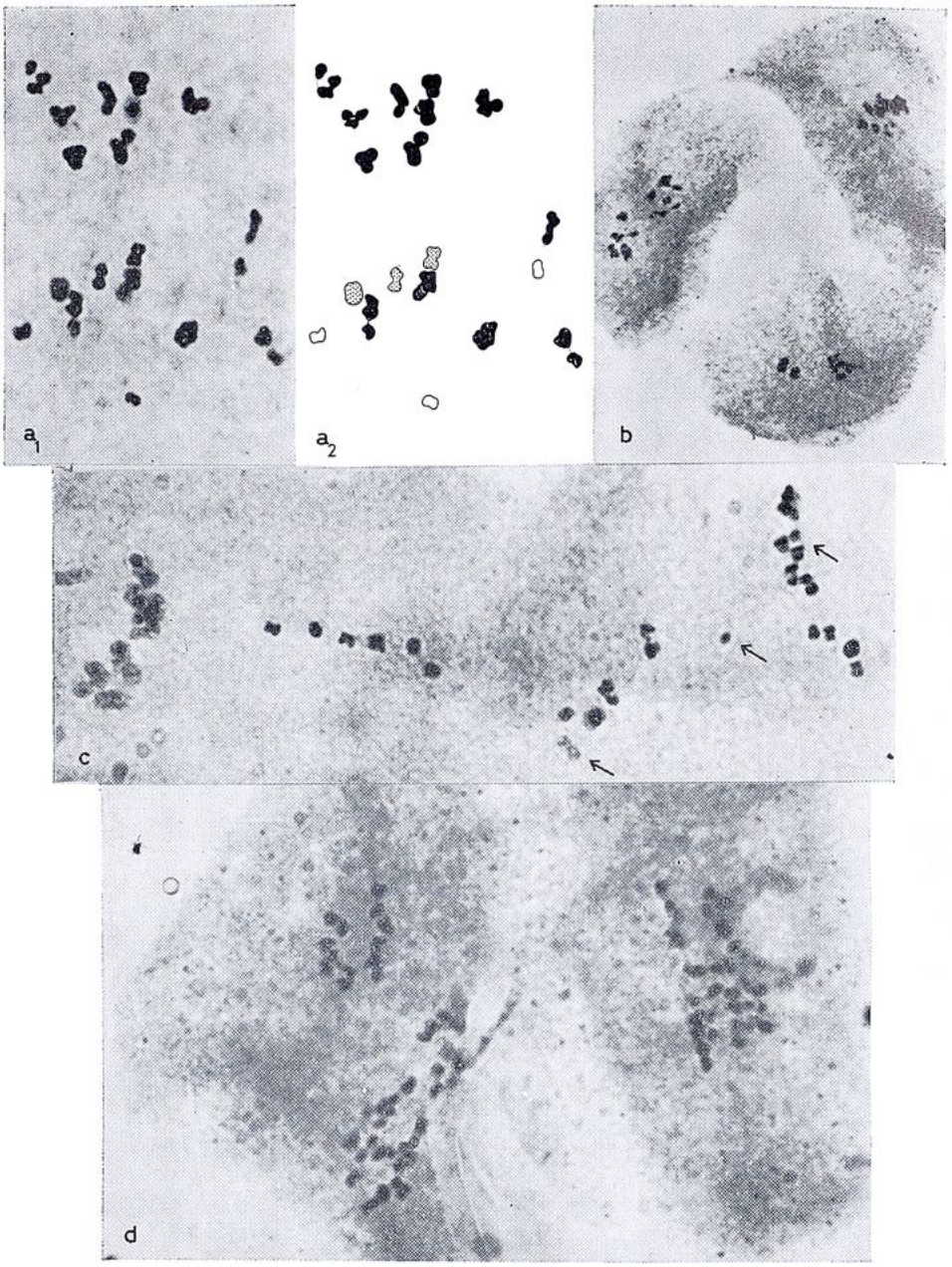

PLATE 7. Complement fractionation and triad formation in *Globba* taxa with 2n = 48: a_1, a_2, metaphase I in *G. cernua* ssp. *crocea* with chromosomes in two subgroups of 8 III and 5 III 3 II 3 I, x 2500; b, triad in metaphase II of *G. curtisii* showing chromosomal subgrouping in all three components, x 1200; c, metaphase II in *G. cernua* (R3d) showing chromosomes on multiple differently orientated equatorial plates—precociously divided chromatids arrowed, x 2500; d, anaphase II in *G. cernua* (R3d) with chromosomes dividing on independant multiple spindles, x 2500.

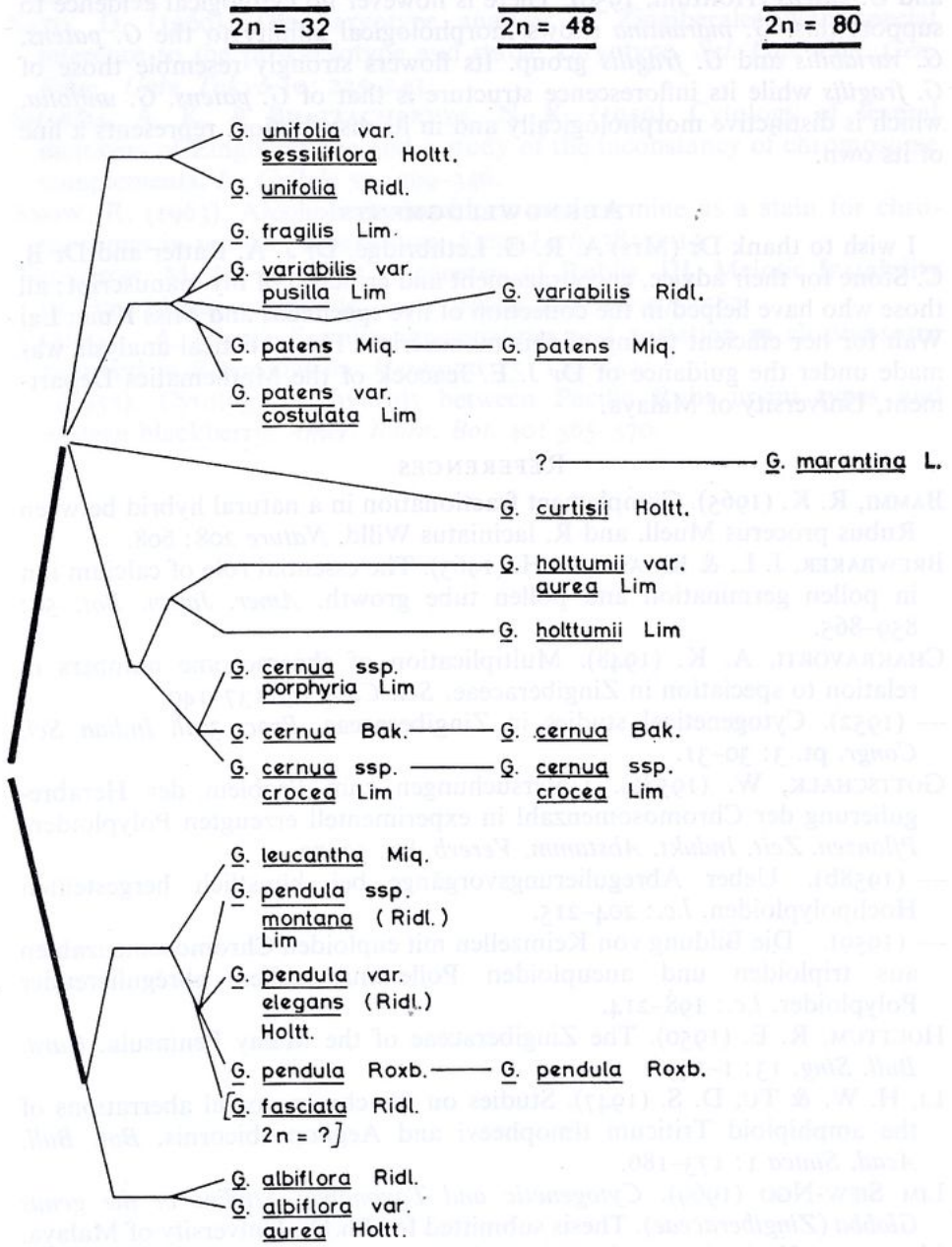

FIG. 1. Schematic representation of a suggested scheme of interspecific relationships in the Malayan species of *Globba*.

and *G. cernua* is indicated by the case of natural hybridization found between the two species. Morphologically intermediate between the two groups is *G. curtisii*, which has been suggested as a possible hybrid between *G. cernua* and *G. patens* (Holttum, 1950). There is however no cytological evidence to support this. *G. marantina* shows morphological affinity to the *G. patens*, *G. variabilis* and *G. fragilis* group. Its flowers strongly resemble those of *G. fragilis* while its inflorescence structure is that of *G. patens*. *G. unifolia*, which is distinctive morphologically and in its distribution, represents a line of its own.

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References

Bammi, R. K. (1965). Complement fractionation in a natural hybrid between Rubus procerus Muell. and R. laciniatus Willd. *Nature* 208: 608.

Brewbaker, J. L. & Kwack, B. H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. *Amer. Journ. Bot.* 50: 859–865.

Chakravorti, A. K. (1948). Multiplication of chromosome numbers in relation to speciation in Zingiberaceae. *Sci. Cult.* 14: 137–140.

— (1952). Cytogenetical studies in Zingiberaceae. *Proc. 39th Indian Sci. Congr.* pt. 3: 30–31.

Gottschalk, W. (1958a). Untersuchungen zum Problem der Herabregulierung der Chromosomenzahl in experimentell erzeugten Polyploiden. *Pflanzen. Zeit. Indukt. Abstamm. Vererb.* 89: 52–79.

— (1958b). Ueber Abregulierungsvorgänge bei künstlich hergestellten Hochpolyploiden. *l.c.*: 204–215.

— (1959). Die Bildung von Keimzellen mit euploiden Chromosomenzahlen aus triploiden und aneuploiden Pollenmutterzellen abregulierender Polyploider. *l.c.*: 198–214.

Holttum, R. E. (1950). The Zingiberaceae of the Malay Peninsula. *Gard. Bull. Sing.* 13: 1–249.

Li, H. W. & Tu, D. S. (1947). Studies on the chromosomal aberrations of the amphiploid Triticum timopheevi and Aegilops bicornis. *Bot. Bull. Acad. Sinica* 1: 173–186.

Lim Siew-Ngo (1969). *Cytogenetic and Taxonomic Studies in the genus Globba (Zingiberaceae)*. Thesis submitted for Ph.D., University of Malaya.

Mahanty, H. (1963). *A karyological study of certain Zingiberaceae with reference to their taxonomy*. Thesis submitted for M.Sc., Univ. of London.

— (1965). *A cytological study of the Scitamineae with special reference to their taxonomy*. Thesis submitted for Ph.D., Univ. of London.

Raghavan, T. S. & Venkatasubban, K. R. (1943). Cytological study in the family Zingiberaceae with special reference to the chromosome number and cytotaxonomy. *Proc. Indian Acad. Sci.* B 17: 118–132.

RIDLEY, H. N. (1924). *The Flora of the Malay Peninsula* IV. Monocotyledons. London.

— (1925). *The Flora of the Malay Peninsula* V. Monocotyledons (concluded) London.

SATO, D. (1960). The karyotype analysis in Zingiberales with special reference to the prokaryotype and stable karyotype. *Sci. Pap. Coll. Gen. Educ., Univ. Tokyo* 10: 225–243.

SHARMA, A. K. & BHATTACHARYYA, N. K. (1959). Cytology of several members of Zingiberaceae and a study of the inconstancy of chromosome complements. *La Cellule* 59: 299–346.

SNOW, R. (1963). Alcoholic-hydrochloric acid-carmine as a stain for chromosomes in squash preparations. *Stain Tech.* 38: 9–13.

THOMPSON, M. M. (1962). Cytogenetics of Rubus. III. Meiotic Instability in some higher polyploids. *Amer. Journ. Bot.* 49: 575–582.

VAARAMA, A. (1949). Spindle abnormalities and variation in chromosome numbers in Ribes nigrum. *Hereditas* 35: 136–152.

— (1953). Cytology of hybrids between Pacific Rubi ursini types and eastern blackberry. *Amer. Journ. Bot.* 40: 565–570.