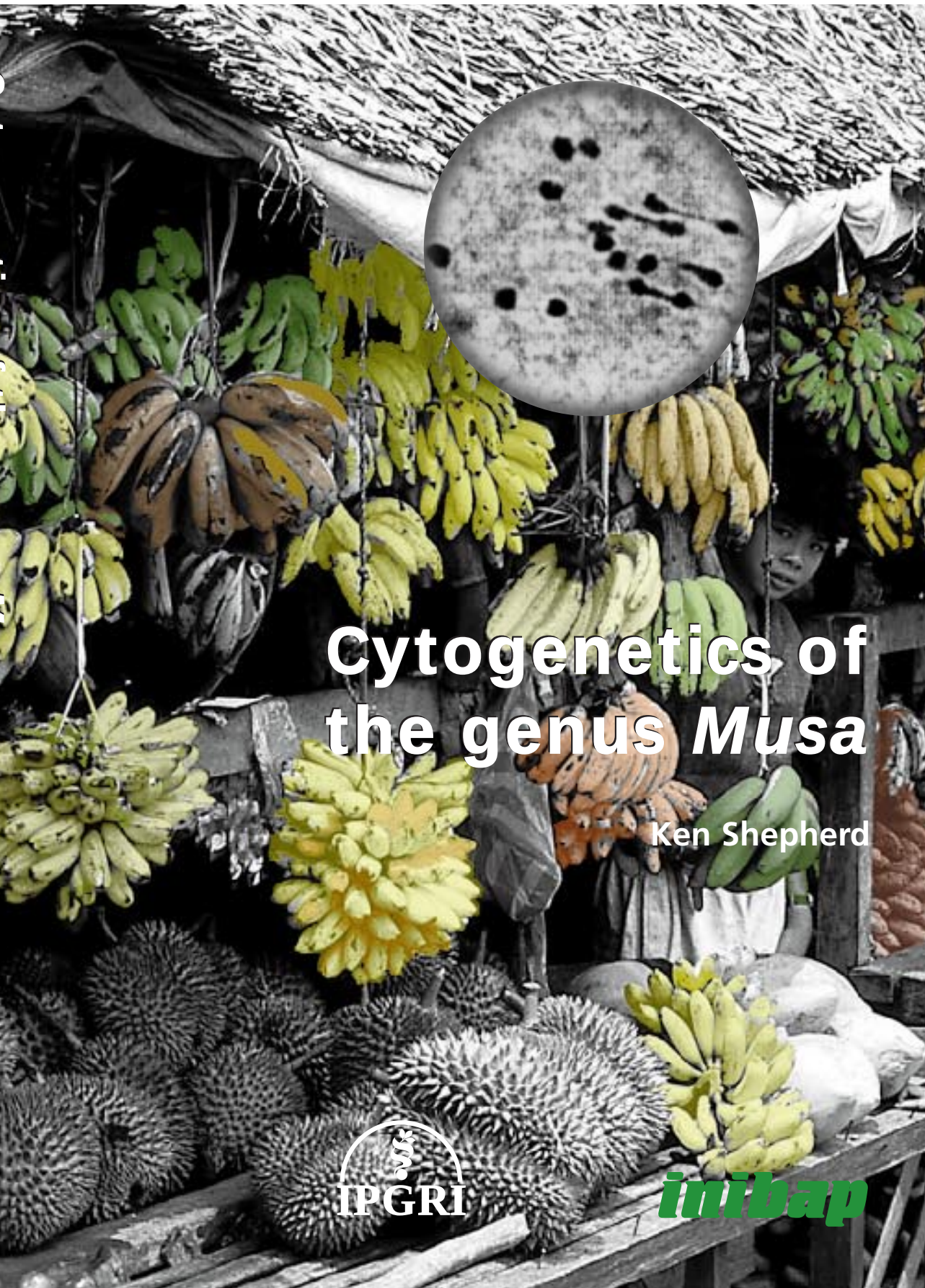

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Foreword

INIBAP is very pleased to present this book on "Cytogenetics of the genus *Musa*" to the *Musa* scientific community. It provides precious information resulting from a lifetime dedicated to banana research. Ken Shepherd has not only devoted his life to studying bananas, but has focused on an area much neglected by other researchers, *Musa* cytology. The publishing of this book is especially timely given the recent upsurge in interest in *Musa* karyology and the new methods that are now available for studying *Musa* chromosomes. We are therefore particularly honoured to be associated with Dr Shepherd's endeavour, and we publish this book with the knowledge that the information it contains will be of great value to *Musa* researchers worldwide for years to come.

INIBAP wishes to give special thanks to Claudine Picq for the technical editing of this publication.

Emile Frison
Director

Author's preface

On the first Sunday morning of February 1950, a 22 years old newcomer landed in Port-of-Spain, Trinidad, appropriately from a banana boat, to start an association with the crop and genus that continued there, in Jamaica and in Brazil, officially until September 1994. But it goes on, since he still has things to say, including information that should have been divulged very many years ago.

At the old Imperial College of Tropical Agriculture (ICTA) he had to follow in a distinguished line, of E.E. Cheesman, K.S. Dodds and N.W. Simmonds, under the last of whom he functioned as a junior for nine years. Among his varied activities, one was dictated by the purchase before his arrival of a new microscope, a Leitz Ortholux and very advanced for its time. He therefore became a serious cytologist, although he had previously dabbled with chromosome counts in *Solanum*.

Both meiotic and mitotic cytological studies enter strongly into this work, although genetic and other aspects of the genus are associated to give a fuller picture. Inevitably it must frequently cite Simmonds' 1962 book on "The Evolution of the Bananas", but the scope is now narrower than his. The concern is not with reproductive isolation under natural conditions but under experimental ones in germplasm collections, where different geographical ranges are not a factor. It is also unavoidable that he disagrees with Simmonds on some points, but especially those on which he has accumulated much additional information. It is the disclosure of the latter, whether on the same species treated in 1962 or on others whose behaviour was not then available, that this effort hopes to offer an important supplement.

Despite the best intentions, the book might never have been written at all were it not for an asset and a pleasure that came to the writer quite late in his professional life, for which he has to thank a young Brazilian colleague (female). This was the use of a computer even in a very elementary way for data storage and writing. It was in the data storage phase that he finally realised how much there was to write. It was the purchase of his very own PC that made it possible. He could no longer think of letting this data run the risk of dying with him. Even so, a part has gone adrift, in the course of his movements from country to country. Only where he has total trust in his memory can reference be made to these results.

By way of general acknowledgements, the author recalls gratefully that, for the first thirty years of his career with bananas, he was either substantially or at least partly supported financially by successive agencies of H.M. Government in London. In preparing this publication he also assumes that he will have the blessings of the Banana Board of Jamaica and of Brazil's Centro Nacional de Pesquisa de Mandioca e Fruticultura Tropical (CNPMF), a unit of the national research system of the country (EMBRAPA).

Ken Shepherd
Cascais, Portugal
January 1997

CHAPTER 1

Methodologies and interpretations

No claim is made that there is an ideal method for any purpose. The one adopted must be very often personal, on the principle that if it works why change it. For this reason, where there is reference here to protocols used by other workers, there is no attempt to be detailed or comprehensive. The aim is to make some general points as well as to outline the procedures that have been adopted by the writer in Trinidad, in Jamaica and in Brazil.

Assessment of pollen samples

In many cases, with experience, a rough estimate of fertility is possible merely on a mental comparison of anther contents of mature flowers with the expectation from a fully fertile form. For more precise study, *Musa* pollen grains are almost visible to the naked eye (haploids in the vicinity of 100-110 μ), but rather sticky. Samples are readily mounted in liquids however, between microscope slide and cover glass, and dispersed by tapping the top of the latter. Various stains are useful in giving a clearer image, these including acetocarmine, orcein in lactophenol and cotton blue in lactophenol.

Counts of good and bad grains can usually be made with some confidence at low microscope magnifications, but there are some materials where bad grains are identifiable only with difficulty or not at all, principally when breakdown occurs very soon after meiosis. Variations in the age of collapse of bad grains can obviously affect the correspondence of sampling results with anther content estimates, but the two evaluations either agree to an acceptable extent or complement one another. Measurements of grain sizes are readily made with the aid of an eyepiece micrometer.

Pollen nuclei are not normally visible without prior fixation, at least in more mature grains, because of the opaqueness of the outer wall.

Handling of pollen mother cells

The first decision concerns the type of material to be fixed. In some past studies this has apparently been by the fixing of whole male flower buds or only stamens from few or several clusters. Presumably the objectives were to guarantee that the material preserved should straddle the meiotic stages, and perhaps to permit a deferment of final preparation until a more convenient time. Dessauw (1987) achieved the latter by preservation in 70% ethanol. The first aim should be unnecessary and the second does

not receive the writer's approval, since it often denies an opportunity of repeating a sampling. In addition, the inclusion of anther wall tissue in the ensuing squashes reduces the degree of flattening of pollen mother cells and thereby casts doubt on their interpretation. Also not recommended is pre-treatment with oxyquinoline (Dessauw, *loc. cit.*), since the drug may destroy useful information on the alignment of meiotic figures.

In general it is better to work with expressed anther contents. Trinidad workers had experimented with various smear methods, but Dodds and Simmonds (1948) had adopted principally the joint fixation of the smeared contents of all five anthers of a bud in 1:3 acetic acid:ethanol, followed by staining with acetocarmine. No details are on record. This author has used such a method almost exclusively, the major amendment being the inclusion of iron in the fixative as a mordant rather than in the stain itself. Initially this was in the form of a few drops of a saturated solution of ferric chloride, but ferric acetate was later used as the low solubility of this compound in glacial acetic acid permitted better standardisation. A fuller account follows for its possible interest to others who might wish to attempt the technique.

Identification of meiotic stages

Cells with meiotic stages may be found at between 20 (rarely) and 35 or more bracts, counting from the outermost, depending on a rate of bract opening which varies between genotypes. However, it is easy with some practice to identify the critical stage by checking the anther contents from successive clusters, starting with buds of about 1.5 cm in length. The apex is nipped off with a thumb nail and the content of all five anthers is expressed, to show the following aspects consecutively with decreasing age:

(a) pollen grains already well-formed – an opaque and granular paste;
(b) very young grains – about three clusters with a content much more liquid;
(c) meiotic stages or pollen tetrads still within the pollen mother cell wall—a translucent, non-granular paste;
(d) contents not expressed or only with difficulty – pre-meiotic.

It may be mentioned that it is possible to identify meiotic material in a similar way in a diversity of plants, to the point of testing single anthers where these are relatively large, such as in certain species of Leguminosae and Commelinaceae for instance. Bananas have the additional great advantage of flower buds disposed in an age series in each cluster. The oldest are the flowers at the right side when the apex of the bud is uppermost.

The first node of type (c) is eliminated, as invariably too old, and the next three are exposed by careful removal of the subtending bracts. There follows the preparation of trial slides to locate meiotic stages between and within clusters. Tests are recommended of single flower buds from the left (younger) side of the outer cluster, from both sides of the next and from the right (older) side of the third. Where flower buds are few, as in some species hybrids particularly, one central bud from each cluster may be tested. Buds in such plants often have poor synchronisation of meiotic stages between pollen mother cells, so that the less precise mode of searching is further justified. The whole anther

content of each bud is expressed, placed on a individual slide and lightly spread with the edge of a cover glass. These smears are fixed for 15-30 minutes **only** in a mixture of 1 part glacial acetic acid (previously saturated with ferric acetate) with 3 parts of commercial alcohol (90-95% ethanol). Then, after partial drying with absorbent paper, the smears are stained in a drop of 1% carmine in 45% acetic acid for about five minutes. A cover glass is placed, the slide is warmed perhaps twice to well short of boiling point and the material is lightly pressed under the cover glass and through some thicknesses of absorbent paper. The pressure in trial slides should not be enough to separate interphase dyads or final tetrads. A magnification of about 250 times is enough to verify the stages present in each squash. In *Musa* generally, stages earlier than late prophase are not normally revealed by carmine and even diplotene and diakinesis are not usually interpretable. Most informative generally are the metaphase and anaphase stages of the first meiotic division.

If all the buds tested are too old, it will be clearly necessary to repeat the tests with younger flower buds, that is the younger end of the third cluster and the older end of the fourth, and so on until the course of the first division in the inflorescence has been defined. Sometimes, one cluster is too old before the next reaches first metaphase. In this case it is possible to wait some hours before making the final smears, meanwhile protecting the inflorescence from desiccation.

The definitive smears

The preparation of the final working slides follows the outline above, except that:

(a) fixation should be for a minimum of three hours and can be up to 20 (such as overnight), although it is more difficult to achieve well flattened cells with dispersed metaphases after the longer period;

(b) the pressure applied in squashing aims first to scatter the cells and ends with a firmer application to flatten them;

(c) the cover glass edges have to be sealed to avoid drying out of the smear; this can be done with a molten mixture of paraffin wax and vaseline or with nail varnish.

One modification tried in Trinidad was the substitution of propionic for acetic acid as a solvent for carmine but this was not profitable. The cells when stained became hardened and the more intense colouring of the chromosomes did not compensate for the darkening of the cytoplasm which also occurred.

Despite the overall success of the method there are yet potential problems. In the first place, intensity of staining can depend much on the source of the stain and on the manner of preparing the solution. The very definition of 1% is optimistic in relation to the solubility found and it is necessary to boil the solvent with the carmine for at least half an hour to achieve the desired result, and yet with filtration afterwards.

Even with the best preparation of the slides, precise assessment is only possible for a small proportion of pollen mother cells at first metaphase in diploids, in fewer triploid cells and in rare tetraploid ones. Frequently, well flattened cells are restricted to the periphery

of the smear. Some materials remain difficult, whether diploid or triploid, because the chromosomes adhere to one another or congregate very closely on the cell equator.

Interpretations of meiotic data

The following observations affect both intraspecific crosses, within *M. acuminata*, and interspecific ones, as related in subsequent chapters. In all cases the practice in this book has been to give most emphasis to the degree of chromosome pairing and to the occurrence and frequency of chromosome structural changes.

Chiasma frequencies

Considering the rather small size of banana chromosomes, although not the smallest among the flowering plants, it is rarely possible in bivalent associations to discriminate between single and double chiasmata linking the same pair of chromosome arms at the first metaphase (M_1). Doubles have occasionally been inferred as highly probable but, in the few cases where late prophase stages have been clearly interpretable, the conclusion was reinforced that these are few in number. As stated below, however, double chiasma situations are often implicit in multivalent figures. For greater consistency, and from a suspicion that multivalents and bivalents may behave differently from one another, chiasmata have been estimated throughout as numbers of arms paired per bivalent only ("Xta" per II). A ring has been scored as 2, a rod as 1 and an unattached pair as 0. The level of accuracy of the means presented should then be sufficient to reveal any important difference between genotypes.

Translocations

The principal problem in the definition of these is that the rather low chiasma frequencies found do not always or even commonly permit a "saturated" expression of such structural heterozygosity. Added to this it may be supposed that the localisation of chiasma is also variable, whether they occur more frequently in sub-terminal or in sub-median positions, between translocated segments or between the exchange points and the centromeres. In the simplest case of a single translocation, the theoretical "ring of four" (IV) appears in a small minority of all relevant pollen mother cells; chains of four are often less common than a trivalent + univalent (III+I) and varying proportions of cells still present two bivalents (II).

Where two independent exchanges affect the different arms of a common chromosome, the full expected "ring of six" has never yet been identified, so far as the author's notes go. Even chains of six (VI) or five (V) may not be so common as two chains of three, these last without any corresponding univalent (I). Lower levels of multivalent association also occur (see especially Table 2.5). Dodds (1943) first referred to this problem in the AA group cultivar Sucrier (type 19). In this case he took an M_1 cell with IV 2III 6II as implying separate rings of VI and IV. The same might have been said of

another cell in his Table 2 with 3III 6II I. Any such conclusion would have as a corollary that it described the **minimal** level of structural hybridity revealed. The author has followed such guidelines and puts stress on the "critical cells" in any plant studied. In this book the level of translocation hybridity disclosed will be stated in terms of "potential rings". Examples will be shown where the types of associations found in a particular plant are conspicuously short of the theoretical expectation.

Where chains of three have been found, the great majority have been simple chains, mostly V-shaped but some linear; only a few revealed crossovers both within and median to an exchange, yielding the "frying-pans" of Dodds or, more rarely, Y-shaped associations. Four linked chromosomes (IV) might be rings or simple chains with either alternating (and potentially fertile) alignment, the same with adjacent chromosomes directed towards a common anaphase pole or were variously complicated by double chiasmata. Associations of V or VI were not only few but were less often totally alternate in presentation on the spindle equator. Figs 2.1 to 2.3 and others illustrate some of the possible forms.

Inversions

Examples are given in several chapters of first anaphase cells (A_1), in both intraspecific and interspecific hybrids, which are typified by chromatid bridges associated with a distinctive fragment (see Fig. 7.2). There can be no reason to doubt that these are the result of crossing over between relatively inverted segments. A problem arises, however, where the bridge has the familiar form but no fragment can be identified. In relation to cv. Lilin (AA group) and its hybrids, Dodds and Simmonds (1948) were careful to be non-committal about these. Later the author was able to identify some cells in similar hybrids where bridges were associated with minute fragments, which were by no means imaginary. These and the association of bridges with a particular translocation, as demonstrated in Chapter 2, now leave scarcely any doubt that the cause in these cases is in fact a quite small and very nearly terminal inversion. In this event, the expected tiny acentric fragment would be quite easily overlooked.

Chromosome counts of new hybrids

The techniques employed for handling root tips of bananas have changed radically from the distant to the recent past, from transverse sections in the 1930's (Cheesman and Larter, 1935) to squashes in the 1950's and subsequently. This author offers no apology for trusting to the simplest methods, starting in Trinidad with that of Tjio and Levan (1950). In this procedure tips are pre-treated with 8-hydroxyquinoline (oxyquinoline), given a "hard" fixation with 1:3 acetic acid:ethanol and then macerated in a heated mixture of 9 parts of 1% orcein in 45% acetic acid: 1 part N.HCl. Finally, the terminal 1.0 – 1.5 mm is squashed in fresh orcein solution and the cover glasses are sealed. The more primitive conditions of a field lab in Jamaica led to further simplifications, when it was found that a joint "soft" fixation + maceration could be substituted for two earlier

steps, and that lactophenol was a much better solvent for orcein, permitting a higher dye concentration and avoiding the necessity of sealing the squashes. No heat source is required. The last method was that used invariably for counts made in Brazil (Shepherd and dos Santos, 1996).

Other c-mitotic agents have been tried in the past in pre-treatments to destroy spindles and to scatter chromosomes. However, much the best results have been achieved by immersing root tips for three to several hours in 8-(hydr)oxyquinoline at 0.002M (about 0.03%) in water, at temperatures of about 20-25ºC. Even these have not been consistently good. The fixation/maceration already mentioned for the author's revised method is for at least four hours, and preferably overnight, in a mixture of 1 part ethanol: 4 parts glacial acetic acid: 5 parts water. Mounting on slides and squashing is in 2% orcein in a lactophenol comprising equal parts by volume of lactic acid, phenol, glycerol and water. Such preparations can take a day or two to "mature" but may then endure for varying periods without drying out, even up to a year or more.

In a genus like *Musa*, with rather small chromosomes, the centromeres of these are not normally revealed by this method. On the other hand, in the better squashes, the translucency of the stain permits the accurate resolution of cells where chromosomes are overlying each other. A word of warning is that orcein, like carmine, can be a variable product and the source of the dye is important for good results.

Trials were also made at the CNPMF in Brazil with a Giemsa procedure, by K.M. da Silva, but the dense, opaque stain precluded the possible resolution of overlaps. It would have been easy to underestimate the chromosome number.

CHAPTER 2

Translocations and inversions in diploid *Musa acuminata*

In addition to the published results mentioned initially, this exposition draws in part on certain unpublished results left behind in Trinidad by K.S. Dodds or by N.W. Simmonds. These are identified in the tables and are gratefully acknowledged. The greater part, however, springs from the writer's own research in Trinidad and Jamaica. Some passing reference is also made to Dessauw (1987) and, through this source, to Hutchison (1966).

Dodds (1943) was the first to make reference to translocation hybridity in the species, in four AA cultivars and in hybrids of two of them with the wild type Calcutta 4 (IR 124 of the Trinidad collection). This was the accession later classified by Simmonds (1956, 1960a) as ssp. *burmannica*. He also noted some incidence of bridges in first anaphase cells of all four cultivars. But, except in the case of 'Lilin', they appeared to be "sticky" bridges, their impeded separation not necessarily a consequence of structural hybridity. Some of these results will be reviewed in a later section.

Dodds and Simmonds (1948b) gave data on crosses of 'Lilin', "Clone A" (ssp. *malaccensis* Selangor IR 53 of this book), Calcutta 4 and Long Tavoy (IR 187), the last also included in ssp. *burmannica*. One of their findings was that an extremely large proportion of 'Lilin' hybrids inherited its modified chromosomes, in preference to those of the other parent. The structure given for Long Tavoy x 'Lilin' hybrids has since been found to be erroneous and is corrected in this chapter.

The first block of results surveyed now, however, relates to the distribution of structural hybridity between wild forms of the species and four AA cultivars. At least seven different translocation groups are shown to be present in *M. acuminata* in the wild.

The story then returns to the theme of the curiously biassed inheritance of the modified structure in 'Lilin' hybrids, with a new hypothesis in way of explanation, one fortified by an example from wild-type backcrosses.

Both published and new data on more diverse AA cultivars are finally brought together, leading to a discussion of male and female fertility in diploids of the species.

Plant materials studied in hybrid combinations

For a more logical presentation of these, it has been necessary to anticipate the results and to separate the forms in the first instance by their known segmental translocation groups. In some instances this involves the splitting of subspecies, and in others their amalgamation, yet only in terms of chromosome structure, **not of taxonomy**. The subspecies names used follow the recent definitions of Shepherd (1990a), with

comments where they differ from those of Simmonds (1956, 1960a). Accessions are listed with their Trinidad-Jamaica introduction numbers (IR), adopted clone names and provenances, where these differ from the clone names.

Standard structure (hereafter abbreviated as ST)

- ssp. *malaccensis* from Central Malaya: IR 53 Selangor, IR 269 Langet, IR 296 Pahang;
- ssp. *microcarpa*: IR 291 Borneo (Sabah);
- ssp. *banksii*: IR? New Guinea, IR 448 Madang from Papua New Guinea; IR 110, IR 430 and IR 433 from Samoa.

This must be the primordial chromosome structure so far as it concerns translocations. Not only is it seen here to be widely distributed in the species but it is evidently identical with that of the related species *M. flaviflora*, *M. ornata*, *M. schizocarpa* and *M. ochracea*, although hybrids with or between these others may reveal inversion hybridity (see appropriate later chapters).

Northern Malayan (NM)

- ssp. *malaccensis* IR 472 Gurum from Kedah, IR 474 Kelantan;
- AA cultivars: Lilin (heterozygous **ST x NM**); Paka (also **ST x NM**) and Sikuzani (homozygous **NM**), the two last from Zanzibar in Tanzania.

The wild accessions here were included with ssp. *siamea* by Simmonds (1956) but, on critical comparison, they differ from it in general morphology as well as chromosomally. More recently, the author has seen similar plants in and from Thailand itself, suggesting that the physical isolation barrier between the two subspecies is in that country rather than further south.

Northern 1 (N1) and Northern 2 (N2)

Northern 1:

- ssp. *burmannica*: IR 124 Calcutta 4 from India;
- ssp. *siamea*: IR 403 Siam from Thailand.

Northern 2:

- ssp. *burmannica*: IR 132 Calcutta 6 from India, IR 187 Long Tavoy from Burma (Myanmar);
- ssp. *siamea*: IR 144 Annam from Vietnam.

It might conveniently be reiterated here that the Calcutta Botanic Garden accession Calcutta 4, in particular, can be assumed to be truly Indian in origin because of a close resemblance to another example received in Jamaica directly from Madras (Shepherd, 1990a). The author does not remember having seen Calcutta 6.

Another Thai accession, IR 476, was not a parent in any intraspecific cross but its hybrids with *M. schizocarpa* (Chapter 5) prove that it also belongs to **N2**.

Malayan Highland (MH)

• ssp. *truncata*: IR 494 Kenayat from the Central Malayan highlands.

This material was earlier regarded by Simmonds (1956, 1960a) as a variant form of ssp. *microcarpa*, but the author could not see this with plants of both in the same collection. The cytological picture is also much against such a taxonomic placing.

Javanese (JV)

• ssp. *zebrina*: IR 205 Buitenzorg from Java; IR 264 'Maia Oa' from Hawaii.

The red-leaved and sometimes red-fruited ornamental 'Maia Oa' was thought to belong here by Simmonds (1962). The author not only agrees but he also encountered a similar and even redder plant in 1985, as 'Monyet' in the Purwodadi collection of East Java.

Pemba (EA)

• ssp. undetermined: IR 307 Pemba from the island of that name in Tanzania.

Three closely similar plant lots were raised in Trinidad from different seed batches. They were unfortunately rather unvigourous there and did not long survive.

cv. Tongat

The clone established in Trinidad was itself found to be heterozygous for a partly inverted translocation.

Hybrids within groups

These were not examined in all cases and the now available data are limited to those in Table 2.1. Otherwise, correspondence has been established by parallel results from crosses with other groups.

Hybrids within the **ST** group showed rare pairing failures at first metaphase and sometimes laggard bridges at first anaphase. The sole instance of a bridge with a substantial fragment is not regarded as having special significance. Such bridges have been seen as a rarity in many plants, as this and other tables show, without any predictability or any possible explanation.

The surviving direct data for correspondence between the three cultivar accessions listed under **NM** is not all that formerly existed. In addition to a study of 'Paka' itself in Trinidad, there were additional plants in Jamaica of 'Paka' x 'Lilin' and at least one of 'Sikuzani' x 'Lilin'. The agreement between the three clones on translocation structure was very clear apart from being reinforced by hybrids with other structural groups. 'Paka', like 'Lilin', is heterozygous for **NM** and reveals anaphase bridges with or without a minute fragment. Many plants were examined of Selangor x 'Paka', and of **N1** or **N2** x 'Paka'; these gave results exactly parallel with the same crosses of 'Lilin'. Moreover, 'Paka' and 'Lilin' showed an approximately equal bias in favour of the inheritance of the modified structure. In hybrids between them **ST** homozygotes would have had a very low

probability of occurrence in two out of three plants. Three plants are in fact reported (see Table 2.2 for the third) and two were homozygous for translocation.

Again, hybrids of forms within **N1** or **N2** showed no multivalents, although both reciprocal pairings were between subspecies.

Table 2.1. First meiotic divisions in hybrids of *M. acuminata* within the identified translocation structural groups: ST – standard, NM – North Malayan, N1 – Northern 1, N2 – Northern 2, JV – Javanese and EA – Pemba. Subspecies are abbreviated to ml – *malaccensis*, mc – *microcarpa*, bk – *banksii*, bm – *burmannica* and sm – *siamea*.

Cross	Metaphases			Anaphases**	
	Cells *	x 2I	"Xta" per II	Cells *	Bridges F m 0
ST x ST:					
ml Langet x ml Selangor	18**T**	0	1.89	6**T**	none
ml Selangor x mc Borneo	20**T**	0.05	1.82	50**T**	none
ml Selangor x bk Samoa 110	50**DS**	0.04	?	56**DS**	none
ml Selangor x bk Samoa 110	20**T**	0.05	1.72	–	
bk New Guinea x ml Selangor	50**DS**	0	1.54	30**DS**	none
bk New Guinea x ml Selangor	70**DS**	0	1.83	80DS	1
bk New Guinea x ml Selangor	20**T**	0	1.72	30**T**	2
and translocation homozygotes of:					
ml Selangor x cv. Lilin	141**DS**	0	?	158**DS**	1
ml Langet x cv. Lilin	20**T**	0	1.68	11**T**	1
bk Samoa 430 x cv. Lilin	40**J**	0	1.60	60**J**	3
bk Samoa 433 x cv. Lilin	40**J**	0	1.61	100**J**	1 2
bk Samoa 433 x cv. Paka	18**J**	0	1.71	17**J**	none
NM x NM:					
ml Gurum 472 x cv. Sikuzani	35**J**	0.06	1.63	61**J**	1
and translocation homozygotes of:					
cv. Paka x cv. Lilin	40**J**	0.10	**1.45**	50**J**	none
- sib	21**J**	0	1.82	–	
and see further sib in Table 2.2.					
N1 x N1:					
sm Siam x bm Calcutta 4	40**T**	0	1.65	100**T**	1 1
bm Calcutta 4 x sm Siam	20**T**	0	1.75	50**T**	1
N2 x N2:					
bm Long Tavoy x sm Annam	20**T**	0	1.78	50**T**	none
sm Annam x bm Long Tavoy	45**T**	0.02	1.67	30**T**	none
JV x JV and **EA x EA**:					
No direct hybrid was made within these structures.					

***DS** indicates data of Dodds or Simmonds, **T** and **J** distinguish results of the author from Trinidad and from Jamaica.

** F = bridges with substantial fragments, m = those with minute ones, 0 = those without visible ones.

Inter-group hybrids

ST x NM

Numerous hybrids are listed in Table 2.2 and they also may be compared with 'Lilin' itself (Dodds, 1943; Dodds and Simmonds, 1948b, and Table 2.7). In all plants one heterozygous translocation was evident and there was also a perceptible frequency of "inversion-type" anaphase bridges. Varying numbers of these were accompanied by visible minute fragments.

Table 2.2. First meiotic divisions in hybrids between translocation groups ST and NM and between N1 and N2; subspecies are abbreviated as in Table 2.1.

Cross	Metaphases					Anaphases**			
	Cells *	IV	III	x 2I	"Xta" per II	Cells *	Bridges F	m	0
ST x NM:									
bk Madang x ml Kelantan	20**J**	10	8	0	?	40**J**		2	5
bk Samoa 430 x cv. Sikuzani	24**J**	6	15	0.04	1.59	72**J**		3	3
bk Samoa 433 x cv. Sikuzani	20**J**	2	11	0.25	1.55	40**J**		3	
and translocation heterozygotes of:									
ml Selangor x cv. Lilin									
(9 plants)	320**DS**	16	109	0.20	?	427**DS**	(Σ82)		
(+12 plants)	172**T**	5	65	0.06	±1.75	40**T**	1	1	5
ml Langet x cv. Lilin									
(5 plants)	123**T**	9	44	0.02	1.79	97**T**			4
bk Samoa 430 x cv. Lilin									
(6 plants)	69**J**	24	30	0.01	1.71	90**J**		7	4
bk Samoa 433 x cv. Lilin									
(19 plants)	234**J**	59	122	0.04	1.71	263**J**	5	19	26
bk Samoa 433 x cv. Paka	40**J**	9	23	0.12	1.68	80**J**		5	3
cv. Paka x cv. Lilin – sib	40**J**	10	18	0.10	1.66	40**J**		2	2
Σ Trinidad	615	30	218			564	1	(Σ 90)	
Σ Jamaica	447	120	227			625	5	34	39
N1 x N2: (Two anomalous **DS** results are omitted.)									
bm Calcutta 4 x bm Long Tavoy	20**T**	7	1	0.05	1.67	25**T**	none		
bm Long Tavoy x bm Calcutta 4	20**T**	15	0	0	**1.90**	50**T**	1		
sm Siam x bm Long Tavoy	20**T**	15	0	0	1.86	50**T**			1
sm Siam x sm Annam	40**T**	33	0	0.02	1.78	52**T**	none		
bm Calcutta 4 x sm Annam	40**T**	28	1	0.02	1.82	90**T**	1		

* **DS** indicates data of Dodds or Simmonds, **T** and **J** distinguish results of the author from Trinidad and from Jamaica.

** F = bridges with substantial fragments, m = those with minute ones, 0 = those without visible ones.

There was a curious overall discrepancy between results obtained in Trinidad, by all workers, and those of the present author in Jamaica. In the former centre, the translocation normally manifested itself as a trivalent plus univalent, these accounting for 88% of all multivalents seen. Yet in Jamaican studies the proportions were of 35% quadri – and only 65% trivalents. Moreover, no certain closed ring IV was seen in the Trinidad analyses listed and these were at least present in Jamaican smears.

Hutchison (1966) and Dessauw (1987) also published first metaphase data on 'Lilin' itself and found quite high quadrivalent levels. The evidence is very strong from these contrasts that environment can play an important part in determining the detailed events of meiosis in diploids. Another highly likely example is to be related in Chapter 3. Simmonds (1962) made reference to this possibility to account for discrepancies between published accounts of meiosis in triploids and here the hypothesis is merely extended to the lower ploidy.

The segregation of the 'Lilin' translocation is examined in a subsequent section where it will be postulated that the type of multivalent is important.

N1 x N2

The story related in the same Table 2.2 is a simpler one, after the exclusion of two sets of early studies, where no multivalent was found at all. One of these was the same hybrid between Calcutta 4 and Long Tavoy whose repeat study is listed. As can be seen, multivalent frequency was still rather low at the second attempt, much less than in the reciprocal cross. In general, and in particular with combinations of ssp. *siamea*, quadrivalents were present in a high proportion of cells and 53 out of a total of 98 were closed rings.

Anaphase bridges were rare. A consequence of the high level of association between the exchange segments, and of alternate presentation on the first metaphase spindles, is that there is often only a slight or even imperceptible reduction in male fertility. It might be surmised that such a translocation could survive within a **N1 x N2** hybrid swarm through many generations without selection pressure in either direction.

ST x N1

Complications now begin to arise, not only in the minimal numbers of segment exchanges but in the relative difficulty in confirming these (Tables 2.3 and 2.4). In the example of Calcutta 4 x Samoa IR 110, the author and his predecessors noted no less than 110 metaphases without finding a single one that demonstrated the presence of two independent translocations. They were absent or very rare in some other samples. But two there certainly were, as potential independent rings of four (confer Fig. 2.1 A)! Pairing failures were more evident than in the combinations previously discussed but apparently not correlated with the lack of multiples. One can only think of variations in the localisation of crossovers, as hinted in Chapter 1, where an excess of sub-median ones hides the existence of differential ends. Here there is also a suspicion of genetic variability between accessions, where ssp. *malaccensis* Langet, ssp. *microcarpa* Borneo

and ssp. *siamea* Siam seemingly yielded the highest quadrivalent numbers, although normally chains rather than rings. Bridges occurred in about 2% of anaphase cells, either with or without conspicuous fragments, and without any pattern of occurrence.

Table 2.3. First meiotic metaphases in hybrids of *M. acuminata* involving the ST and NM structures with the N1 and N2 ones. Subspecies are abbreviated and data sources are indicated as in Table 2.1.

Cross	Cells	Translocations*					x 2I	"Xta" per II
		4	3	2	1	0		
ST x N1:								
ml Selangor x bm Calcutta 4	57**DS**			5	22	30	0.21	1.57
ml Selangor x bm Calcutta 4	25**T**			3	17	5	0.04	1.62
bm Calcutta 4 x ml Selangor	68**T**			2?	40	26	0.28	1.68
bm Calcutta 4 x ml Langet	40**T**			**1**	23	16	0.05	1.81
ml Selangor x sm Siam	20**T**			7	13	1	0.20	1.51
bm Calcutta 4 x mc Borneo	20**T**			7	7	6	0.05	1.62
sm Siam x mc Borneo	20**T**			5	11	4	0.05	**1.39**
bm Calcutta 4 x bk Samoa 110	30**DS**			**0**	9	21	0.03	?
bm Calcutta 4 x bk Samoa 110	80**T**			**0**	26	54	0.05	?
bm Calcutta 4 x bk New Guinea	55**DS**			**0**	15	40	0.09	?
bm Calcutta 4 x bk New Guinea	40**T**			**4**	14	22	0.15	?
ST x N2: (One plant with anomalous **DS** results is omitted.)								
bm Calcutta 6 x ml Selangor	20**DS**		**0**	3	11	6	0.05	?
ml Selangor x bm Long Tavoy	20**T**		**0**	6	9	5	0	1.62
ml Pahang x bm Long Tavoy	20**T**		**0**	3	12	5	0.15	1.51
bm Long Tavoy x ml Pahang	22**T**		2	6	10	4	0.09	1.64
bk Samoa 110 x bm Calcutta 6	20**T**		1	4	13	2	0.05	1.58
bm Calcutta 6 x bk New Guinea	22**DS**		0	8	3	11	0.09	?
bm Calcutta 6 x bk New Guinea	20**T**		**0**	2	6	12	0.10	?
bm Calcutta 6 x bk New Guinea	+ 24**T**		4	12	7	1	0.08	1.69
sm Annam x ml Selangor	25**T**		3	4	15	3	0.16	1.77
sib	15**T**		0	5	7	3	0.07	1.68
sm Annam x mc Borneo	20**T**		**5**	11	4	**0**	0.05	1.69
NM x N1:								
bm Calcutta 4 x cv. Lilin	338**DS**		+				?	?
			(numbers unknown)					
bm Calcutta 4 x cv. Lilin	21**T**		3	13	4	1	0.05	1.72
NM x N2:								
bm Long Tavoy x cv. Lilin	100**T**	3	39	38	16	4	0.03	1.65
sib	23**T**	1	10	9	3	0	0.26	**1.46**
sib	44**T**	6	16	12	8	2	0.23	1.57
sm Annam x cv. Lilin	20**T**	4	13	3	0	0	0	1.78

* Numbers of cells with multivalents indicating each situation.

Table 2.4. Further first meiotic data from hybrids of *M. acuminata* of the ST and NM structures with the N1 and N2 ones. Subspecies and data sources are abbreviated as in Table 2.3.

Cross	Metaphases						Anaphases**			
	Cells	Multivalents*					Cells	Bridges		
		VI	V	IVR	IVC	III		F	m	0
ST x N1:										
ml Selangor x bm Calcutta 4	57**DS**			0	9	23	69**DS**		none	
ml Selangor x bm Calcutta 4	25**T**			0	7	16	-			
bm Calcutta 4 x ml Selangor	68**T**			3	6	35	-			
bm Calcutta 4 x ml Langet	40**T**			3	13	9	50**T**	1		
ml Selangor x sm Siam	20**T**			0	10	1	50**T**			1
bm Calcutta 4 x mc Borneo	20**T**			0	14	7	47**T**			3
sm Siam x mc Borneo	20**T**			0	9	12	50**T**		none	
bm Calcutta 4 x bk Samoa 110	30**DS**			0	2	7	50**DS**	3		
bm Calcutta 4 x bk Samoa 110	80**T**			1	6	19	20**T**		none	
bm Calcutta 4 x bk New Guinea	55**DS**			0	1	14	63**DS**	1		
bm Calcutta 4 x bk New Guinea	40**T**			3	5	14	20		none	
	455**T**			10	82	171	419	5	0	4
ST x N2:										
bm Calcutta 6 x ml Selangor	20**DS**			0	6	11	50**DS**		none	
ml Selangor x bm Long Tavoy	20**T**			1	10	10	9**T**		none	
ml Pahang x bm Long Tavoy	30**T**			0	4	14	50**T**	1		
bm Long Tavoy x ml Pahang	22**T**	1	1	2	7	15	50**T**			1
bk Samoa 110 x bm Calcutta 6	20**T**	1		1	10	11	60**T**	1		2
bm Calcutta 6 x bk New Guinea	22**DS**	1	2	1	8	4	55**DS**	2		1
bm Calcutta 6 x bk New Guinea	20**T**			2	3	5	-			
bm Calcutta 6 x bk New Guinea	+ 24**T**	3	1	7	22	6	-			
sm Annam x ml Selangor	40**T**		6	2	24	11	72**T**		none	
sm Annam x mc Borneo	20**T**	1	7	5	16	4	-			
	238**T**	7	17	21	110	91	346**T**	4	0	4
NM x N1:										
bm Calcutta 4 x cv. Lilin	338**DS**		1	0	24	634	795**DS**			?87
Note: Data calculated from Dodds and Simmonds (1948b).										
bm Calcutta 4 x cv. Lilin	21**T**	**0**	**0**	0	1	38	50**T**	2	?0	6
NM x N2:										
bm Long Tavoy x cv. Lilin	100**T**	5	11	0	41	148	32**T**	1		7
sib	23**T**	**0**	**1**	0	2	51	30**T**	2	1	2
sib	44**T**	2	12	0	5	71	50**T**	10	5	
sm Annam x cv. Lilin	20**T**	1	3	0	3	50	50**T**	1		6
	187**T**	8	27	**0**	51	320	162**T**	4	11	20

* Chains of VI and V, rings and chains of IV and various III.

** F = bridges with substantial fragments, m = those with minute ones, 0 = those without visible ones.

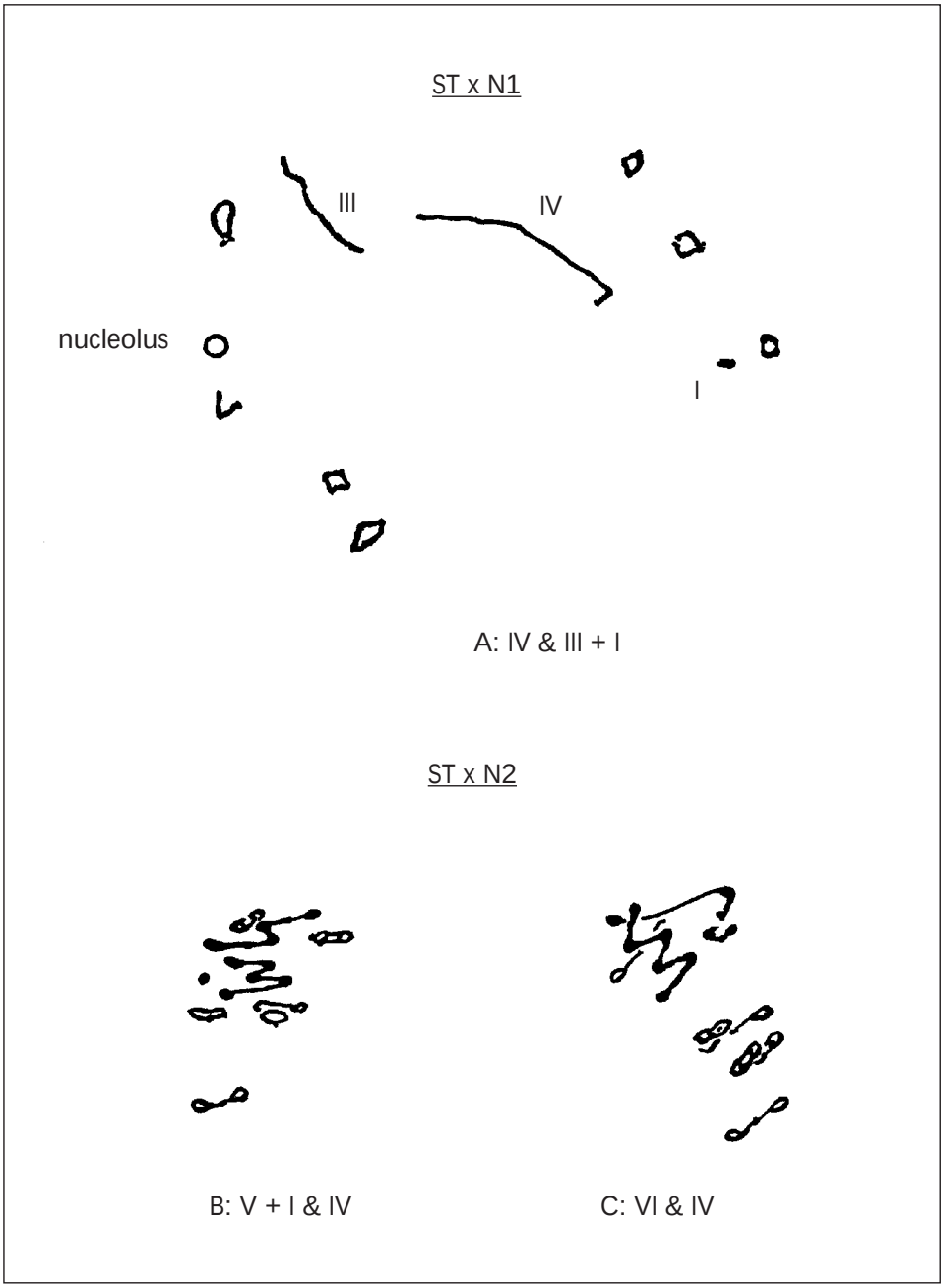


Figure 2.1. Some critical meiotic cells in hybrids within *M. acuminata.*

A: Prophase in ssp. *siamea* Siam x ssp. *microcarpa* Borneo; B: M_1 in ssp. *burmannica* Calcutta 6 x ssp. *banksii* Samoa 110; C: M_1 in Calcutta 6 x ssp. *banksii* New Guinea. Approximate scales vary in the region of x1600.

ST x N2

The same tables affirm the above problems further magnified. Six out of eleven samples from eight hybrids failed to show in a single cell the three translocations that had to exist. In two of these however, the first sample of Calcutta 6 x New Guinea and the discordant one of Annam x Selangor, cells were seen with groups of either six or five, or with two III and without any corresponding univalents. The last situation is equally indicative of a group of six. Critical cells in the others ranged from a complement of VI IV 6II to one of IV 2III 6II (Figs 2.1 B & C; 2.2 A & B). Again rare bridges occurred at first anaphase in some plants.

NM x N1 and NM x N2

In each of these cases the expectation was of one extra translocation over and above those found in the respective hybrids of **N1** or **N2** with **ST**. Also to be established were the potential ring situations. From the results summarised in Tables 2.3 and 2.4, Dodds and Simmonds (1948b) judged **NM x N1** correctly, as three translocations tending to rings of six and four, although they saw no association of six and only one of five. Strangely, the plant 1590 of Dodds (1943) was not reconsidered and a repeat study by this author verified the absence of the third translocation (Table 2.5).

As for **NM x N2**, one exchange was missed in the old examination of Long Tavoy x 'Lilin', although Dodds and Simmonds (*loc. cit.*) stressed the difficulty of interpretations where full associations were so uncommon. It is in part to illustrate this aspect that Table 2.5 is included, to list numbers and types of metaphase cells found by the author in repeat studies of three plants each of **NM x N1** and **NM x N2**. He did find critical cells for three translocations in two plants of the former and for four exchanges in all three of the latter, but they were few in number. In 426A-5 only one cell in 23 indicated the theoretical structure of two rings of six. The analysis of large samples of cells is therefore very desirable. Examples of the "two rings of VI" structure are illustrated in Figs 2.2 C, D & E.

MH Hybrids

Pollen mother cells in the few virtually male-sterile hybrids raised in Jamaica were commonly difficult to handle, with their first metaphases clumped and "sticky". Even the slender cytological evidence obtained, from a hybrid with **NM**, has been regrettably mislaid. It was sufficient, however, to indicate a difference of **four** translocations from that structural group, from memory forming "two rings of VI" once more. In the unworkable smears of hybrids with **N1** and **N2** there were also suggestions of frequent multivalents; no correspondence was envisaged of **MH** with either Northern structure.

JV Hybrids

As Tables 2.6 and 2.7 set out, hybrids of **ST x JV** were heterozygous for a ring of four, sometimes with some pairing deficiencies at first metaphase. Inversion bridges with fragments were always common at first anaphase and there was also a variable

Table 2.5. Details of the author's repeat data on first meiotic metaphase of NM x N1 and N2 plants in Trinidad (critical cells in bold).

T*	Cells	Numbers with each type of association
NM x N1 (Calcutta 4 x 'Lilin'):		
	Plant 2/1590 (confer Dodds, 1943) – 21 cells (not in Tables 2.3 and 2.4):	
3	**0**:	
2	4:	4 x IV III 7II I;
1	10:	8 x IV 9II; 2 x III 9II I;
0	7:	7 x 11II.
	Plant 162-11 – 10 cells only:	
3	2:	**2** x **3III 6II I**;
2	7:	**2** x **2III 8II**; 4 x 2III 7II 2I; 1 x 2III 6II 4I;
1	1:	1 x IV 9II;
0	0.	
	Plant 265-16 – 11 cells only:	
3	1:	**1** x **3III 6II I**;
2	6:	**4** x **2III 8II**; 2 x 2III 7II 2I;
1	3:	3 x III 9II I;
0	1:	1 x 11II.
NM x N2 (Long Tavoy x 'Lilin') – all repeats of Dodds and Simmonds (1948b):		
	Plant 426A-4 – 100 cells:	
4	3:	**1** x **VI 2III 5II**; **1 x V 2III 5II I**; **1 x 4III 5II**;
3	39:	2 x VI IV 6II; 2 x VI III 6II I; 2 x V IV 6II I; 6 x V III 6II I; 16 x IV 2III 6II; 1 x IV 2III 5II 2I; 10 x 3III 6II I;
2	38:	2 x V 8II I; 14 x 2III 8II; 12 x IV III 7II I; 10 x 2III 7II 2I;
1	16:	8 x IV 9II; 7 x III 9II I; 1 x III 8II 3I;
0	4:	2 x 11II; 2 x 10II 2I.
	Plant 426A-5 – 23 cells:	
4	1:	**1** x **4III 5II**;
3	10:	1 x V III 5II I; 1 x IV 2III 5II 2I; 6 x 3III 6II I; 2 x 3III 5II 3I;
2	9:	2 x 2III 8II; 1 x IV III 7II I; 6 x 2III 6II 4I;
1	3:	3 x III 9II I;
0	0.	
	Plant 426A-12 – 44 cells:	
4	6:	**1** x **VI 2III 5II**; **5 x V 2III 5II I**;
3	16:	1 x V IV 6II I; 4 x V III 6II 2I; 1 x IV 2III 6II; 1 x IV 2III 5II 2I; 9 x 3III 6II I;
2	12:	1 x VI 8II; 1 x V 8II I; 1 x V 7II 3I; 3 x 3III 8II; 1 x IV III 7II I; 5 x 2III 6II 4I;
1	8:	1 x IV 9II; 4 x III 9II I; 3 x III 8II 3I;
0	2:	1 x 11II; 1 x 10II 2I.

* T = translocations evident.

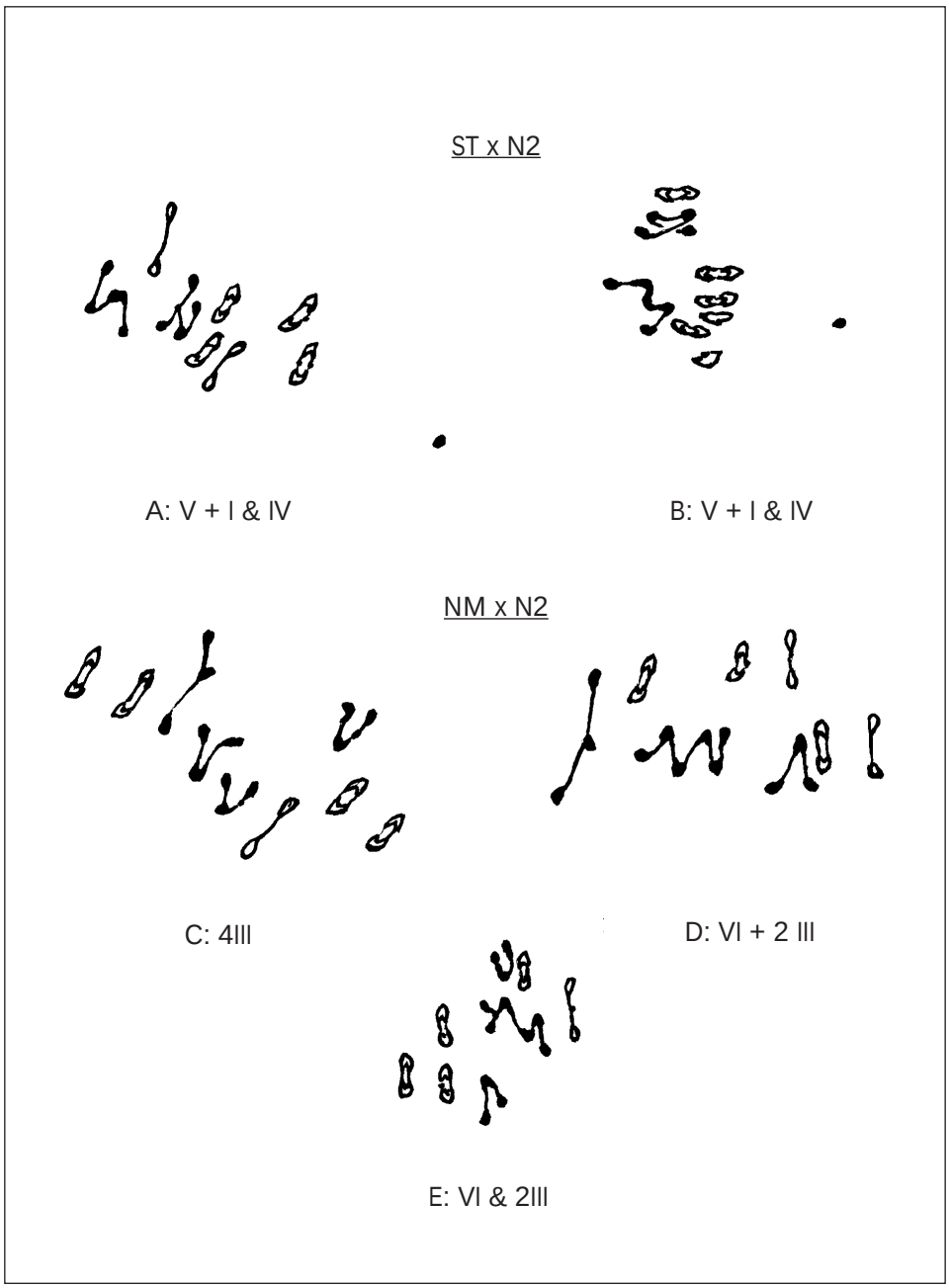


Figure 2.2. More critical meiotic cells in hybrids within *M. acuminata.*

All M_1 stages: A: ssp. *siamea* Annam x ssp. *malaccensis* Selangor; B: ssp. *siamea* Annam x ssp. *microcarpa* Borneo; C & D: ssp. *burmannica* Long Tavoy x cv. Lilin; E: ssp. *siamea* Annam x cv. Lilin. Approximate scales vary in the region of x1600.

Table 2.6. First meiotic metaphases in hybrids of *M. acuminata* involving the JV and EA structures with other ones. Subspecies are as in Table 2.1 with the addition of zb – *zebrina*; data are results the author in Trinidad (T) or Jamaica (J)

Cross	Cells	Translocations *					x 2I	"Xtra" per II
		4	3	2	1	0		
ST x JV:								
ml Selangor x zb Buitenzorg	25**T**				7	18	0.20	?
ml Selangor x zb Buitenzorg	+ 35**T**				27	8	0.17	**1.48**
zb Buitenzorg x ml Selangor	20**T**				11	9	0.35	1.52
zb Buitenzorg x mc Borneo	20**T**				10	10	0.05	1.80
zb Maiaoa x ml Selangor	20**T**				5	15	0	1.62
Note: this hybrid is the plant SH 125 below.								
and 13 translocation heterozygotes of:								
SH 125 x ml Langet:	195**J**				146	49	0.03	1.68
NM x JV:								
SH 125 x ml Kelantan	20**J**			11	7	2	0	1.64
SH 125 x cv. Sikuzani	4**J**			3	1	0	0	
zb Buitenzorg x cv. Sikuzani	20**J**			13	7	**0**	0.15	1.53
N1 x JV:								
bm Calcutta 4 x zb Buitenzorg	20**T**		4	10	5	1	0.15	1.77
sm Siam x zb Buitenzorg	12**T**		3	5	4		0.4	**1.48**
N2 x JV:								
sm Annam x zb Buitenzorg	25**T**	2	13	4	3	3	0.28	1.51
bm Long Tavoy x zb Buitenzorg	7**J**		3	4			0	
ST x EA:								
Pemba x ml Selangor	20**T**				13	7	0	1.73
Pemba x mc Borneo	30**T**				16	14	0.10	1.71
Pemba x cv. Lilin								
(single heterozygote)	20**T**				15	5	0	1.67
NM x EA:								
Pemba x cv. Lilin								
(double heterozygote)	24**T**			2	17	5	0.04	**1.49**
N1 x EA:								
bm Calcutta 4 x Pemba	14**J**		6	8			0	1.73
N2 x EA:								
sm Annam x Pemba	47**T**	9	25	11	2		0.02	1.63
JV x EA:								
Pemba x zb Buitenzorg	**1T**			**1**				
zb Buitenzorg x Pemba	20**T**			12	8		0.20	?

* Numbers of cells with multivalents indicating each situation.

occurrence of fragmentless bridges. Plant SH 125, of 'Maiaoa' x Selangor, became important in illustrating a potential relationship between the two forms of structural hybridity (see later in this chapter) and for showing that **NM x JV** crosses were typically heterozygous for two independent "rings of IV" and for one inversion at least. Actual rings of four were quite few.

In the same tables two **N1 x JV** hybrids undoubtedly had three translocations in the heterozygous state and critical cells contained V and IV, IV and 2III or 3III with a single univalent. "Rings of VI and IV" were the minimal level of change (Fig. 2.3 A). So far as **N2 x JV** was concerned, critical cells became yet more "critical". The small number of cells of Long Tavoy x Buitenzorg failed to reveal additional complexity to that in **N1 x JV**. On the other hand, Annam x Buitenzorg had the expected quota of four changes, although one of the individual decisive cells showed less. The maximal association was of V and 2III with one univalent only (Fig. 2.3 B) but another cell had two IV + one III. The only structure which could have given such a joint result was that of "VIII & IV".

EA Hybrids

The few hybrids which established this last of the identified structures of the wild species are also summarised in Tables 2.6 and 2.7. Once more there is a single relative translocation from **ST**, which is independent from **NM**, in this case without any evidence of a conspicuous inversion. In the only cross studied with **N1** no group of VI or V was detected, but six critical cells with either IV 2III 6II or 3III 6II I made it clear that the minimal potential was for "VI & IV". This was well confirmed by two plants of **N2 x EA**, Annam x Pemba, where the additional exchange converted the system into "two rings of six". No less than nine critical cells had either VI 2III 5II or V 2III 5II I (Fig. 2.3 D & E). **EA** also differed from the **JV** structure, where a maximal association of 2IV 7II was found to include some closed rings.

cv. Tongat (TT)

This is another area where important results have been misplaced. Apart from a meiotic analysis of the cultivar itself many of its hybrids were studied in Trinidad or Jamaica, including those with **ST** Selangor, **NM** 'Lilin' and **N2** Long Tavoy.

As already indicated, 'Tongat' itself was found to be heterozygous for one translocation, usually expressed in Trinidad as metaphase cells with trivalents, and for a submedian inversion within the exchange set. Again, the inheritance was biassed but, as an added difficulty in matching, it was in this case the modified chromosomes that tended to be eliminated in hybrids. At least, **TT** structure was confirmed as being different from **NM** and from **N2**.

Exceptionally, a repeat study was made of 'Tongat' in Bahia, where 30 M_1 cells comprised three with a IV, twenty with a III and seven without either form of multivalent, a result evidently similar to the Trinidad one. 27 A_1 cells included eleven with bridge and fragment.

Table 2.7. Further first meiotic data from hybrids of *M. acuminata* of the JV and EA structures with others. Subspecies and data sources are abbreviated as in Table 2.6.

Cross	Metaphases						Anaphases**			
	Cells	Multivalents*					Cells	Bridges		
		VI	V	IVR	IVC	III		F	m	0
ST x JV:										
ml Selangor x zb Buitenzorg	25**T**			2	3	2	joint:			
ml Selangor x zb Buitenzorg	+ 35**T**			6	11	10	100**T**	24		1
zb Buitenzorg x ml Selangor	20**T**			4	4	3	28**T**	8		1
zb Buitenzorg x mc Borneo	20**T**			7	2	1	50**T**	10		11
zb Maiaoa x ml Selangor	20**T**			1	2	2	52**T**	11		1
Note: this hybrid is the plant SH 125 below.										
∑ Trinidad	115			20	22	18	230	53	0	14
SH 125 x ml Langet	195**J**			39	59	48	255**J**	55	1	7
NM x JV:										
SH 125 x ml Kelantan	20**J**			4	13	12	50**J**	11	**0**	3
SH 125 x cv. Sikuzani	4**J**				4	3	-			
zb Buitenzorg x cv. Sikuzani	20**J**			5	13	15	18**J**	3	3	
N1 x JV:										
bm Calcutta 4 x zb Buitenzorg	20**T**	1	1	0	8	25	50**T**	29		
sm Siam x zb Buitenzorg	12**T**		1	0	7	14	100**T**	44		
N2 x JV:										
sm Annam x zb Buitenzorg	25**T**		3	0	13	39	50**T**	8		5
bm Long Tavoy x zb Buitenzorg	7**J**				3	12	50**J**	15		4
ST x EA:										
Pemba x ml Selangor	20**T**				1?	12	17**T**		1	
Pemba x mc Borneo	30**T**				4	12	47**T**	2		
Pemba x cv. Lilin (single heterozygote)	20**T**				12	3	29**T**			1
NM x EA:										
Pemba x cv. Lilin (double heterozygote)	24**T**			1	7	13	40**T**			3
N1 x EA:										
bm Calcutta 4 x Pemba	14**J**				6	28	25**J**		2	1
N2 x EA:										
sm Annam x Pemba	47**T**	6	6	1	18	88	100**T**	1	4	6
JV x EA:										
Pemba x zb Buitenzorg	**1T**					2	50**T**	12		3
zb Buitenzorg x Pemba	20**T**			6	15	11	50**T**	13	5	2

* Chains of VI and V, rings and chains of IV and various III.

** F = bridges with substantial fragments, m = those with minute ones, 0 = those without visible ones.

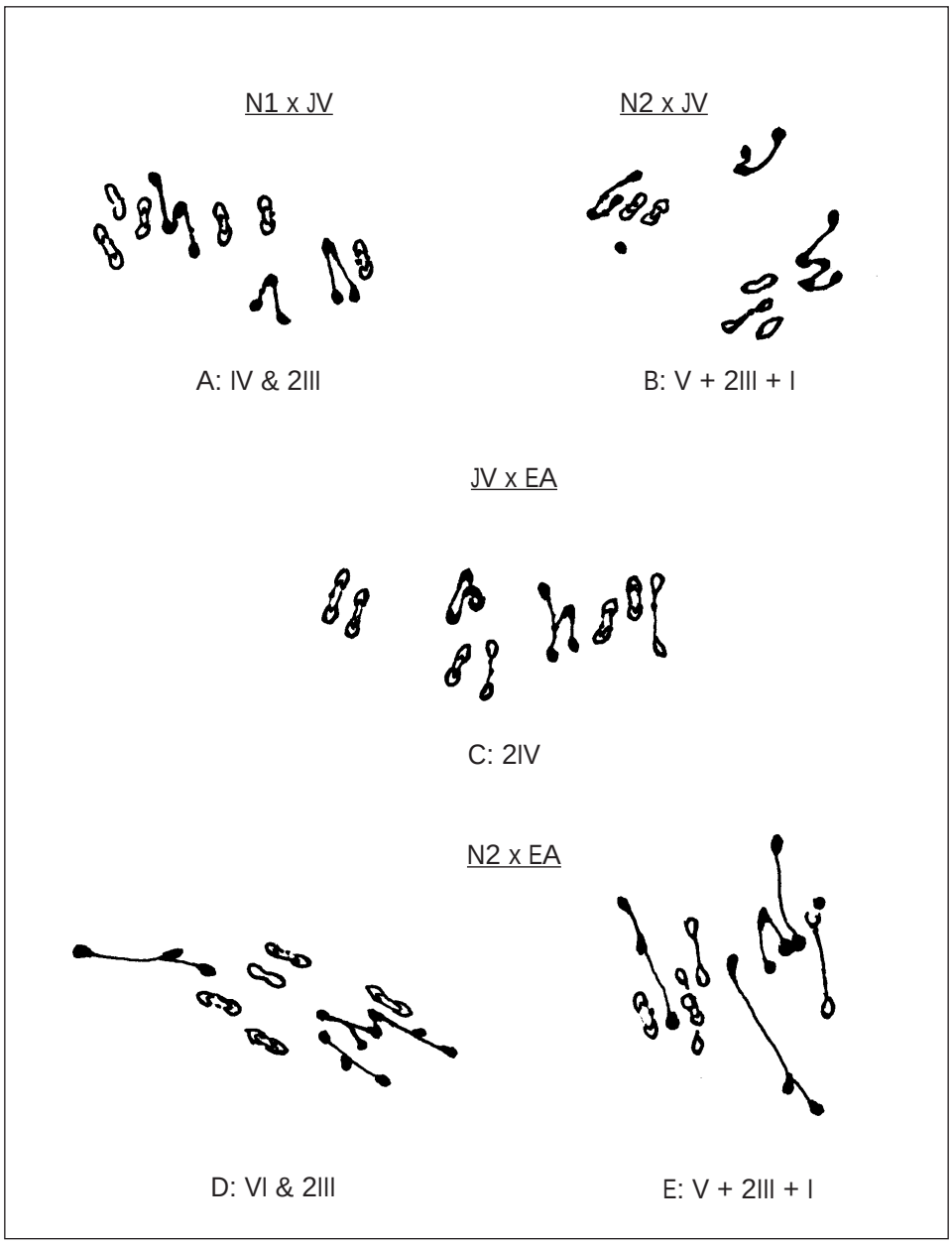


Figure 2.3. More critical meiotic cells in hybrids within *M. acuminata.*

All M_1 stages: A: ssp. *burmannica* Calcutta 4 x ssp. *zebrina* Buitenzorg; B: ssp. *siamea* Annam x ssp. *zebrina* Buitenzorg; C: ssp. *zebrina* x ssp. undefined Pemba;
D & E: ssp. *siamea* Annam x ssp. undefined Pemba. Approximate scales vary in the region of x1600.

Matchings attempted of **TT** with **JV** in Jamaica are listed in Table 2.8, where only one out of four plants had two independent translocations evident, in six critical cells with a high incidence of quadrivalents. It may be suggested that this is another instance of an environment favouring crossovers between exchange segments. Not one anaphase cell among fifty displayed two bridges with fragments, however, an occurrence that would have been expected. The cultivar clone is perhaps only known from the island of Borneo, and particularly from Sabah. It remains an open question whether or not it derived its chromosomal modification from a yet unidentified wild parent or during its history of cultivation. Despite the relatively simple chromosome structure, 'Tongat' is very nearly male-sterile.

Summary of potential rings in wild hybrids

Group	x	ST	NM	N1	N2	JV	EA
ST		•	IV	2IV	VI+IV	IV	IV
NM		IV	•	VI+IV	2VI	2IV	2IV
N1		2IV	VI+IV	•	IV	VI+IV	2IV
N2		VI+IV	2VI	IV	•	**VIII+IV**	2VI
JV		IV	2IV	VI+IV	**VIII+IV**	•	2IV
EA		IV	2IV	VI+IV	2VI	2IV	•
MH			**2VI?**				

Table 2.8. First meiotic divisions in certain 'Tongat' (TT) hybrids. Data from the author's studies in Jamaica.

Material	Plants	First metaphase*				First anaphase**				
		Total	Cells with:			Total	Numbers with:			
		cells	IVR	IVC	III	cells	1u	2u	BF	1u & BF
Buitenzorg x TT	1	16	6	5	3	14	1	1	3	0
sib	1	20	9	9	0	0				
Note: no cell had two multivalents to show the TT translocation.										
SH 125 x TT	1	20	7	9	3	50	7	1	14	3
Note: six cells had either 2IV or IV+III.										
sib	1	20	5	6	0	17	0	1	9	0
Note: once more, no cell had two multivalents.										

* Rings or chains of IV and various III.
** Odd univalents or pairs, bridges with fragments and simultaneously with a bridge and fragment plus an odd univalent.

Inheritance of translocations/inversions in AA

'Lilin' (PL)

Dodds and Simmonds (1948b) found that nine out of ten plants of family 206, ssp. *malaccensis* Selangor x PL, inherited from the heterozygous male parent what we are

now calling the **NM** translocation. Also among 26 plants analysed of family 265, from ssp. *burmannica* Calcutta 4 x PL, 15 appeared to be hybrid for rings of VI and IV and 11 had at least the possible ring of VI. The latter finding also could be critical for the same three translocations, although the authors did not claim this and perhaps the segregation might be better left as inconclusive. As was already related, the earlier plant 1590 of this same parentage was a clear exception to this amazing segregation. These authors had struggled to find explanations for the bias and thought of gametic or zygotic lethal genes. However, the author's results do not lend support to such hypotheses.

For simplicity in the investigation of these strange facts, it is obviously better to deal with plants which are either heterozygous or homozygous for one exchange structure, be the latter **ST** or **NM**. From this viewpoint, a new cross of Selangor x PL was first added to the 1948 data in Tables 2.9 and 2.10, as well as with some comparable hybrids of ssp. *malaccensis* Langet x PL. These additional plants gave joint totals of 17 to possibly 22 heterozygotes and six homozygous **ST**.

The next stage was an analysis of two successive backcross generations of the Selangor **ST** as female parent with pollen from heterozygotes, on the principle that, if lethals were involved, the first cross had already replaced the original **ST** chromosomes by fresh "untainted" ones and this process was then twice repeated. But the outcome was much the same as before, of 23 heterozygotes to five **ST**.

Now, based on a provisional ratio approximating to 4 **NM** gametes: 1 **ST** produced by heterozygous male parents, as found by combining the previous two sets of data, we could test whether the inheritance was also biassed on the female side. For this purpose a backcross was made in the other direction, of 206 x PL. If transmission were random in megaspore mother cells, then (a) homozygotes would be half of the plants in family 3006 and (b) 80% of them would be **NM**. In fact 3006 showed a significant excess of homozygotes, 31 among 44 plants, but of structure undefined at that point. So homozygotes were tested again in crosses with Selangor and all 20 tested turned out to have been **NM**. There was a poor fit in both aspects. On the other hand, if the segregation were equally biassed in both parents of 3006, we would get a ratio of 16+1 homo-: 8 heterozygotes and almost all of the former would be **NM**. This is what was found.

Dodds and Simmonds (1948b) also commented on the presence of first anaphase bridges in their heterozgous plants derived from PL, but did not go so far as to claim an associated inversion because of the lack of identifiable fragments. Yet a linkage of anaphase 1 bridges to the translocation is abundantly evident in Table 2.10, where some at least of the bridges in **NM x ST** heterozygotes were accompanied by an identifiable minute fragment. It can only be surmised that one of the exchange segments contained a small subterminal inversion. The heterozygous plants from crosses within ssp. *malaccensis* manifested bridges at a frequency of 0.14 per cell, as well as univalents at 0.29 per cell. However, cells which had both irregularities were markedly rare, at virtually the same low rate as bridges in homozygotes. The conclusion has to be that the relevant bridges and the univalents excluded from a set of four were in fact mutually exclusive events. The univalents therefore generally contain one of the relatively

Table 2.9. First meiotic metaphase data from 'Lilin' (PL), from ST x PL hybrids and from backcross derivatives of these latter.

Material	Heterozygotes					Homozygotes	
	N-Cells	Multivalents			None	No multivalents	
		IV	III	x 2I	x 2I	N-Cells	x 2I
Studies in Trinidad:							
PL *	1 – 103	1	35	?	?	–	
Selangor x PL (206)*	9 – 320	16	109	?	?	1 – 141	0
Selangor x PL (1638)**	12 – 172	5	65	0	11	5 – 100	5
Langet x PL	5 – 123	9	44	0	3	1 – 20	0
Subtotals	17 – 295	14	109	0	14/172	6 – 120	5
Frequencies		0.05	0.37		0.08		0.04
Selangor x 206 (987)	15 – 117	2	48	0	11	3 – 60	2
Selangor x 987	8 – 51	1	25	2	7	2 – 40	3
Subtotals	23 – 168	3	73	2	18/92	5 – 100	5
Frequencies		0.02	0.43		0.20		0.05
206 x PL (3006)	13 – 135	1	37	0	4	31 – 602	15
Selangor x homozygotes of 3006	20 – 253	0	133	4	21	0	
Subtotals	33 – 388	1	170	4/171	25/217	31 – 602	15
Frequencies		0.00	0.44	0.02	0.12		0.02
Studies in Jamaica:							
Samoa 430/433 x PL	25 – 303	83	152	3	8/68	4 – 80	0
Frequencies		**0.27**	0.50	0.01	0.12		

* Derived from Table 5 of Dodds and Simmonds (1948b).
** Five additional plants had reduced pollen contents and were therefore very likely heterozygotes.

inverted segments. Should the I be the **ST** chromosome consistently, there is a clear mechanism for a favoured inheritance of **NM**. Perhaps the **ST** unit has one or both arms rather short, with the inversion further impeding crossing over.

Mathematical estimates for a mechanism of unequal inheritance must then depend principally on the frequency of the III + I configuration of the translocation set at M_1, in relation to cells with only pairs. In the 463 M_1 cells analysed by the author of crosses **ST** x (**ST x NM**) these accounted jointly for 96% of the total. 57% had only pairs and the probability of segmentally balanced interphases must have been equal to 0.5, rather less in viable microspores as a result of breakage deficiencies caused by crossovers within the inversion. There is no reason to suppose that the survivors are not an equal mixture of **ST** and **NM**. 39% had III + I and 76% of the trivalents classified had a V-form alternate alignment, such that half of the consequent interphase cells and potential spores would be segmentally homozygous. This outcome could have been prejudiced a little by the aberrant anaphase behaviour of excluded univalents.

In summary, from every 100 pollen mother cells there might be expected, from the 57 with 11II, about 50 **ST** and 50 **NM** viable microspores. From 39 with III 9II I there might

Table 2.10. First meiotic anaphase data from 'Lilin' (PL), from ST x PL hybrids and from backcross derivatives of these latter.

Cross	Plants	Cells	Univalents*	Bridges**	U & B
Heterozygotes for translocation:					
PL ***	1	196	52	44	3
Selangor x PL (206)***	9	427	119	82	8
Selangor x PL (1638)	3	40	10	5	0
Selangor x 987	8	179	72	6	0
Selangor x various 3006	-	613	176	74	2
Langet x PL	5	97	24	4	0
Sub-totals Trinidad		1554	453	215	13
Frequencies			0.29	0.14	0.008
Expected for independence					0.047
Samoa 430/433 x PL (Jamaica)	22	353	100	58	5
Frequencies			0.28	0.23	0.014
Expected for independence					0.047
Homozygotes:					
ssp. *malaccensis* x PL	6	267	2	2	0
Frequencies			0.007	0.007	
ssp. *banksii* x PL	4	160	2	5	0
Frequencies			0.012	0.031	

* Based only on "odd" univalents; pairs have been ignored.
** Restricted to bridges with minute fragments or none.
*** Derived from Table 6 of Dodds and Simmonds (1948b).

arise 30 balanced interphases and 60 spores; are these last all or nearly all of **NM** structure? These figures illustrate a possible basis for the preferential inheritance of **NM** but do not yet explain the observed magnitude of the effect. The actual data would require a much higher incidence of V-form trivalents.

The mentioned tables also repeat some heterozygotes from Table 2.2, studied in Jamaica, of ssp. *banksii* from Samoa x PL, now along with the few homozygous **ST** plants. It has to be stressed, however, that the ratio of hybrid to **ST** now seen is a Trinidad result, since the seeds were produced there. It is the high quadrivalent frequency that is a Jamaican manifestation, together with the interesting evidence that this seems to lead to the expectedly more frequent bridges at anaphase. It is unfortunate that backcrosses were not raised from these as well; perhaps the inheritance bias might have disappeared or have been much reduced.

There is yet another interesting corollary to the behaviour of **NM** in its hybrids, to the extent that these show a predominance of trivalents over tetravalents. Should this chromosome race extend its geographic area either to the south or to the north, then this translocation would be preferentially selected and become more widely established at the expense of pre-existing ones. Perhaps it has already done so in the **NM** region itself.

Javanese (JV)

As shown above under hybrids of this translocation, and in Table 2.7, the "backcross" of SH 125 x Langet yielded **ST x JV** plants with a high frequency of quadrivalents, many of them closed rings. Thirteen of these were examined and there was appreciable between-plant variation, such that it was possible to make a fortuitous subdivision into classes with many or with few IV (Table 2.11). The frequencies of arm pairs in bivalents were obviously independent of the multivalent forms or numbers. Very strikingly, on the other hand, the predominant multivalent form determined the level of occurrence of bridges with substantial fragments at first anaphase. These were much commoner in plants where IV's were numerous. Again the suggestion is that in cells with a III, the excluded univalent tended to have a relatively inverted piece. In this case, however, there is no subsequent study to link this univalent with either the **ST** or the **JV** structure.

Table 2.11. First meiotic divisions in translocation heterozygotes of SH 125 x Langet ([JV x ST] x ST). Plants in Jamaica are in groups with many or few quadrivalents and then in a sequence of declining frequencies of IV + III

Plant	Metaphases						Anaphases		
	Total cells	Cells with: IV*	III*	Other	x 2I	"Xta" per II	Total cells	Cells with: u	BF
– 9	14	12	2	**0**	0	1.63	4	0	1
– 20	20	17	1	2	1	1.63	25	1	8
– 30	20	16	1	3	0	1.73	40	9	18
– 6	12	8	2	2	0	1.64	3	0	2
– 19	20	13	3	4	0	1.69	25	0	5
– 16	20	12	1	7	0	1.70	40	4	7
Subtotals	106	78	10	18	1	1.68	137	14	41
Frequencies		0.74	0.09	0.17	0.01			0.10	0.30
– 22	13	5	6	2	0	1.69	20	5	4
– 23	13	1	10	2	1	1.69	17	9	0
– 13	20	2	13	5	3	1.62	25	25	0
– 17	10	4	2	4	0	**1.86**	11	2	5
– 18	9	4	0	5	0	1.69	8	0	3
– 29	4	0	2	2	0	1.65	12	6	1
– 25	20	4	5	11	1	1.76	25	6	1
Subtotals	89	20	38	31	5	1.70	118	53	14
Frequencies		0.22	0.43	0.35	0.06			0.45	0.12
Totals	195	98	48	49	6	1.69	255	67	55

* Types of multivalent: IV: 42 rings (23 alternate) and 56 chains (36 alternate), III: 44 out of 48 alternate (V-form).

Structural change in some other AA cultivars

Three others of these were treated by Dodds (1943), using a classification by "Type" numbers which may have masked their identities. Taking them in increasing order of complexity, and with reference to Dodds' original data, we have the following.

'Palembang' (Type 21, IR 56): one translocation was identified, as III + I only, in 16 out of 36 first metaphases; no bridges were recorded in 79 first anaphases. Male fertility was nevertheless low and some diploid pollen was produced.

'Bande' (Type 22, IR 11): this diploid was already present in the West Indies when the Trinidad collection was started; its Asian provenance is not known to the author. One first metaphase cell out of 16 was said to have two sets of III + I, one had a IV and six had a III. Of 23 first anaphases six had a bridge with fragment and one of these possibly two, apart from laggard univalents. Diploid grains were again evident in the very sparse pollen.

'Sucrier' (Type 19, IR 65): three out of 23 first metaphases showed configurations critical for rings of VI and IV, either IV 2III 6II or 3III 6II I. Two of the IV's were in fact rings, in different cells. 46 first anaphase cells included no less than 22 with one bridge and fragment and seven with two of them. There have been attempts elsewhere to "classify" this cultivar by subspecific origin but no one has considered previously the cytological picture, nor has anyone apparently given weight to the nearly waxless brownish pseudostems. A hybrid involving ssp. *siamea* or ssp. *burmannica* is a strong possibility.

Among other cultivars the author's only detailed record on hand is for the Indian 'Anai Komban' in Jamaica. Again one heterozygous translocation was found, but represented by no less than 16 multivalents among 20 first metaphases. Thirteen of these were IV's including two rings; only seven IV's were alternate in orientation as were the three III's. Inversion-type bridges with fragments were seen in 23 out of 50 cells at first anaphase; all but one of the fragments were small but conspicuous enough. A further author's note, unsupported now by precise data, is that 'Jari Buaya' is also heterozygous for one exchange. Both cultivars once more are virtually male-sterile.

Another useful source of information on AA cultivars is Dessauw (1987), who offered data on frequencies of various associations of chromosomes in four Papua New Guinea clones introduced to Guadeloupe from EMBRAPA's collection in Brazil. He stated that there were either one or two translocations heterozygous in each of them, although there is sadly no mention of critical cells to corroborate this. One clue comes from the absence of any association higher than that of IV. 'Bie Yeng' and NBB11 (SF265), the former without any ring IV, presumably had only one exchange each. No more than one per cell was found of any multivalent type and their summed frequencies were no greater than in 'Lilin' (<0.86/cell). Up to two multiples were recorded in the same cell in 'Towoolie', with a total frequency of 0.95, and in S/N 2 with the very high total of 1.76 multiples per cell, which might suggest three exchanges rather than the two claimed. Dessauw made only brief mention of first anaphase irregularities and without numerical data. He found all five of his AA cultivars to be at least partly male-fertile, with the greatest fertility in 'Lilin' at 67%.

In review we have some information so far on ten different AA cultivars and only one of these has been homozygous in the segmental arrangement of its chromosomes, namely 'Sikuzani', although two others have a single translocation identifiable with that of a wild-type hybrid. A single heterozygous translocation is the common case among the others,

rising to a maximum of three. But gametic fertility in several has been found to be much below the expectation from this cause alone. 'Sikuzani' was unusual in this respect in its pollen mother cells. In those of young buds these demonstrated a quite high level of asynapsis and a consequent low level of pollen production. As the bud aged, first divisions reverted progressively to a normal situation where high male fertility resulted.

Structural hybridity and fertility

Hybrids of wild types

In general, hybrids between wild forms of *M. acuminata* have revealed levels of both male and female fertility compatible with their structural hybridity, either demonstrated or postulated. Fertility may be moderately to very high where only one simple exchange is present but is progressively reduced with two or three of these. The most sterile seen by the author were undoubtedly the few hybrids raised which were heterozygous for **MH**. Another unusual case is taken separately in Chapter 8.

Male fertility tends to follow the same pattern in crosses between wild accessions and the cultivars with the **NM** structure. One practical consequence was that even relatively complex hybrids of this type could serve efficiently as male parents in genetic improvement in Jamaica, providing that pollen was applied in excess of the amount necessary to fertilise the available ovules. 'Tongat' exceptionally was in practice a most difficult breeding parent, because pollen in most of its hybrids was not only again sparse but also performed very feebly in effecting fertilisations. Another notable exception in the 1960's is that recounted in some detail in the next sub-section. From the available evidence it should be surmised that extreme male sterility in diploid cultivars arises principally from causes other than structural hybridity.

Male sterility in hybrids of ssp. banksii Samoa with 'Lilin':

The hybrids in question were those in Tables 2.1 and 2.2 that segregated for the **NM** translocation; it was lamented in an earlier section that backcross progenies were not raised. But these would have been difficult in the specific case because the plants were all in a conspicuous degree male-sterile. In two studies of pollen mother cells, there were either restitution cells or spindle irregularities at the first division, but it became clear that this was not the major cause of the problem.

The answer came from studies of smears of young pollen in several different plants, either soon after meiosis or at an expected intermediate stage of development. In the youngest post-meiotic buds there was a general collapse of the grains, such that few or very few survived up to the half-way stage, approximating to that of the first mitotic division of the gametophyte. Both nuclear and cellular degeneration were observed to be continuing in the oldest samples examined.

Obviously no claim can be made that this is the universal or even a common mode of operation, of factors determining genetic rather than chromosomal pollen sterility of AA

cultivars and their hybrids. Unexpected male sterility had also been encountered in family 3006, part of the earlier story of the inheritance of **NM** in crosses with **ST** Selangor, but these examples were not investigated.

Female fertility in AA cultivars and hybrids

In the first place it is almost a matter of definition that even a diploid banana cultivar ought to be female-sterile; human selection must have favoured any genetic variation that promoted this. Possible mechanisms of action must logically be divided into those that inhibit the production or development of an embryo sac and those which prevent its fertilisation.

From the evidence on the male side in four AA cultivars (Dodds, 1943), meiotic errors should not have been a major general contributor, except in a genotype like 'Sucrier' ("Type 19"). Yet Dodds (1945) found that the structurally more simple clone 'Bande' ("Type 22") also showed over 80% of breakdowns before the first mitotic division of the megaspore. He further disclosed that from two to four competing embryo sac mother cells might be found in the same ovule of some diploid accessions, but these multiples were only frequent in 'Lilin' ("Type 32"), where they accounted for rather more than 40% of ovules.

In the same report, a study of ovules in 'Lilin' two days after pollination revealed an almost total absence of pollen tubes, despite apparently normal growth of these in the styles. Further discussion of these aspects is reserved for Chapter 10.

In practice, female fertility is extremely low in most AA cultivars. For 'Lilin' it is practically non-existent. In 'Tongat', in Trinidad and Jamaica, it corresponded at best to less than one good seed per fruit and there were periods of total sterility, perhaps but not certainly associated with the morphological immaturity seen in stigmas of newly opened female flowers. A wide range of AA cultivar accessions was pollinated at the CNPMF in Brazil in varying degree. Among the most productive of seeds, sometimes at **three or more** per fruit, were 'Gwanhour' and 'Kumburgh' from Papua New Guinea, 'Madu' and 'Sinwobogi' from the (now) FHIA collection in Honduras. Many others were more sparsely or occasionally fertile, but seeds were always scarce in relation to the numbers of ovules available.

CHAPTER 3

M. balbisiana and *M. acuminata*

These two species are generally recognised to be central, separately or jointly, to the evolution of edible *Musa* cultivars (Simmonds and Shepherd, 1955). The objective now is to re-examine what is their degree of reproductive isolation, one from the other. For convenience, and to avoid the frequent repetition of the two species names, the now familiar genomic designations will be used throughout. A is a haploid chromosome set from *M. acuminata* and B is one from *M. balbisiana*, such that an F_1 hybrid is AB and triploid backcrosses are alternatively AAB and ABB.

Once again, the story to be told starts a great many years ago, with some hybrids studied by workers in Trinidad in the 1940's (Dodds and Pittendrigh, 1946; Dodds and Simmonds, 1946, 1948a). The earliest data have been treated in summary form by Simmonds (1954, 1962). He concluded that, although crossing was easy in both directions, seed germination was variable and there was probably a reciprocal difference in vigour of hybrids; those from AA x BB were said to be relatively weakly. It was also inferred by Simmonds in these later reviews that when AB hybrids yielded viable seeds in experimental backcrosses, the resulting plants were pentaploids. However, the AB parents were not listed individually and, in fact, this conclusion was **not** always in accord with the results published earlier and listed in a later section.

These old ICTA data related to crosses with three subspecies of AA, if we include 'Lilin' with one of them, but with only one BB accession among the many maintained in the Trinidad collection of the era. Simmonds' (1954) own comment could therefore have been relevant, that "To what extent the frequency of diploid progeny is characteristic of a whole cross rather than merely of a clone is not known".

Simmonds (1962) also referred to "natural AB hybrids" in an introduction of BB-type seeds from Java. In the author's memory, this was a single plant ("IR 294A") among the ten raised, and not "others". This AB yielded numerous seeds in backcrosses with the parent species and these, as well as yielding pentaploids, on this occasion gave a substantial proportion of triploid plants! Specimen plants of such synthesised AAB and ABB were used by Simmonds and Shepherd (1955) as standards of comparison for the corresponding triploid cultivar groups. Moreover, of course, their ready production lent important weight to the notion of the evolution of hybrid triploid cultivars from AB.

The synthesis of hybrid triploids from AB hybrids had no practical interest at the time as a method of genetic improvement. However, when the Brazilian programme started in 1982, there was immediate interest in the direct generation of AAB from AB. As a consequence there was a further survey of AB hybrids, of such origins as were then available, as potential sources of AAB triploids. Some initial results have been reported (Shepherd *et al*., 1987a) and another "AB" hybrid was identified as a potential source of

synthetic triploids. The plant IAC 2 from the Instituto Agronômico de Campinas was said to be a hybrid of AAB 'Maçã' ('Silk') with *M. balbisiana*. The major emphasis of this chapter, however, is not on AAB production but on the large-scale production of diploid backcross plants, and sometimes fertile ones, from certain AB wild-type combinations. Two distinct BB accessions have given very different results in this respect.

In addition to crosses between the wild species, a further source tested of initial hybrids was one long ago suggested by Cheesman and Dodds (1942). They commented that the diploid hybrids of 'Bluggoe' (ABB) with AA were "slender plants... differing in no essential from the F_1 between *M. acuminata* and *M. balbisiana*", and "one of them backcrossed by *M. acuminata* again gave diploid progeny (four individuals examined)". Later, it was forgotten that these phenotypically AB plants could generate diploid or nearly diploid backcrosses. A survey of meiosis and of hybrid production in 'Bluggoe', as in other ABB cultivars, is reserved for Chapters 10 and 11, but some diploid and near-diploid hybrids form part of the present account.

Plant materials

Trinidad and Jamaica

- *M. balbisiana*: accession IR 100 from Ceylon (now Sri Lanka).
- *M. acuminata* ssp. *malaccensis*: IR 53 from Selangor (also formerly known as "Acuminata A" or Clone A); AA cultivar Lilin;
- *M. acuminata* ssp. *burmannica*: IR 124 (Calcutta 4), IR 132 (Calcutta 6), both from the Calcutta Botanic Gardens but which seem to correspond to South Indian forms of the subspecies;
- *M. acuminata* ssp. *banksii*: IR no longer recalled, from New Guinea; IR 110 from Samoa.

AB group: IR 294A, 'Guindy' as IR 32 or Type 20 of Trinidad and 'Kisubi' from East Africa, these both evidently synonymous with 'Ney Poovan' of India.

Brazil

- *M. balbisiana*: code 06 from the Instituto Agronômico de Campinas (IAC), its Asian provenance unknown; code 07 Butuhan received at the CNPMF in Bahia as seeds from the Philippines; several seedling genotypes of this accession were employed in crosses, perhaps with some minor genetic variability between them;
- *M. acuminata*: these were also seed introductions, from Jamaica, but only one seedling was raised from each of IR 291, 296 and 448:
- ssp. *microcarpa*: code 01 = Borneo IR 291 of Trinidad and Jamaica;
- ssp. *zebrina*: code 02 = Buitenzorg IR 205;
- ssp. *burmannica*: code 03 = Calcutta 4 IR 124;
- ssp. *banksii*: code 04 = Madang IR 448;
- ssp. *malaccensis*: code 05 = Pahang IR 296, code 12 = AA cultivar Lidi (synonym of Lilin).

Pollen of several other AA cultivars was tried in the pollination of *M. balbisiana* bunches, but the only AB hybrid obtained contributes nothing useful to this account.
ABB group: three accessions of 'Bluggoe', codes BL, FC and FV.

Results in Trinidad and Jamaica

Available records of meiosis in AB hybrids from the above sources are set out in Table 3.1. These include not only the plants appearing in early publications from Trinidad, but also two later crosses of the same Ceylon accession of BB (SH 123 and SH 124) and the natural hybrid IR 294A, also studied there. 'Kisubi' was examined in Jamaica, with startling differences from 'Guindy'. Pairing failures were least frequent in plant number 1 of SH 62, Ceylon x 'Lilin' and most common in IR 294A, paralleled only by Dodds' finding for the AB cultivar 'Guindy'. On the whole, these values were not indicative of any high degree of chromosome homology between the species and the frequencies of arms with chiasmata were also low, similar to those in most hybrids of *M. laterita* with other species (Chapter 4). Agarwal (1983) also studied meiosis in AB cultivars in India and reported a range of univalent frequencies from none to 6.11 ('Kunnan' and 'Ney Poovan', respectively). He also noted the presence of trivalents in two of his six clones, but none in 'Ney Poovan'.

The degree of chromosome structural differences between wild forms of the species could well have been variably concealed by the infrequency of crossovers or by their relative locations. There was a conspicuous case in the reciprocal crosses SH 124 and SH 6, where translocation configurations were seen only in the former, as six first metaphase cells out of twenty with a trivalent plus univalent. Logically, they were more conspicuous in hybrids of the Calcutta accessions (Fig. 3.1 A & B). Bridges with a fragment were also noted in six of the seven hybrids that provided a sampling of first anaphase cells.

In two respects, but especially in multivalent frequencies, there is again strong evidence of an environmental effect on meiotic behaviour, as previously mentioned in Chapter 2 with respect to 'Lilin' and its hybrids. In Jamaica, the writer found a lower incidence of univalents in 'Kisubi' than was previously reported for 'Guindy' in Trinidad, despite the inclusion in the later sample of one totally asynaptic cell. Moreover, neither Dodds nor Agarwal (*loc. cit.*) noted trivalents in this cultivar, yet the Jamaican smears abounded with them: six out of twenty cells had two (Fig. 3.1 C) and eight more had one each, for an overall mean of 1.0 per cell. Not a single quadrivalent was seen! With regard to the presence of multivalents in this clone and others of the group, it seems very possible that the known AB cultivars originated only in India and, if local forms of ssp. *burmannica* were involved in their evolution, either the **N1** or **N2** translocation structure would have been incorporated. The 'Kisubi' result is not such a strange one.

The full record for backcrossing, from the data still available (Dodds and Pittendrigh, 1946; Dodds and Simmonds 1948a), is as follows:

SH 6	Ceylon x Selangor	6 pentaploids;
SH 15	Calcutta 4 x Ceylon	sterile;
SH 17	Samoa x Ceylon	9 pentaploids;

SH 21	Calcutta 6 x Ceylon	3 triploids;
SH 62-1	Ceylon x 'Lilin'	sterile;
SH 62-2	"	1 diploid.

These few cases cannot be regarded as a valid survey of AB hybrids as a whole, but instances of triploids and even a diploid were related as occurring among backcross plants as well as pentaploids. The diploid was indeed overlooked by this author until he started to write the current chapter.

Production and assessment of AB hybrids in Brazil

Ease of crossing the wild species and the hybrids resulting

Seeds were not copious in the original crosses attempted and their germination was quite variable, although better where the Butuhan accession was the female parent.

Table 3.1. Meiosis in F_1 hybrids between *M. acuminata* and *M. balbisiana*.
D and S indicate data of Dodds and/or Simmonds; T and J separate the author's results obtained in Trinidad and Jamaica.

		First metaphases*				First anaphases *			
SH	**Cross**	**Cells**	**T**	**x 2I**	**Arm pairs per II**	**Cells**	**BF**	**2BF**	**Other B**
	Female *M. acuminata*:								
124	Selangor x Ceylon	20**T**	1	1.55	1.30	50**T**	8	0	1
15	Calcutta 4 x Ceylon	20**DS**	**2**	1.85	1.02	50**DS**	4	0	1
21	Calcutta 6 x Ceylon	20**S**	2-3	2.30	0.91	34**S**	2	0	3
	Female *M. balbisiana*:								
6	Ceylon x Selangor	25**D**	0!	0.72	1.22	0			
123	Ceylon x Calcutta 4	20**T**	2	1.50	1.16	joint –			
		+23**T**	2	**2.96**	0.93	42**T**	2	0	3
32	Ceylon x New Guinea	31**DS**	1	2.48	?	11**DS**	4	0	1
62	Ceylon x 'Lilin' (four sibs):								
	– 1	28**D**	1	0.18	?	32**D**	0	0	5
	– 3	13**D**	0	0.7	?	0			
	– 4	14**D**	1	0.6	?	0			
	– 5	12**D**	1	0.8	?	0			
	Accession IR 294A	25**T**	0	**4.84**	0.77	50**T**	**17**	0	5
	Cultivar AB:								
	Guindy IR 32	32**D**	**0**	4.38	0.63	0			
	Kisubi	20**J**	**2**	2.85	0.86	14**J**	0	0	1

* T = translocations identified and BF = bridges with fragments.

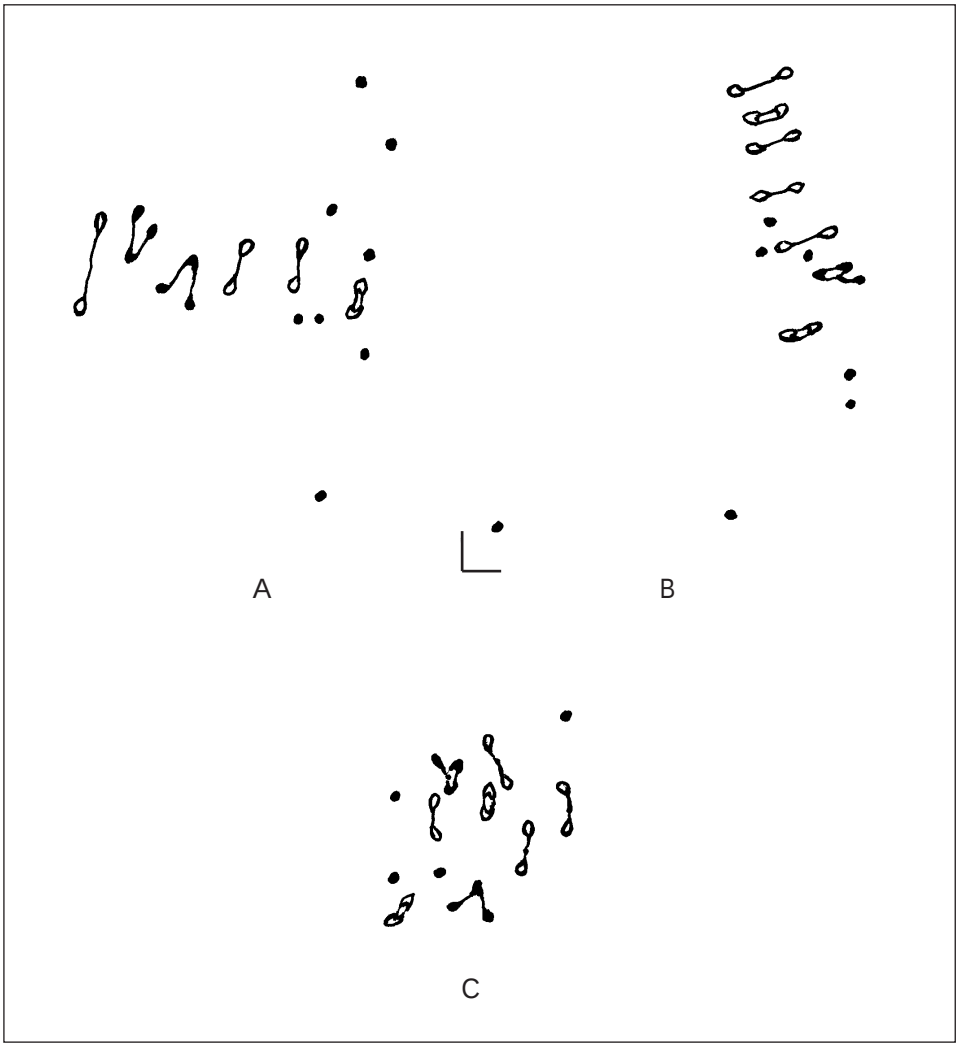


Figure 3.1. First metaphases in AB hybrids Ceylon x Calcutta 4 (A & B) and 'Kisubi' (C).

A: 2 alternate III, 4 II (one ring) and 8 I; B: one frying-pan III, 6 II (two rings) and 7 I;
C: 2 III (one alternate and one frying-pan), 6II (two rings) and 4 I. Approximate scale x1400.

However, in both respects, the situation was much clouded by the frequent presence of spurious plants among those raised (Table 3.2). Numerous plants of AA x BB were identified as AA and many of BB x AA were BB once more. Sundry later attempts to cross Butuhan and other BB female flowers with AA cultivars resulted virtually solely in BB plants. It is hardly believable that these were always errors in pollination; it must at least be conjectured that some wild forms are capable of generating diploid seeds without fertilisation, after cross-pollinations in some degree "illegitimate". This could

Table 3.2. Outcome of crosses attempted in Brazil between two accessions of *M. balbisiana* * and five of *M. acuminata* **.

Cross	Seeds		To	Spurious		Genuine AB plants		
	sown	germ.	field	AA	BB	Nº	Vigour	♀ fertility
Crosses with BB code 06:								
0106	99	32	30	30				
0601	none!							
0206	118	3	1			1	poor	none
"	400	43	19		18	1	slow & poor	unknown
0602	100	3	2			2	both poor	unknown
0306	134	6	5	1		4	all poor	unknown
0603	85	1	1		1			
0406	cross not made							
0604	100	1	1			1	died early	
Crosses with BB code 07:								
0107	100	26	7	3		4	rather poor	slight in one
0701	200	121	20		20			
0207	69	0						
0702	106	66	23			23	poor to good	none
0307	22	3	0					
0703	140	17						
	& cultures	+	25		3	22	mostly good	some in all
"	150	4	3		3		no record	no record
0407	?	?	10	7		3	two good	none
0704	78	10	9			9	mostly good	none-slight
"	?	83!	20			20	mostly good	none-slight
0507	cross not made							
0705	156	50	23		10	13	mostly good	none-slight

* BB: 06 = IAC and 07 = Butuhan;
** AA: 01 = Borneo, 02 = Buitenzorg, 03 = Calcutta 4, 04 = Madang, 05 = Pahang.

have been an echo of Vakili (1967), who recovered diploid BB from BB x BBBB crossings, or of Jamaican experience in the 1960's, where AA hybrids with AAAA similarly yielded many **segregating** diploids. Vakili also considered pollination errors as an explanation but these could not have been a common factor in the current instance, among a great many separate pollinations. Also there was no suggestion of the presence of haploid grains in pollen samples examined from the tetraploids in Jamaica.

The major difference found in vigour of AB offspring was between the two BB accessions employed, codes 06 and 07. No hybrid in either direction of BB-IAC (06) was vigorous (Table 3.2). One of the combination 0206 evidently yielded a few backcross seeds, since a solitary plant from these is on record; it was diploid.

On the other hand, AB hybrids derived from BB-Butuhan (07) were frequently moderately to highly vigorous and rather often female-fertile, in most cases after open pollination in an area where both A pollen and natural pollinators were abundant. Data compiled by a field assistant (cf Table 3.3) even hinted at the occasional presence of very sparse pollen, but this may not be an entirely reliable observation. Of five combinations with different subspecies of *M. acuminata*, that with Calcutta 4 stood out for fertility; it will occupy the principal place in the disclosures of following sections.

Table 3.3. Some characteristics of selected hybrids of BB Butuhan (07) x *M. acuminata* in their first field generation in Brazil.

Cross and plant	Height (m)	Suckers	Bunch angle *	Stalk length (cm)	Hands-Fruits	Fruit length (cm)	♂ **	Good seeds/ fruit
0702:								
01	2.5	4	0	34	7- 87	7	12	0
02	1.8	8	-15	15	3- 24	4	xx	0
11	1.6	4	15	9	4- 32	3	xx	0
12	2.0	5	0	27	5- 40	4	xx	0
16	1.3	2	0	13	4- 43	3	12	0
0703:								
02	2.1	4	0	22	4- 46	8	xx	2.5
03	2.1	5	15	38	7-112	9	xx	5.8
04	2.4	7	15	36	8-125	9	xx	6.9
05	2.5	7	30	20	8-117	9	xx	4.3
10	2.6	7	30	50	8-138	9	xx	4.8
11	2.1	5	45	30	6- 91	10	xx	8.7
12	2.5	9	45	38	8-151	11	xx	3.8
13	2.3	6	15	21	6- 88	13	xx	3.9
14	1.3	2	30	34	5- 63	5	xx	0.9
		This was generally a relatively weak plant.						
15	2.7	8	0	44	8-126	9	xx	5.3
0704:								
01	2.9	6	0	36	8-122	9	xx	0.9
02	2.7	3	0	23	8-112	6	xx	1.0
04	3.0	6	15	20	6- 70	6	xx	0.6
06	2.7	3	0	33	6- 73	5	xx	0
09	3.0	4	15	32	7- 93	5	xx	0
0705:								
01	3.0	1	90?	26	5- 60	4	12	0.1
02	2.5	3	15	34	7- 87	5	12	0
03	2.8	3	15	29	8-111	6	12	0.4
05	2.7	4	0	27	8-122	8	xx	0.3
07	2.9	2	0	28	6- 69	4	12	0

* Estimated as degrees positive below the horizontal and negative above.

** Open-pollinated bunches, denoted as xx, were usually more fertile than those hand-pollinated with 'Lidi' (12).

Table 3.3 also gives some indication of the variability encountered within distinct combinations of 07 with AA, this without considering the occasional very weak plants. Segregation in the Butuhan parent was possibly involved but some of the divergences must surely have been contributed by the A pollen. They affected all the quantitative characters listed and usually also the relative female fertility. The expected correlation of fruit size with seed content was not always conspicuously manifest.

Behaviour of diploid and near-diploid hybrids of 'Bluggoe'

A very high proportion of the crosses made were with pollen of Calcutta (03). This was because of the known greater "potency" of this A source in the procurement of seeds from cultivars, even when compared in Brazil with other fully fertile wild forms. The AB aspect of the hybrids in the field, as claimed by Cheesman and Dodds (1942), was universally confirmed but there was appreciable variation in vigour, plant size and female fertility (Table 3.4). It may be surmised from the known meiotic behaviour of the 'Bluggoe' type that the maternal haploid cells consisted generally of recombinants from two possibly differentiated "B" chromosome sets, but may perhaps have included segments of one or more "A" chromosomes (Chapter 9). "A" chromosomes, or parts of them, should certainly have been a component of the trisomic plants with 23 chromosomes, yet these too included a few that were vigorous and rather female-fertile (Table 3.5).

A small number of selected "AB" genotypes of both chromosome numbers was replanted clonally and, among them, BL03-14 was conspicuous again in its plant size and vigour, forming a massive clump.

An important point to be stressed with all these hybrids is that, despite the occasionally impressive fruit length, there was no hint of parthenocarpy among them. This has seemed throughout to be an A genome characteristic lost in the AB F_1's. Variations in fruit size in the hybrids were very clearly determined in the hybrids by the relative development of maternal tissues other than parthenocarpic pulp.

Backcross ploidy in AB x AA

As already stated, the majority of AB plants was left for open pollination but, both generally and in the particular case of the AB family 0703 (Tables 3.3 and 3.4), these open bunches clearly gave more seeds than those hand-pollinated by 'Lidi'.

Seed germination rates were far from high, which may have owed much to a poor greenhouse environment at the time. There was indeed an evident overall difference between open-pollinated seeds from the 0703 F_1 and the usually later ones derived from 'Lidi' backcrosses, 12% and 23% respectively, as well as the contrasts between individual seed lots.

Reliable chromosome determinations could not be made for all seedlings, either from lack of time or from lack of plant vigour. However, pentaploids were easily identifiable by their physical aspect and almost all possible triploids were verified in root tip squashes, whether or not by exact counts. With a practised eye, hyperdiploid aneuploids were also

Table 3.4. Some characteristics of diploid hybrids of ABB 'Bluggoe' clones crossed with AA Calcutta 4 in Brazil.

Cross and plant	Crop	Height (m)	Suckers	Bunch angle *	Stalk length (cm)	Hands-fruits	Fruit length (cm)	♂ **	Good seeds/ fruit
Area 1:	Planted 1983-84:								
BL03- 07	1	1.7	12!	45	54	6- 80	9	12	1.2
BL03- 07	2	2.4		60		5- 74		12	1.0
BL03- 07	+					5- 75		12	0
BL03- 07	+					5- 69		12	5.6
BL03- 14	1	2.6	5	60	33	5- 81	12!	xx	11.3
BL03- 14	2	3.8!		75				12	0.4
BL03- 25	1	1.4!	8	30	22	4- 46	7	12	3.3
BL03- 25	2	2.5		60		6-100		12	1.7
BL03- 25	+					8-160		12	0.4
FC03- 14	1	2.2	9	60	51	5- 69	6	12	1.0
FC03- 14	2	2.5		75		5- 75		12	0.5
FC03- 14		+				5- 74		12	0.2
FC03- 32	1	2.6	4	90!	55	8- 111	8	12	0
FC03- 32	2	3.2		90!		7- 90		12	0
FC03- 32	+					6- 83		12	0.5
FC03- 47	1	2.0	5	45	25	6- 70	10	12	3.9
FC03- 47	2	3.1		15		7-?			
FV03- 01	1	2.3	10	?	36	4- 53	11	xx	3.1
FV03- 01	2	?		90!		4- 60		12	0
FV03- 01	+					5- 80		12	1.0
Area 2/3:	Planted 1984:								
BL03- 23	1	1.1!	6	45	23	3- 27	5	12	?
BL03- 23	2	2.3		30		3- 28		xx	1.2
FC03- 82	1	2.4	14!	45	37	4- 57	4	12	0.3
FC03- 82	2	3.5		45		5- 78	4	12	0.1
FC03- 82	+			45		5- 71	4	12	0.8
Area 4:	Planted 1984:								
BL03-B19	1	2.0	5	30	38	5- 66	5	xx	5.9
BL03-B19	2	?		15		5- 70			
BL03-B19	+			30		5- 70			
FC03- 93	1	1.7	6	30	28	3- 31	6	xx	1.8
FC03- 93	2	?		30		3- 34			
FC03- 99	1	1.6	9	75	27	4- 49	5	12	0
FC03- 99	2?	1.5!		45		3- 38		xx	0.7
FC03- 99	+					3- 32		xx	0
FC03-103	1	1.6	8	75	23	4- 41	5	12	0
FC03-103	2	2.1		60		5- 72		12	0.2
FC03-103	+					5- 78		12	0.1
FC03-388	1	2.0	10	30	25	5- 70	7	??	?
FC03-388	Bunches pollinated later gave seeds in unknown numbers.								
FV03- 13	1	1.1!	8	45	33	3- 26	6	xx	0
FV03- 13	2	1.9		45		3- 31		04	4.2
FV03- 13	+					4- 35	7	xx	13.4
FV03- 13	+					5- 55		xx	2.8

* Estimated as degrees below the horizontal. ** xx = open-pollinated; 12 = 'Lidi'; 04 = Madang.

Table 3.5. Some characteristics of trisomic hybrids of Bluggoe accessions (2n=23) crossed with AA Calcutta 4 (03) in Brazil.

Cross and plant	Crop	Height (m)	Suckers	Bunch angle *	Stalk length (cm)	Hands-fruits	Fruit length (cm)	♂ **	Good seeds/ fruit
Area 1:	Planted 1984:								
FC03- 16	1	1.5	1!	75	30	4- 33	7	12	0
FC03- 41	1	1.0!	4	45	15	4- 40	3	12	0
FC03- 42	1	2.1	6	75	44	7- 84	8	xx	3.0
FC03- 42	2	3.3		75		5-?		12	0.3
FC03- 42	2 bis					6-101		12	0.2
Area 2/3:	Planted 1984:								
BL03- 20	1	2.7	14!	60	30	6- 71	5	12	0.3
BL03- 20	"1 bis"	2.4		75		5- 66		12	0.1
BL03- 20	2					6- 75		12	0.5
Area 4:	Planted 1984-85:								
BL03- 56	1	1.4	3	30	17	3- 10	3	xx	?
BL03- 84	1	1.6	6	30	38	4- 52	4	xx	0
FC03- 95	1	1.2	2	75	21	4- 50	4	12	0
FC03- 95	2	1.6		60		5- 57		xx	0.5
FC03-111	1	1.6	6	75	36	5- 53	5	12	0
FC03-111	2	2.3		60		4- 58		xx	1.2
FC03-114	1	1.8	4	15		4- 53	6	12	0
FC03-125	1	1.1!	4	0	22	2- 15	2	xx	0.2
FC03-148	1	1.7	4	15	28	5- 51	7	xx	0.2
FC03-148	2	2.9		45		6- 93	6		
FV03- 45	1	1.8	4	15	20	7-100	11	xx	+
FV03- 45	2	2.0		45		7			
FV03- 57	1	2.3	5	0	22	4- 64	7	xx	?
FV03- 63	1	1.6	4	no other data					
FV03- 63	2	2.2		45	31	5- 70	6	xx	?

* Estimated as degrees below the horizontal. ** xx = open-pollinated; 12 = 'Lidi'.

often recognisable by their narrower and thicker leaves. These have been placed jointly with confirmed exact diploids in Tables 3.6 and 3.7, as "±2x". Additionally, it is thought that the greater part of the unthrifty plants, unclassified in these tables, might also have been of this nature.

In any case, as Table 3.6 shows, plants that were approximately diploid constituted much the most numerous class among backcrosses of the Butuhan-07 hybrids. Pentaploids were notably frequent from certain individual F_1's rather than occurring evenly; triploids were very rare.

Regarding backcrosses of the 'Bluggoe' F_1's, it was fortuitously possible to divide the individual AB's into three rather distinct classes (Table 3.7). Some plants yielded a predominance of pentaploids, some gave diploids and pentaploids in about equal numbers and the remainder generated principally diploids. Once more, few triploid

Table 3.6. Germination and ploidy classification of backcross progenies of AB hybrids derived from BB Butuhan in Brazil.

Cross	Plant	♂	Seeds		Numbers of plants per ploidy level			
		*	sown	germ	±2x	3x	5x	?
0107	07	41	?	?	7	0	0	?
0703	01	xx/12	292	65	40	0	6	19
	02	xx	117	45	6	1	30	8
	03	xx	200	31	25	1	0	5
	04	xx/12	296	47	27	0	1	19
	09	xx/12	198	23	13	0	3	7
	10	xx/12	300	67	39	0	11	17
	11	xx/12	250	30	25	1	0	4
	13	xx/12	423	80	49	1	1	29
	15	xx/12	293	61	45	0	2	14
	17	xx/12	212	22	16	0	0	6
	18	xx/12	275	37	25	0	5	7
	19	xx/12	282	41	19	0	3	19
	20	xx/12	500	63	45	2	1	15
	21	xx/12	271	72	6	0	41	25
	others	xx/12	1250	78	34	2	12	30
	sums	xx	3882	468	269	5	58	136
		12	1277	294	145	3	58	88
		xx/12	5159	762	414	8	116	224
0704 x	three	xx	?	?	5	0	1	?
0705 x	two	xx/12	?	?	6	0	0	?

* xx = open-pollinated; 12 = 'Lidi'; 41 = hybrid Calcutta 4 x Madang.

backcrosses were identified, although it may be argued that a plant like BL03-14 could be a source of AAB plants in practice. This is because the visual separation of 3x from 5x is normally easy. It is also possible, as judged from the very limited data, that the backcross seeds from this parent might be easier to germinate (Table 3.7).

Where chromosome counts were achieved, exact "diploids", with counts of 22 in cells of high quality, did not even comprise the majority of the "±2x" class, as Table 3.8 shows in detail. One of the two remarkable seedlings with 21 chromosomes survived for some appreciable time in a pot but died before reaching a size for field planting. Plants with 23 chromosomes were common and possibly constant numbers up to 27 were recognised. Quite evidently, meiosis in the F_1 parents was far from regular and aneuploid megaspores were at least sometimes functional.

Another surprisingly frequent component was of plants with an inconstant chromosome number. Elsewhere, the author and co-workers have disclosed many instances of mitotic instability in triploid cultivars but found none in AA diploid cultivars (Shepherd and da Silva, 1996; Shepherd, 1996). Apparently this is not necessarily the case for the more unbalanced hybrids. Details of counts made in the parent AB hybrids

Table 3.7. Germination and ploidy classification of backcross progenies of AB hybrids derived from 'Bluggoe' in Brazil.

♀ AB	♂ *	Seeds sown	Seeds germ	Numbers of plants per ploidy level ±2x	3x	5x	?
A. **Seedlings predominantly pentaploids**:							
BL03- 14	xx/12	222	128		4	97	27
FC03- 47	12	273	80	1	3	20	56
FC03- 99	xx	24	10	2	1	5	2
FC03-103	12	27	14	1	0	11	2
FV03- 01	xx/12	245	56	0	0	43	13
B. **Seedlings a mixture of diploids and pentaploids**:							
BL03- 20	12	67	10	4	0	4	2
FC03- 42	xx/12	244	54	25	0	18	11
FC03- 82	xx	56	36	13	0	22	1
C. **Seedlings very predominantly more or less diploids**:							
BL03- 07	12	649	132	88	1	1	42
BL03- 25	12	521	25!	15	1	1	8
BL07-B19	xx	300	50	40	1?	0	9
FC03- 77	12	88	13	7	0	0	6
FC03- 82	12	82	19	14	0	0	5
FV03- 13	04/xx	594	167	85	6	5?	71

* 04 = Madang; xx = open-pollinated; 12 = 'Lidi'.

Table 3.8. Numbers of AB x AA backcross plants in Brazil with more or less reliable chromosome counts at or near diploid level.

AB parents	2n constant 21	22	23	24	25	26 & 27	2n inconstant 17 22	18 22	19 25	20 22	21 22	21 23	21 24	22 23	22 24	22 25	23 24	23 25	24 25
Wild crosses: (150 plants – 25 inconstant)																			
0107		2	1									1		1					
0703	1	68	39	6	1	1			1	3		2		10	3	2			
0704		1	1	1															
0705		3											1		1				
subtotals	1	74	41	7	1	1			1	3		3	1	11	4	2			
♀ **BL/FC/FV**:																			
F_1 2n = 22: (135 plants – 32 inconstant)																			
	1	40	43	11	4	1	1	1		1	2	1	1	8	9		6	1	1
												and one with 26-28							
F_1 2n = 23: (10 plants – only one inconstant)																			
		5	2	2										1					

from the 'Bluggoe' type are unfortunately no longer available for verification but examples of apparently inconstant numbers have been found in other triploid x AA crosses (Chapter 10).

Vigour and field performance of AB x AA backcrosses

Altogether, 130 plants went into larger pots, these having Calcutta (03) as the original AA parent. 99 were believed to be exact diploids, one as already said had a count of 2n-1 and 30 had revealed inconstant numbers including cells with 22 chromosomes. Table 3.9 summarises their subsequent history in the hardening shed and in the field, but with inevitable slight inaccuracies; a few plants were surely lost because their planting was delayed by lack of field space. A single diploid plant from 0704, open-pollinated, also reached the field but remained small and finally died.

On a broad survey, those derived from BB-Butuhan x 03 were rather more viable than those from 'Bluggoe' x 03; about one-third of exact diploids reached flowering stage in the former as against one-sixth in the latter.

The detailed observations made on flowering plants form a not easily digestible mass of data and have accordingly been reproduced for reference as Appendix 3.1. Among vegetative characters, there was great variation in general vigour, plant height and robustness and freedom of suckering (from one to seven were noted at first flowering). Bunch stalks ranged from short and slender to long and stout, bunch angles were from sub-pendulous to nearly erect; fruit numbers were from very few to many, their lengths from minute to moderate. Assessments of female fertility, from none to many seeds, may have been affected to some degree by lack of pollination, in the place and period. More important is that some degree of male fertility had reappeared in aproximately half of the plants, although anther content was usually very slight and only once more than 20% of the expectation in a fully fertile plant.

Also conspicuous was the reappearance of A characteristics that are recessive in the AB F_1's. Some observed segregations are listed in Table 3.10, where only the pig-

Table 3.9. Survival and vigour of AB x AA backcross plants in Brazil.

AB parent:		**0703**			**ABB x 03**	
Backcross 2n:		**21**	**22**	**±22***	**22**	**±22***
Potted on:		1	62	17	37	13
	died in pots	1	6	2	7	0
	abnormal		4	1	1	0
	weak		9	4	3	2
	vigorous		43	10	26	11
To field:			40	10	22	10
	died young		7	5	10	3
	abnormal		2	1	1	
	slow or weak		11	1	5	5
	vigorous		20	3	6	2

* 2n inconstant but at least cells recorded with 22 chromosomes.

Table 3.10. Segregations in AB x AA backcross plants in Brazil of some phenotypic characters which separate AA (score 1) from BB (score 5).

Genotypes:	AA	BB	AB	Backcross plants				
Scores:	1	5		1	2	3	4	5
Character:								
Petiole margins	open	closed	3	20	5	4	3!	1!
Peduncle pubescence	dense	none	5	13	5	2	1	8
Male bract scar	high	flat	3?	10	1	16	1	4!
Colour of base inside of bract	yellow	red	5	15		6*		9
Perigonium anthocyanin	none	strong	5	24				6

* One corner was yellowish as in some AAB clones, the remainder was red.

mentation of the inner bract face appears to be a possible monogenic one. For this character, the score 3 corresponds also to some AAB clones and is most likely a modified B character. More dramatic yet was the appearance of a single backcross plant very susceptible to yellow Sigatoka (*Mycosphaerella musicola*).

Interspecific isolation between AA and BB in summary

Up to this point in time, data exist on the breeding behaviour of combinations of five distinct isolates of *M. balbisiana* with different numbers of subspecies of *M. acuminata*. The outcome has been strikingly varied, as follows:

- BB – Ceylon: F_1 can be vigorous; diploid backcrosses seem to be uncommon and pentaploids were the most common component of the few produced;
- BB – Java, AB – IAC 2: F_1's vigorous and female-fertile but only triploids and pentaploids occurred among the backcross plants;
- BB – IAC: F_1 little vigorous and little fertile;
- BB – Butuhan: F_1 often vigorous and female-fertile, quite productive of diploid backcrosses with A pollen; backcross plants varied in vigour but tended towards male and female fertility.

In some cases, an evident possibility exists for the transfer of specific genes from BB to AA, although a comprehensive exploration of this approach, as an enrichment of AA germplasm, would be a major undertaking. In the case of gene transfer from ABB to AA, only one positive B source has been investigated, that of the Bluggoe cultivar. At that it has proved to be a slightly more "reluctant" donor than the Butuhan form of wild BB. Nevertheless, it will be shown in Chapter 10 that some other ABB clones can also produce diploid F_1 hybrids when pollinated by diploid AA forms. These would equally be worthy of study.

CHAPTER 4

Rhodochlamys and *M. flaviflora*; their hybrids with AA and BB

This theme covers an intricate mixture of published and hitherto unpublished facts. Simmonds (1954, 1962) has covered some of the ground but within a very broad canvas of numerous species combinations. The result in the author's view was so condensed as to partly conceal major considerations that he himself hinted at.

For crosses with *M. acuminata*, particularly, this once more concerns his 1954 comment (page 70) that: "To what extent the frequency of diploid progeny is characteristic of a whole cross rather than a clone is not known. Certainly, different clones of various parentage within the cross *M. acuminata* x *M. ornata* showed markedly different frequencies of diploids in their progeny, just as siblings within one family also showed variation in this respect."

When the early part of the research was performed, in the 1940's, there were in fact fewer accessions available than later of *M. acuminata*, of well-defined geographical provenance. For the most part Acuminata A or Selangor became the "standard bearer" for the species and not a truly representative one, as it now seems. Calcutta 4 tended to be used alternatively in crosses because there was still doubt as to its best taxonomic definition. In the case of *M. balbisiana* also, as in other combinations (Chapter 3 etc.), no effort was made to seek differential behaviours between accessions. Only a small number of hybrids was raised and maintained of this species with section Rhodochlamys and the only one on record of *M. flaviflora* x *M. balbisiana* (SH 84) has no surviving data.

The intention here is to present more detail on the published crossing behaviour of the taxa, and to add information on additional hybrids, most especially those of *M. ornata* and *M. flaviflora*. Although important gaps remain in the story, the mutual affinities of the species will be made clearer in consequence; there can even be postulated a strange origin for one species. Otherwise, emphasis is given to possible gene transfer between the species in a breeding programme, rather than to possible natural introgression.

Relevant results come from Trinidad, Jamaica and even some from Brazil and cover a period of over forty years.

Plant materials

Rhodochlamys

In brief definition, these are slender plants of rather short to very short stature with erect inflorescences.

M. ornata: despite Simmonds' (1954, 1962) suggestion of a natural range, it is the writer's recollection that this type was only ever found once in the wild, and this in the 19th century. It has been disseminated widely by way of Botanic Gardens either as vegetative offshoots or as self-pollinated seeds. Variability is not obvious. Introduction IR 1 of the Trinidad/Jamaica collection was already present in the Trinidad Botanic Garden; plants in Brazil, diploid code 08, were acquired in São Paulo State from the Instituto Agronomico de Campinas.

M. velutina: this is described by Simmonds (*loc. cit.*) as native to Assam, where it is probably widespread but little visible in times of drought, to which it has scarcely any tolerance. By its short stature, rapid growth and hermaphrodite basal flower clusters it seeds readily and quickly. In favourable conditions, the furry, red fruits may dehisce at no more than seven to eight months from seed germination. The species is well adapted to the monsoon climatic zone it occupies. IR 212 of Trinidad had later to be replaced and the few hybrids studied must have reflected its lack of dry season vigour.

M. laterita: Simmonds (*loc. cit.*) reports this species only from south-western Burma (Myanmar) but the writer found it in a Thai collection in 1985, whence came the Brazilian specimen. It is also drought intolerant and is marked out by its habit of suckers travelling 1 m or more underground before emerging. The accession most studied was IR 225 of Trinidad/Jamaica.

M. sanguinea: The Trinidad stock was lost in the early 1950's when very few hybrids of it had been achieved.

Eumusa

These are plants of greater and more diverse stature and more robust, with horizontal or pendulous fruit bunches.

M. flaviflora: Three seed accessions entered the Trinidad collection, but from only two areas. These were IR 209 or "Mariani" and IR 241 and 242F, reported by Simmonds (1952) as Assam A and B. For several years their status was in doubt because of their general resemblance to *M. acuminata*, in their plant habit and in the horizontal female phase of the inflorescence. Simmonds then treated them provisionally as belonging to that species, although his results published then, and his others unpublished of the time, revealed lower than expected seed yields in crosses between the new form and a few clones of the better known species. Although a taller plant than any Rhodochlamys, *M. flaviflora* has a common characteristic with at least some of its species in the rich yellow colour of the male flowers. The species hybrids raised and studied in Trinidad were all derived from the Mariani accession, those in Jamaica from IR 241.

M. acuminata: The wild forms used of this species, including those at the CNPMF in Bahia with one exception, were from the Trinidad/Jamaica collection, as follows:

- ssp. *malaccensis*: IR 53 from Selangor, formerly cited as Acuminata A or "Clone A"; IR 296 (CNPMF code 05) from Pahang; IR 474 from Kelantan, with the NM translocation;
- ssp. *burmannica*: IR 124 (code 03) Calcutta 4; IR 132 Calcutta 6 both from the Calcutta Botanic Gardens; IR 187 Long Tavoy from Burma (Myanmar);

- ssp. *siamea*: IR 144 from Annam; code 60 Pa Rayong from Thailand;
- ssp. *microcarpa*: IR 291 (code 01) Borneo (Sabah)
- ssp. *zebrina*: IR 205 (code 02) Buitenzorg from Bogor;
- ssp. *banksii*: IR? from New Guinea; IR 110 from Samoa; IR 448 (code 04) from Madang.

M. balbisiana: One hybrid with *M. ornata* used the accession Brachycarpa (IR 83 from Java) others were with the accession IR 100 from Ceylon (now Sri Lanka).

Vigour, meiotic behaviour and fertility of F_1 hybrids; performance of F_2 or backcross plants

As a preliminary note, hybrids of *M. laterita* as female were difficult material for meiotic study. Either all or the greater part of the pollen mother cells at first metaphase tended to have bivalent or higher associations both "sticky" and clumped on the equator of the spindle. This accounts for the often small numbers of cells listed in Tables 4.1 and 4.2 and very likely led to an underestimate of multivalent frequencies in some cases.

Relationships within Rhodochlamys

The information available is mainly derived from Simmonds (1954, 1962) with expansion of the meiotic data and with some minor refinements from old unpublished records.

In both combinations studied which involved *M. ornata*, there was an evident reciprocal difference in viability and vigour. In relation to *M. ornata* x *M. velutina*, hybrids of *M. velutina* x *M. ornata* were less easily obtained and more difficult to establish as young plants, improving later. The case of *M. laterita* x *M. ornata* was more extreme; the only plant obtained was inviable, while the reciprocal hybrids were finally vigorous after an uncertain juvenile phase. For a third combination, *M. laterita* x *M. velutina* was probably more vigorous than the reciprocal.

At the first meiotic division (Table 4.1), pairing failures were least evident in hybrids of *M. ornata* with *M. velutina* in either direction, but these were heterozygous for probably two inversions. Univalents were particularly conspicuous in *M. ornata* x *M. laterita*, this presumably accounting for the near absence of bridges with fragments at first anaphase. This and the low chiasma frequencies (as ring bivalents) must be responsible also for the absence or scarcity of multivalents at metaphase in all the *M. laterita* hybrids, since this species will later be shown to differ by at least two translocations from the **ST** structure of *M. acuminata*. In the one hybrid of *M. sanguinea*, as female parent with *M. velutina*, pairing failures were in intermediate frequency and no bridges were seen at anaphase.

This last plant was also slightly female-fertile, in contrast to the nearly sterile hybrids with *M. laterita*, and Simmonds (*loc. cit.*) confirmed that the backcross seedlings tested were diploids. Of the F_1 hybrids of *M. ornata* and *M. velutina*, SH 69 which had *M. ornata* as female parent was much more fertile than SH 60 with *M. ornata* as male, the former

Table 4.1. Meiosis in hybrids within Rhodochlamys and between it and *M. flaviflora*. D and S indicate data of Dodds or Simmonds; other entries are the author's results obtained in Trinidad.

SH	Cross	First metaphases *				First anaphases *			
		Cells	T	x 2I	"Xta" per II	Cells	BF	2BF	Other B
	Both parents Rhodochlamys:								
69	*ornata* x *velutina*	20**D**	0	0.30	1.30	49**D**	19	0	1
						+79**S**	21	0	6
60	*velutina* x *ornata*	21**D**	0	0.19	±1.5	47**D**	20	1	0
53	*ornata x laterira*	21**D**	**0**	5.35	?	23**D**	1	0	1
70	*velutina* x *laterita*	15**D**	**0**	1.67	0.97	13**D**	1	0	1
71	*laterita* x *velutina*	9	**1**	0.78	1.24	3	0	0	0
75	*sanguinea* x *velutina*	21**D**	0	0.67	?	50**D**	0	0	0
	From Simmonds (1954, 1962) this may have been the reciprocal cross.								
	One parent *M. flaviflora*:								
104	*flaviflora* x *velutina*	20	?1	0.35	?	50	13	0	6
	Three "possible" IV's seen but these unlikely								
129	*velutina* x *flaviflora*	20	0	0.20	1.57	50	19	0	6
77	*flaviflora* x *laterita*	20	1	1.40	?	50	14	0	15
	repeat	10	**2**	0.8	1.3	50	11	0	4
105	*laterita* x *flaviflora*	12	1	2.00	1.02	53	14	**4**	2
88	*flaviflora* x *sanguinea*	20	0	1.10	1.21	50	23	0	0
79	*ornata* x *flaviflora*	20	0	0.05	**1.86**	50	0	0	1
	No univalents were found at first anaphase.								
101	*flaviflora* x *ornata*	20	0	0.05	**1.93**	50	0	0	0
sib	"	20	0	0	?	50	0	0	0
sib	"	20	0	0	?	50	0	0	5
	The univalent pair at first metaphase appeared to result from precocious disjunction of a II.								

* T = translocations identified and BF = bridges with fragments.

producing both pollen and up to thirty seeds or more per fruit. Simmonds (1953) described SH 69 (*o x v*) as "the most fertile yet found in bananas". 95 backcross plants all gave diploid chromosome counts, as did five plants secured from the reciprocal F_1. Two pentaploids were indeed found later among more than forty F_2 plants he placed in the field. This still contrasted with the occurrence of many pentaploids in backcrosses of other species hybrids, a phenomenon that was given great significance at the time.

About a quarter of the field plants "died before flowering or showed symptoms of unbalance". Of the vigorous ones, about a half seemed to be at least as female-fertile as

Table 4.2. Meiosis in hybrids of Rhodochlamys with *M. acuminata*. D, P and S indicate data of Dodds, Pittendrigh or Simmonds; other entries are the author's results obtained in Trinidad.

		First metaphases *				First anaphases *			
SH	**Cross**	**Cells**	**T**	**x 2I**	**"Xta" per II**	**Cells**	**BF**	**2BF**	**Other B**
1	*ornata* x Selangor	32**DP**	0	1.30	1.18	100**DP**	25	0	2
102	*ornata* x Selangor	20	0	0.28	**1.75**	24	8	1	1
46	*ornata* x 'Lilin'	11	1	2.1	1.07	50	10	0	2
		Metaphase figures were clumped and sticky.							
14	*ornata* x Calcutta 4	23**D**	2	1.83	1.06	56**D**	15	1	8
26	*ornata* x Calcutta 6	20**S**	**2**	1.15	?	52**S**	17	4	2
28	*ornata* x New Guinea	20**DS**	0	1.65	1.05	50**DS**	8	2	2
31	*ornata* x Samoa	20**DS**	0	0.90	1.1 ?	50**DS**	15	3	9
54	Selangor x *velutina*	25**S**	0	1.08	?	51**S**	**8**	**1**	0
		Inversion bridges were unexpected here.							
50	*laterita* x Selangor	6**D**	1	0.7	?	21**D**	0	0	0
		+ 5	**2**	0	?				
66	Selangor x *laterita*	20	2	0.20	?	50	0	0	0
52	*laterita* x Calcutta 4	20**S**	**1!**	0.25	?	50	0	0	0
67	Calcutta 4 x *laterita*	15	**3**	0.00	**1.68**	50	0	0	0

* T = translocations identified and BF = bridges with fragments.

the F_1 and these usually "bore more or less abundant pollen", indicating that "there is no effective reproductive isolation between the species". "About a quarter or more of the plants broadly resembled *M. ornata* (but had paler bracts) but none approached *M. velutina* with its characteristic colour, hairiness and dehiscence of the fruits. Indeed these three characters did not even appear individually."

Hybrids of Rhodochlamys with *M. flaviflora*

In hybrids with *M. velutina* and *M. laterita*, *M. flaviflora* again performed better as the female parent! Vigour of SH 129 (*M. velutina* x *M. flaviflora*), although fair, was less than that of the reciprocal SH 104. Both were partly female fertile, however, either in self-pollination or in backcrossing with the parent species. *M. flaviflora* x *M. laterita* (SH 77) was only fairly vigorous while the reciprocal SH 105 was a poor plant. Only the former produced good seeds on pollination; these were very few and the only backcross plant assessed was a pentaploid.

From reference to Table 4.1, the first meiotic metaphase was much more regular in either hybrid of *M. flaviflora* with *M. velutina* than in the *M. laterita* hybrids. The latter displayed heterozygosity again for translocation with up to two exchanges detectable,

despite relatively low chiasma numbers. As occurred in hybrids of *M. ornata* x *M. velutina*, all four of these hybrids of *M. flaviflora* had frequent bridges with fragments at first anaphase, apparently implying two heterozygous inversions.

The revelation of this series of crosses was that hybrids of *M. flaviflora* and *M. ornata*, in either direction (SH 79 and SH 101), yielded highly vigorous and highly fertile F_1's. Estimates of pollen quality gave values upwards of 95% sound! At the first meiotic division (Table 4.1), frequencies of arms paired were exceptionally high, univalents were rare and **structural hybridity of either type was totally absent**. These plants did not behave in any way as species hybrids! A cross between the two F_1's gave an average of 43 good seeds per fruit in one bunch pollinated.

No F_2 generation was raised with any of these *M. flaviflora* hybrids, but backcrosses were planted of SH 104 and of both SH 79 and SH 101 with each parent species; seed set was moderately prolific in all cases. The numbers of valid progeny and general comments follow:

- SH 104 x *M. flaviflora*: 56 plants were almost all tall and strong, but some suckered poorly and twelve had chlorotic symptoms. Male fertilities were here based on pollen smears and not one out of the 28 plants assessed presented less than about 65% sound; some were nearly fully fertile.
- SH 104 x *M. velutina*: 27 planted included two weaklings and 13 more or less chlorotic. Male fertilities were estimated on anther contents as: two plants nearly sterile, eight with contents about 25%, six about half-fertile and five more up to 75%. This backcross was therefore less fertile than the other.
- SH 79 x *M. ornata*: 41 planted of which one died;
- SH 79 x *M. flaviflora*: 42 planted of which two died and three were weak;
- SH 101 x *M. ornata*: 44 planted and all survived, though a few were weak;
- SH 101 x *M. flaviflora*: 44 planted, three died and six were weak.

Most plants of the last four of these families were extremely vigorous, free suckering and highly fertile. Total pollen abortion was noted, however, in six plants of F_1 x *M. ornata* and one of F_1 x *M. flaviflora*. Ovule abortion was an occasional event in SH 101 and recurred in five plants of SH 101 x *M. ornata*. Apart from these plants and the few weaklings recorded, there was no suggestion of reproductive barriers between the parent forms.

In all six combinations, segregations were examined for a group of interspecific differential characters, more of them in the case of derivatives of SH 104. A scale has been imposed on the observed phenotypes, ranging from 1 = Rhodochlamys to 5 = *M. flaviflora*. Class frequencies are shown for a number of differential characters in Appendix 4.1. Allowing for an undoubted element of subjectivity, it is evident that inheritance was not simple as a general rule. In F_1's x *M. ornata*, particularly, segregations were sometimes less complex than in crosses with Mariani. The remarkable tendency in general was for a proportion of backcross plants to express phenotypes closer to the wrong parent! This could only be a further indication of polygenic inheritance, either of the characteristics themselves or of modifiers.

In backcrosses with *M. velutina*, in contrast with the F_2 of *M. ornata* x *M. velutina* reported by Simmonds (1952), some plants did occur with the fruit pubescence or dehiscence of the Rhodochlamys species, once jointly in a generally low scoring segregate, but the typical fruit colour of the species did not reappear. It should be further noted that, as in the case of the F_2 of *M. ornata* x *M. velutina*, a few plants of SH 104 x *M. flaviflora* displayed bract colours in some degree reminiscent of *M. ornata*; some others had bracts resembling those of SH 79 or 101.

Only one instance, excluded from Appendix 4.1, appeared to offer monogenic ratios. This is a common and distinctive characteristic of both *M. velutina* and *M. ornata*, where the base colour of the inner face of the male bracts, in parts without anthocyanin, is a dull white, labelled as "ivory", as distinct from the normal range of yellows from creamy to even orangeish. Sometimes, when the anthocyanin pigment came close to the base in mature bracts, the base colour was more evident in slightly immature ones. The F_1 hybrids and the backcrosses to Rhodochlamys always echoed the Rhodochlamys parent. Only a very few backcross plants with *M. flaviflora* presented intermediate scores but a contemporary note emphasised that these were slight quantitative differences and never qualitative. The major gene discrimination was therefore clear enough. From the backcrosses of the two interspecific hybrids, there came the following segregations:

SH 104 x *M. flaviflora*:	24 ivory	:	21 yellow
SH 79/101 x *M. flaviflora*:	39 ivory	:	29 yellow
Totals	63	:	50

Despite the slight excess of ivory in both types of cross, the fit for a 1:1 segregation is evident. This is one of the rather few clear instances of a simple dominant gene that the author has encountered in the genus.

A hybrid swarm of *M. flaviflora* and *M. velutina* was in fact found by Simmonds (1956, 1962), growing alongside *M. flaviflora* in Assam. There were indications that natural backcrossing and introgression were occurring. For Simmonds (1962, page 65) the example "serves to emphasise the significance of *M. flaviflora* as a a connecting link between Eumusa and Rhodochlamys". The writer sees these relationships as more complex, as shall be discussed at the end of this chapter.

The hybrid *M. flaviflora* x *M. sanguinea* revealed rather low chromosome pairing at meiosis (Table 4.1), as well as the expected inversion or inversions. No record is available of its relative fertility.

Hybrids of Rhodochlamys with *M. acuminata*

Data on the first meiotic divisions, where reference is made, are to be found in Table 4.2.

M. ornata and *M. acuminata* in Trinidad and Jamaica

Simmonds (1954, 1962) stated that crossing of *M. ornata* with *M. acuminata* was easy and seed germination was good; this is in part contrary to the CNPMF experience related below. Hybrids in Trinidad could be vigorous except that in one instance, of Calcutta 4 with *M. ornata*, those with ssp. *burmannica* as female parent were narrow-leaved and

weak. This was confirmed in Jamaica and there was a very similar deficiency in ssp. *siamea* x *M. ornata*. The phenomenon clearly parallels the reciprocal differences found in crosses within Rhodochlamys, suggesting a cytoplasmic effect, but one that was later seen in Brazil to be very variable in intensity.

Meiotic cytology was studied only with *M. ornata* as female parent, with wild representatives of three different subspecies of *M. acuminata* as well as with 'Lilin'. Heterozygosity appeared for the expected numbers of translocations, based on known differentiation within *M. acuminata* (Chapter 2). Pairs of univalents were common, but for the quite exceptional case of the repeat cross of *M. ornata* x Selangor (SH 102). They were usually more numerous than in hybrids of *M. ornata* with *M. velutina*, but always many fewer than in *M. ornata* x *M. laterita*. Ring bivalents were also very frequent in SH 102 while in other hybrids there were many less. Bridges with fragments were always found at first anaphase including those recorded for SH 102, a fact confirming that this plant was indeed of the correct parentage. In five of the seven cases, there was at least one cell with two of them, indicating two inversions as in the hybrids already discussed of *M. ornata* with other Rhodochlamys species. Two unusual A_1 cells in *M. ornata* x 'Lilin' had a laggard trivalent, one of them flanked by a fragment corresponding to each persistent chiasma (Fig. 4.1). Evidently, one of the inversions derived from *M. ornata* is in a chromosome homologous to one of the **NM** quartet of AA.

Another plant of *M. ornata* x 'Lilin' was unexpectedly triploid. Only five clear first metaphases were seen, with from seven to ten trivalents and balancing numbers of bivalents and univalents. In 21 other cells, univalents ranged up to a maximum of seven. The mean for all 26 cells was of 3.62 univalents, so that the surprisingly high mean of seven to eight trivalents per cell can be assumed. A few cells underwent "cryptic restitution", where nuclear size increased without condensation of the chromosomes.

In the author's view, Simmonds (1954, 1962) overstressed the massive production of pentaploids in backcrosses of F_1's; this was in fact a characteristic of one particular combination with Selangor (Dodds and Pittendrigh, 1946). Of greater significance must be the occurrence of balanced diploids in the second generation and the following backcross data found in old files portrayed high diploid frequencies:

- F_1 *ornata* x Calcutta 4: 57 diploids, no polyploids;
- F_1 *ornata* x Calcutta 6: 46 diploids and 1 pentaploid;
- F_1 *ornata* x New Guinea: 46 diploids, no polyploids;
- F_1 *ornata* x Samoa: 14 diploids, 2 pentaploids.

No F_2 or backcross plants were raised in the field, but it seems likely that hybrids with ssp. *burmannica* could have given rise to either natural or contrived introgression between the taxa, and that a similar result would be possible experimentally with ssp *banksii*.

M. ornata and *M. acuminata* at the CNPMF in Brazil

Here, a greater range of crosses was tried but the restrictions of available time have left uneven results. Some hybrids were studied more intensively than others and there were no analyses of meiotic behaviour.

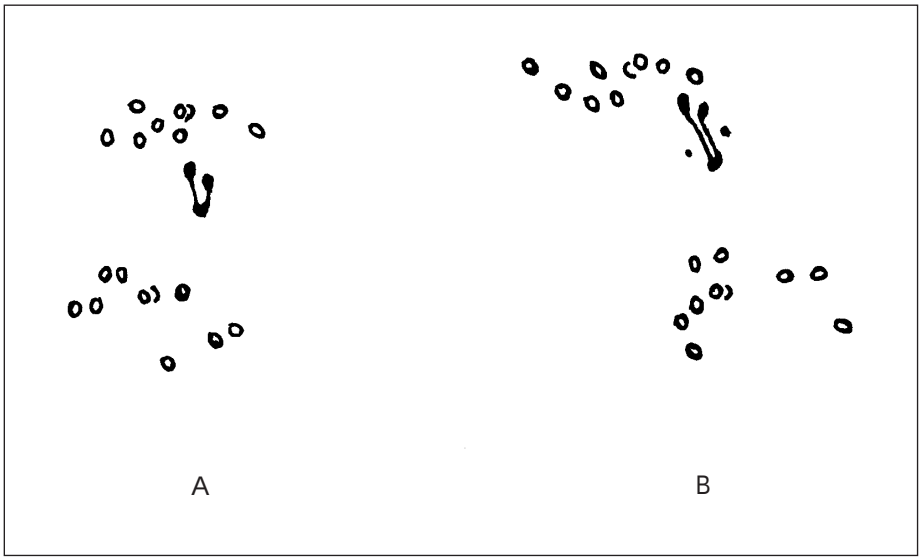


Figure 4.1. Persistent trivalents at first anaphase in *M. ornata* x 'Lilin'.

A: lagging III but without evidence of inversion; B: lagging III and evident inversion fragments (one minute) associated with each side of the figure. Approximate scale x2000.

In general, crosses with *M. ornata* as female were **not** easy to achieve, although this could well have been a consequence of a dry climate at the time. Many bunches failed to set seeds, including some self-pollinated ones. Germination capacity was very variable and often poor, whether in the greenhouse or in the embryo culture routine. In the latter process, of a total of 314 apparently sound seeds, from all but one of the lots received in the laboratory, 37% lacked both embryo and endosperm and a further 43% with embryos were also without endosperm. Only 17 plantlets resulted. As a contrast, the other lot was of 120 seeds all with fair to good endosperm content, although nine were without an embryo. A total of 67 or 56% of these germinated! In all, hybrids were obtained for planting from four combinations with different subspecies, to add to three interesting reciprocal combinations. For three out of the seven, some physical characteristics of F_1 plants were recorded at the CNPMF during 1984-85. However, only those grown in relatively good environmental conditions are included in Table 4.3.

With* M. ornata *as female

Male parent ssp. *malaccensis* Pahang (0805): five of the seven hybrids planted were surprisingly unvigorous. The other two were from 2.0-2.5 m in stature; inflorescences seen were of 18 fruits in four female hands and of about 30 fruits in five hands; male flowers were five and eight per cluster, respectively, and had pollen at about 15-20% of potential content.

Male parent ssp. *burmannica* Calcutta 4 (0803): the only plant of this cross was only semi-vigorous and was suckering poorly when inspected in the field, quite different from the performance of SH 14 in Trinidad.

Male parent ssp. *siamea* Pa Rayong (0860): the only plant was extremely vigorous and formed a large clump. Three plants seen on the same date, of about 2.5 m in stature, bore semi-erect bunches with between 12 and 42 fruits in three to five hands. All contained seeds in variable quantities but pollen found was less than 10% of the potential.

Male parent ssp. *zebrina* Buitenzorg (0802): one of the four hybrids died and three varied in vigour up to very good. They flowered first at about 1.5 m with sub-erect inflorescences of 23-30 fruits in four to five hands. The two best were female-fertile and had estimated pollen contents of 15-20 and 25%. One of these was recorded as having an exceptionally tall although slender plant at 3.7 m, among its many ratoon followers (Table 4.3).

This hybrid was "backcrossed with *M. acuminata*" in open pollination; A pollen was abundant in the area as were insect pollinators (small stingless bees). Of 262 seeds sown 26% germinated and the plants recovered appeared to be all more or less diploids. Counts in root tip cells of twelve comprised seven with 22 chromosomes and **five with 23 or 24**. These Brazilian plants furnished the first record of aneuploid offspring from the second generation of such hybrids; they were not noted in the early studies. One seemed to be inconstant in chromosome number, with counts ranging from 22 to 24.

With* M. ornata *as male

Female parent ssp. *malaccensis* Pahang (0508): this was the most extreme case yet seen of possible cytoplasmic incompatibility. The several plants in the greenhouse all had their leaf laminas very much reduced, irregularly so as far as the midribs; such lamina pieces as were more developed left space still for chlorotic streaks.

Female parent ssp. *microcarpa* Borneo (0108): these plants were of normal appearance and seventeen were planted, but the second lot of seven were in a relatively poor field environment and are not treated here. All of the first ten were vigorous but they presented a varying number of chlorotic streaks in the older leaves. They also varied appreciably in plant height, in the ratio of circumference to height, an indication of relative slenderness, in the angle of bunch (measured as degrees above the horizontal) and and in numbers of hands and fruits. Details are given in Table 4.3. In the case of numbers of fruits per hand, it was noted that in a hand with up to six fruits these were uniseriate, as in *M. ornata*. Where there were eight or more, they were at least partially biseriate.

None of the ten had a pollen content better than sparse and eight of them bore not a single seed, among 363 fruits pollinated by Borneo and 731 open pollinated. A ninth plant was slightly female-fertile, with 21 good seeds among 162 fruits, but plant number 10 was more fertile yet with an average of 3.2 good seeds per fruit in three bunches pollinated by Borneo. Thirty plants of this backcross were examined in a greenhouse, but without chromosome counts. Except for four possible aneuploids once more, they had the expected aspect of diploids.

Table 4.3. Some phenotypic parameters of *M. ornata* - *M. acuminata* hybrids at the CNPMF in Brazil.

Plant	Plant characteristics*					Pollinated bunches*		
	Crop	Ht(m)	Cf/Ht	Suckers	Angle	No.	Hands	F/Hand
0802 - *M. ornata* x Buitenzorg:								
1	1st	1.6	0.15	++	-75			
	2nd	**3.7**	0.11		0	2	5.5	7.0
0108 - Borneo x *M. ornata*:								
1	1st	2.8	0.10	+	?			
	2nd	2.9	0.10		-15	6	5.8	5.8
2	1st	1.8	0.16	8	0			
	2nd	2.3	0.13		-30	3	5.0	6.3
3	1st	1.4	0.16	7	-60			
	2nd	2.0	0.14		-45	5	5.8	5.7
4	1st	1.8	0.12	5	-30			
	2nd	1.9	0.12		-45	6	4.5	5.0
5	1st	2.3	0.12	8	-60			
	2nd	**1.7**	**0.16**		0	3	4.7	6.0
6	1st	2.1	0.10	8	0	2	6.5	7.5
7	1st	**3.0**	**0.08**	6	?			
	2nd	**3.2**	**0.08**		-30	3	5.0	6.3
8	1st	1.8	0.13	11	-30			
	2nd	1.8	0.16		0	6	5.7	6.5
9	1st	**1.2**	0.16	10	-30			
	2nd	2.0	0.15		-60	4	5.8	7.0
10	1st	1.5	0.15	**16**	-30			
	2nd	1.9	0.15		-15	8	6.8	7.0
0408 - Madang x *M. ornata*:								
1	1st	not recorded						
	2nd	**3.1**	**0.12**		-75	(5	30)	
2	1st	1.6	0.17	**16**	no further record			
	2nd	1.7	0.18		-15	(5	28)	

* Ht = plant height to point of emission, Cf = circumference at 30cm from the ground, bunch angles are in degrees above the horizontal; F/Hand = mean number of fruits per hand.

Female parent ssp. *banksii* Madang (0408): two of the three F_1's in the field were in good growing conditions and both became very large and vigorous clumps; the first also became quite tall (Table 4.3). This one was also conspicuously the more fertile with a pollen content of about 50% of that of a fully male-fertile plant; seeds were also numerous. Only a few backcross plants were inspected, these from open pollinations, and they again seemed to be diploids or nearly so.

M. velutina and *M. acuminata*

The only combination ever raised to flowering was of Selangor x *M. velutina*. Simmonds (1954, 1962) says that hybrids were relatively easily obtained in both directions, but these were weak in the juvenile phase. The survivor was fairly vigorous at maturity. His meiotic data revealed a fairly high pairing frequency, with an absence of multivalents but with a moderate frequency of bridges and fragments at anaphase.

As Simmonds (1962) also reports, there was appreciable female fertility in this hybrid, but of the spurious kind found with *M. ornata* x Selangor. In this instance, all 102 backcross plants produced were pentaploids.

M. laterita and *M. acuminata*

Again, Simmonds (1954, 1962) states that hybrids were easily obtained, adding however that those with Calcutta 4 were less vigorous than the ones with Selangor. The hybrid of *M. laterita* x Kelantan in Jamaica was also vigorous. From memory, an extra point of some interest is that no hybrid of *M. laterita* with species of sections Rhodochlamys or Musa, whether in Trinidad, Jamaica or Brazil, displayed the habit of offshoots wandering underground.

At the first meiotic division, it is significant that pairs of univalents were much less frequent than in hybrids of *M. laterita* with other Rhodochlamys species or with *M. flaviflora* (Tables 4.1 and 4.2). In the plants reliably recorded for arms paired, these were also conspicuously more numerous in hybrids with *M. acuminata*. Translocation hybridity was more readily interpretable in crosses with the species as male parent, because of the technical difficulties mentioned at the head of this main section. There appeared to be two heterozygous in the hybrids with Selangor, three in that with Kelantan, but only three again in Calcutta 4 x *M. laterita*! One is missing and the possibility must be considered that these two parents have their modified structures partially in common, at least in relation to the chromosome arms involved.

A pollen assessment by the writer from SH 50 (*M. laterita* x Selangor) was of anthers about 20% full with mostly sound grains, but with a wide size range; actual fertility may have been about 15%. The three interspecific hybrids tested in Trinidad were all at least slightly female-fertile but with poor germination rates; the writer's information confirms Simmonds (1962) in that the backcross hybrids obtained were solidly diploids, as follows:

- *laterita* x Selangor: 1 good seed per fruit, no plant raised;
- *laterita* x Calcutta 4: up to 3 good seeds/fruit, 9 x 2x;
- Calcutta 4 x *laterita*: up to 9 good seeds/fruit, 47 x 2x.

A small number of backcross plants of SH 67 (Calcutta 4 x *M. laterita*) were screened in the field in Jamaica. They varied appreciably in both vigour and fertility; details have been lost but individuals could have been selected that excelled in both aspects.

M. laterita x Calcutta 4 was repeated at the CNPMF. The three plants raised varied in size and vigour, and in fertility. The best made a large clump with plants up to about 1.8 m; pollen in the male clusters of twelve flowers was again in the region of 15-20% and seeds were also evident in the bunches of few hands and six to eight fruits per hand in two rows.

Hybrids of *M. flaviflora* with *M. acuminata*

Records have survived from only two instances of attempts to secure hybrids of this combination with the first species as male parent. The combination of Selangor x Mariani (SH 81) was successful but in Calcutta 4 x Mariani the plants were scarcely viable. This difference in behaviour matches the parallel crosses with *M. ornata*.

Even SH 81 was less vigorous than the reciprocal hybrid SH 80. The latter displayed almost wholly regular metaphases at the first meiotic division but univalent pairs were quite frequent in the former. These also occurred in varying numbers both between and within other pairings of *M. flaviflora* female with four different subspecies of *M. acuminata* (Table 4.4), except for one plant of the cross with ssp. *malaccensis* from Kelantan. They were conspicuosly frequent in one plant out of three analysed with ssp. *microcarpa* (Borneo) as male parent.

This lessened homology was also reflected in fewer arms paired and, in the crosses with Long Tavoy and Annam, in a deficiency of translocation multivalents. Presumably

Table 4.4. Meiosis in hybrids of *M. flaviflora* with *M. acuminata*.
T and J separate the author's results in Trinidad and Jamaica.

		First metaphases *				First anaphases *			
SH	**Cross**	**Cells**	**T**	**x 2I**	**"Xta" per II**	**Cells**	**BF**	**2BF**	**Other B**
80	*flaviflora* x Selangor	20**T**	0	0.00	1.58	joint:			
		+20**T**	0	0.10	1.62	62**T**	25	2	4
sib	"	20**T**	0	0.10	?	100**T**	29	0	10
81	Selangor x *flaviflora*	125**T**	0	0.62	1.4 ?	50**T**	8	0	11
-	*flaviflora* x Borneo	20**J**	0	0.35	1.43	50**J**	12	1	6
sib A	"	30**J**	0	0.40	1.41	50**J**	23	3	2
sib B	"	40**J**	0	**1.50**	**1.15**	50**J**	19	3	4
		This plant was unexpectedly almost male-sterile.							
-	*flaviflora* x Kelantan	20**J**	1	0.20	1.59	37**J**	20	3	3
		2 fragments were "minutes" of NM structure.							
sib A	"	7**J**	1	0	1.6	40**J**	11	2	2
		1 anaphase had 3BF; 3 fragments were "minutes".							
sib B	"	20**J**	1	0.30	1.42	48**J**	18	5	1
		Only one fragment was of "minute" type.							
sib C	"	16**J**	1	0.56	1.42	16**J**	6	1	1
		In the cell with 2BF one fragment was "minute".							
100	*flaviflora* x L. Tavoy	20**T**	**1!**	1.05	1.17	50**T**	9	0	1
112	*flaviflora* x Annam	30**T**	**2**	0.80	1.30	50**T**	21	2	6
98	*flaviflora* x B'zorg	20**T**	1	0.70	1.35	50**T**	16	**16**	8
		This material was unusually difficult and two other sibs in Jamaica gave no useful M_1's.							

* T = translocations identified and BF = bridges with fragments.

for the same reason, bridges with fragments at anaphase varied in numbers. Cells with two of them were exceptionally common in the hybrid with Buitenzorg, but none had the three or more theoretically expected of this cross, in relation to the **JV** structure shown in Chapter 2. A single cell with three was finally discovered among 87 from two new and additional hybrids examined in Jamaica.

Male fertility was much the highest in the two Borneo hybrids that had relatively good chromosome pairing; counts from pollen smears revealed 60% and 50% of sound grains in these two, compared with less than 10% in the deficient one.

There was also variability in the female fertility of the F_1 plants assessed in Trinidad. The hybrids with Long Tavoy and Annam were totally female sterile in pollinations attempted. Seeds were few in backcrosses of the Buitenzorg combination and at least some pentaploid offspring resulted. Most interesting was again the difference in behaviour between SH 80 and SH 81. The former was much more fertile and is only known to have yielded diploids in the second generation; the corresponding result from SH 81 was a mixture of diploids and pentaploids.

Backcross plants were examined in the field from both SH 80 and SH 81, the latter restricted to the F_1 x Selangor combination. The numbers planted and their relative levels of vigour (scored on a range of 0 = dead or feeble to 4 = excellent) were as follows:

- SH 80 x Mariani: 42 plants of which five died or were very weak but six were excellent (mean score 2.2)
- SH 80 x Selangor: 46 plants of which four soon died and five remained feeble but seven were excellent (mean score 2.1)
- SH 81 x Selangor: 28 plants of which six soon died and one was both poor and chlorotic; only one was marked as excellent (mean score only 1.7).

Male fertility of many plants was generally estimated on the basis of pollen quantity and varied appreciably:

- SH 80 x Mariani: six high to very high, five fairly high, six moderate, three low and three very low or virtually nil;
- SH 80 x Selangor: fifteen high, nine moderate and five low;
- SH 81 x Selangor: only two high, six moderate, seven low and one very nearly sterile.

The SH 81 backcross was therefore clearly less fertile on average than the others. Microscope examination of some pollen samples from SH 80 x Mariani did not always agree with the above estimates, but early pollen abortion was a likely cause of discrepancies. Four of the seventeen samples contained giant grains.

As Appendix 4.1 shows, there were once more several instances of phenotypes varying in the wrong direction. On mean expressions there were some surprising differences between the reciprocals in their Selangor backcross progenies; subjectivity may have played some part in this. The general impression is of polygenic inheritance of the differential characters studied; despite this conclusion, glabrous peduncles may have been a simple dominant with pubescent ones recessive. This has been suspected in some intraspecific crosses within *M. acuminata*, but is not the case, apparently, in *M. flaviflora* x *M. velutina*.

Hybrids of Rhodochlamys with *M. balbisiana*

Simmonds (1954, 1962) regarded hybrids of BB with *M. ornata* as "weak or even inviable as seedlings or at maturity". Crosses with *M. laterita* were slightly easier and with *M. sanguinea* barely possible. As for the first, the writer's experience with a few plants of BB Butuhan x *M. ornata* at Cruz das Almas was in good agreement; they were very slender dwarfs with depauperate inflorescences if any.

Yet it was possible to study meiosis in Trinidad in a few plants, as was done by Dodds and Simmonds (1946) for BB x *M. laterita*, where they stressed the presence of pollen mother cells with giant nuclei and no evidence of the meiotic process. But the current wish is to examine chromosome behaviour in these and for crosses with two other species of Rhodochlamys (Table 4.5). So far as the limited results go, chromosome pairing was least poor in the sole hybrid with *M. ornata* and was very much restricted in the others. There can be little doubt that this reduced homology caused the difficulty found in identifying translocations in the *M. laterita* hybrids.

Table 4.5. Meiosis in hybrids of Rhodochlamys with *M. balbisiana*. D and S indicate data of Dodds and/or Simmonds; other entries are the author's results obtained in Trinidad.

		First metaphases *				First anaphases *			
SH	**Cross**	**Cells**	**T**	**x 2I**	**"Xta" per II**	**Cells**	**BF**	**2BF**	**Other B**
7	BB B'carpa x *ornata*	22**DS**	0	1.77	?	13**DS**	2	0	0
89	BB Ceylon x *velutina*	20	1	3.20	0.92	40	0	0	0
90	*velutina* x BB Ceylon	20	1	3.75	0.84	34	0	0	0
51-1	BB Ceylon x *laterita*	25**DS**	**0**	4.52	±0.7	?			
sib 3	"	50**DS**	**2**	4.94	?	?			
sib 4	"	21**DS**	**1**	4.62	0.56	?			
sib	"	14	**2**	4.86	0.61	12	1	0	0
138	*laterita* x BB Ceylon	10	1	2.8	0.9	8	0	0	2
55	BB Ceylon x *sanguinea*	20**DS**	0	4.70	0.64	20**DS**	0	0	0
sib	x	30	0	**9.18**	**0.18**	-			
		Only one ring II was seen; the A_1 was chaotic.							

* T = translocations identified and BF = bridges with fragments.

Interspecific affinities or isolation and the taxonomic scheme

Thus far, the treatment of this chapter has followed pre-existing taxonomic lines, starting with section Rhodochlamys and continuing with its relationships with section Musa, as represented successively by *M. flaviflora*, by *M. acuminata* and by *M. balbisiana*. It now remains to scrutinise the information reviewed above in relation to this preliminary background.

In the first place there has to be considered the status of *M. ornata*. It has been shown that, except fortuitously for a quite small number of backcross plants, it is not isolated in any way from the morphologically very different *M. flaviflora*, neither in

chromosome structure, where they have inverted segments in common, nor in general chromosome homology, nor in the vigour and fertility of two hybrid generations. Both species tend to make better hybrids when they are employed as female parents in crosses. *M. ornata* possesses an unusual bract pigment dominant gene in common with *M. velutina*. In the F_2 of the hybrid between these latter species and in backcrosses, especially that of (*M. flaviflora x velutina*) x *M. flaviflora*, bract colours reminiscent of *M. ornata* reappeared.

These facts call to mind the remark of Simmonds (1962): "Thus the ill-known Rhodochlamyds in the **Flora of British India** may owe some of their confusion to hybridity rather than to imperfect description; further, it is not impossible that one or more of the four species assumed here to be "good" may be of hybrid origin". Here he has correctly predicted, with near certainty, the nature of *M. ornata*, as a relic of a hybrid swarm between *M. flaviflora* and *M. velutina*!

Simmonds' (*loc. cit.*) suggestion of *M. flaviflora* as a link between the sections surely holds good for *M. velutina*, on the evidence now available. This conclusion so far excludes any attempt at hybridisation of the latter with a wide range of forms of *M. acuminata*, and in both directions. It may be predicted that some would be fertile in the sense of providing a diploid second generation, but it is also unlikely that such hybrids would be as fertile as SH 104 or 129. On the most pessimistic estimate, it may be that gene transfer from the ornamental species to the more useful one, in terms of the pursuit of genetic improvement of edible bananas, might only be possible indirectly.

In the case of *M. laterita*, on the contrary, *M. flaviflora* affords no semblance of an "inter-sectional" link. The former in effect is as much removed from the latter as it is from the other members studied of section Rhodochlamys, whether in structural hybridity, in homology of the chromosomes or in fertility of the hybrids. *M. laterita* is more allied indeed to at least one subspecies of *M. acuminata*, one moreover which is more nearly sympatric and which may even share a common chromosome structural change.

The relationships of *M. sanguinea* are less well known for want of appropriate hybrids, but it appears to fall closer to *M. velutina* than to any other species here discussed.

On all the evidence submitted, the status of Rhodochlamys must be revised from the phylogenetic scheme as proposed by Simmonds (1962, page 72); it cannot any longer be thought of as a single evolutionary divergence. Rather, instead of the whole section figuring on one side of *M. acuminata*, beyond *M. flaviflora*, it appears to be divided into at least two very distinct parts, one of which certainly belongs "on the other side" of *M. acuminata*, away from *M. flaviflora*, *M. ornata* and *M. velutina*!

Furthermore, it is not possible to regard the Rhodochlamys species most intensely studied as quite distant genetically from *M. acuminata*. Surely they are less so than such forms of section Musa as *M. balbisiana*, *M. itinerans* and *M. basjoo*, for instance, and others even more remote. For the first of these three named, we have found in Chapter 3 that the degree of affinity may depend partly on the geographical origins of the specific parents in crossing, as has also been found in Rhodochlamys hybrids. Information of this kind is lacking for the others (Chapter 6). They too would merit further research involving a wide range of hybrids with as many accessions as possible of *M. acuminata*.

CHAPTER 5

M. schizocarpa and *M. acuminata*

Simmonds (1956) first described *M. schizocarpa*, as a distinct species of section Musa native to Papua New Guinea, soon after his visit there as part of a collecting mission in 1954-55. However, it may have been present earlier and lost in the ICTA collection in Trinidad. Perhaps this was the basis of his remark (1962), that one species was "discovered (or rediscovered)" at the later time. Up to that year, data were inexistent as to its place in the section, although Simmonds placed it close enough to *M. acuminata* in his evolutionary diagram.

Argent (1976) has distinguished two forms, the shorter-statured of them probably extending into West Irian. He also reported the existence of natural male-fertile hybrids between this species and *M. acuminata* ssp. *banksii*, which he believed to be most likely F_1 hybrids. He concluded that this must be a not uncommon occurrence since the species are often in mutual contact. This is of course very relevant to the context of this chapter. Very recently, data have been reported on reaction to yellow Sigatoka disease of some other such hybrids, collected in Papua New Guinea as part of an activity organised in 1988-89 by the then International Board for Plant Genetic Resources (Daniells *et al.*, 1996).

The plants in this study were obtained from two seed lots sent by Simmonds to Trinidad in 1955, but enormous difficulties arose because of the material's extreme susceptibility to Panama disease, despite the relatively *Fusarium*-unfriendly nature of the soils there. Not more than two or three plants survived to their first flowering and not one matured a fruit bunch. Even so, a few immature but viable seeds were recovered from the only inflorescence that was pollinated. The hybrids, including one with *M. flaviflora*, were lost before much of a useful study could be made.

Pollen from the new species was used more successfully on other forms and it was a matter of accident, rather than intent, that the surviving hybrids were all with forms of *M. acuminata*. This was also in the era of the transfer of banana research from ICTA in Trinidad to the Banana Board in Jamaica; accordingly, almost all of the hybrid seeds were first sown in 1959 at the Bodles Banana Breeding Station, where research on the resulting plants was accomplished between 1960 and 1965.

Plant materials

M. schizocarpa: The accessions were IR 440 and 441 in the Trinidad/Jamaica register, but only brief mention can be made of plants from the latter number.

M. flaviflora: The sole hybrid lot was with pollen of IR 209 from Mariani.

M. acuminata: Seeds and hybrids arose from the application of pollen of *M. schizocarpa* on ssp. *banksii* IR 448 from Madang, ssp. *microcarpa* IR 303 from Sarawak,

ssp. *malaccensis* IR 53 from Selangor ("Clone A"), ssp. *burmannica* IR 124 or Calcutta 4 and from two distinctive collections of ssp. *siamea* from Thailand, IR 403 or "Siam" and IR 476 or "Thai".

Hybrids raised in Trinidad

Selangor IR 53 x *M. schizocarpa* IR 441

Pollination number 8133 of May 1957 yielded an average of 11.6 good seeds per fruit and 66 out of 100 germinated. Only five went to the then restricted field space; two died, two contracted *Fusarium* wilt and the fifth was maintained clonally for a time as SH 152. It was a slender, rather unvigorous plant even shorter in stature than its female parent. It was noted as having anthers about one-third full and a similar estimate was made of good grains in a sample examined. 31 cells at first meiotic metaphase included twelve pairs of univalents; arm pairs per cell averaged 1.51. 36 first anaphases appeared regular out of 48 seen, four had a lagging bivalent and laggard univalents showed a mean of 0.27; no bridge was observed. These values are within the range found in Jamaica for plants from the cross IR 53 x IR 440.

Calcutta 4 IR 124 x *M. schizocarpa* IR 440

This was a sampling of seeds from the same pollination number 8192 later raised in Jamaica. Seeds were very numerous and 43/50 germinated, from which five were put out, all fair to excellent in vigour. One was selected and transferred to Jamaica clonally as SH 153. It was almost male-sterile. Physical data obtained from it in Trinidad have been excluded, since they were surely obtained in a different environment. It is however included with the newer plants in respect of its meiotic behaviour and female fertility, studied in Jamaica.

M. schizocarpa IR 440/441? x *M. flaviflora* IR 209

No record remains of numbers of seeds or germinations, nor of field performance except that there were at least two plants, one of which was replanted clonally but lost. An attempt was made to study meiosis in pollen mother cells but first metaphases were unclear. It seemed that chiasma frequency was high. Not surprisingly for a hybrid with this male parent (Chapter 4), bridges with fragments were present at first anaphase.

Hybrids raised in Jamaica

Numbers and vigour of F_1 plants

The families planted and their general behaviour are summarised in Table 5.1. They were in an area previously abandoned for the pollination of 'Gros Michel', although the spread of attack by *Fusarium oxysporum* f. sp. *cubense* (hereafter abbreviated to *Foc*) in that

Table 5.1. Summarised performance of F_1 hybrid families in Jamaica of six forms of *M. acuminata* crossed with pollen of *M. schizocarpa*.

Subspecies Clone	IR	Family	Planted	Early death	Later death	Total *Foc**	Data** taken	General vigour
banksii								
Madang	448	8211A	3	0	0	0	3	very good
microcarpa								
Sarawak	303	8411	17	2	1	2	12	very good
malaccensis								
Selangor	53	8412	35	7	3	10+	14	poor to fair
burmannica								
Calcutta 4	124	8192	31	6	0	11	21	survivors good
siamea								
Siam	403	8429	67	3	13	30	19	poor to fair
Thai	476	8423	14	0	0	0	14	very good

* *Fusarium oxysporum* f. sp. *cubense*. ** Most frequently for estimated pollen content and female fertility but sampled also for other characteristics, as shown in subsequent tables and in Appendices 5.1 and 5.2.

cultivar had been relatively slow by Bodles standards. The final distribution of the pathogen might still not have been very uniform, but it must be at least suspected that the pathogen was the unrecorded cause of some of the plant deaths noted. Also, root and rhizome infections could have contributed to the lack of vigour in families with IR 53 and IR 403. In the family with IR 124, on the other hand, the sharp line of demarcation between sick plants and good ones strongly suggests that the cross segregated for resistance. The most conspicuously vigorous combinations were those not or scarcely affected by the pathogen, those with IR 448, IR 303 and IR 476.

Genetic variability within the F_1 families

Ranges within families for some quantitative characters are set out in Appendices 5.1 and 5.2. As conceded in these, there were no reference plants of the parent *M. acuminata* forms placed in the same field environment as the hybrids. Even the data included from other sources are either incomplete or very approximate. Nevertheless, it is evident that no F_1 was uniform in its quantitative data, either for vegetative or inflorescence parameters. Since *M. schizocarpa* itself has hermaphrodite basal flowers and must be commonly self-pollinated in nature, the assumption is that most of the variability sprang from the *M. acuminata* parents. Ssp. *banksii* must also be commonly self-pollinated, from anthers in the basal hands, and the cross of IR 448 should therefore have been more uniform than the others. It was unfortunate that this cross could not have been better represented in number of plants.

As a general observation, the relatively massive plant size of the male parent was not well reflected in the first vegetative generation of the hybrids, but became conspicuous in the second. Not only was plant height much augmented but also length of the leaf lamina, so much so that the length/breadth ratio of these was substantially modified. One characteristic which remained more or less constant between crops was male bract shape.

There was a lamentable omission in the study of qualitative characters; no note survives of the inheritance or not of hermaphrodite basal flowers in the F_1's. From memory, however, there was no need for emasculation of these in the production of backcross seeds. Provisionally it is assumed that anthers were rare or absent. Relative uniformity or variability for other qualitative features defies simple tabulation and apparent segregations are outlined in the following account for the various crosses.

IR 448 x IR 440:

– only ratoons were examined but the three plants were closely similar in most details; plant number 2 suckered rather poorly;

IR 303 x IR 440:

– peduncle pubescence: present in both parents but hybrids varied from densely pubescent to glabrous;

– male rachis colour: purple flush in IR 303 variably restricted in the hybrids;

– inner male bract face pink-flushed in IR 303, varying from strongly to weakly pigmented in the hybrids.

IR 53 x IR 440:

– redness of vegetative parts (characteristic of IR 53): intense in four, moderate in seven and little in four;

– peduncle pubescence: dense in IR 53, moderate in one hybrid but usually sparse or virtually absent;

– male bud scored as fat in four and slender in ten;

– male bud colour: reddish in five, purple in three and bluish in five;

– male flower teeth: orangeish in three and yellow in seven;

– stigma colour of these: orange in five, yellow in eight, quite pale in one;

– additionally, one plant presented a strange, twisted bud with the bract tips not appressed.

IR 124 x IR 440:

– peduncle pubescence: diverse classifications from densely hairy to glabrous;

– male rachis: cushions purple-rimmed in three, green in seven;

– male flower teeth: orange in four, orange-yellow in three, yellow in three (one quite pale);

– stigma colour: bright orange in six, pale orange in three and very pale in one.

IR 403 x IR 440: `

– peduncle pubescence: dense in fifteen, short and moderate in five, sparse in five;

– male bract colour: sharply and conspicuously segregating with twelve having dark bracts and fifteen quite pale;

– the inner face colour was rather variable within the "dark" class.

IR 476 x IR 440:

– peduncle pubescence: unclassifiable but varying in length and colour;

– male bract yellow tip spot: from very pronounced to moderate;

– male bract inner face colour: strong in three, moderate in eight and weak in two;

– free tepal apiculus: minute, triangular and transparent in nine, variably larger in five;

– pink spot on ovaries: conspicuous in five, absent in nine.

Meiosis in pollen mother cells

An important general result was that *M. schizocarpa* quite evidently shares the Standard (ST) arrangement of *M. acuminata* in respect of its chromosome arms. Further, although bridges and fragments were registered in some plants of all crosses, they were neither frequent nor consistently present and do not suggest any large or significant differential inversion.

For convenience then, at least for the first metaphase stage, hybrids with different translocation types are here analysed separately. Where multivalents are indicated, it must be stressed also that these are Jamaican data. As mentioned in Chapters 2 and 3, there exists at least a suspicion that multivalent formation was more frequent in the Bodles environment than it was in Trinidad.

Table 5.2, therefore, shows first metaphase events in crosses with **ST** clones, together with the relatively uncomplicated first anaphases that ensued. Chiasma frequencies were consistently at a high level in hybrids with ssp. *banksii* and ssp. *microcarpa*,

Table 5.2. Meiosis in pollen mother cells of F_1 hybrids with *M. schizocarpa* of forms of *M. acuminata* having ST translocation structure.

Subspecies & IR		First metaphases *				First anaphases *			
	Plant N°	Cells	T	x 2I	"Xta" per II	Cells	U	BF	Other B
banksii IR 448									
	sum 3	60	0	0.07	1.74	126	0.01	5	2
Notes: a) the two pairs of univalents in plant 1 appeared to be early separations of bivalents; a further sample of 30 cells had none; b) bridges with both large and small fragments implied distinct very small inversions.									
microcarpa IR 303									
	sum 9	180	0	0.04	1.71	296	0.05	5	5
Note: two anaphase cells had numerical discrepancies between poles (12-10).									
malaccensis IR 53:									
	# 2	20	0	0.45	1.54	36	0.97	0	1
	#24	20	0	0.95	1.45	50	0.66	1	2
	#27	20	0	0.15	1.70	50	0.20	0	0
	#31	20	0	0.90	1.35	50	**1.42**	0	0
	#33	20	0	0.55	1.40	50	0.58	0	0
	sum 5	100	0	0.60	1.49	236	0.75	1	3
Notes: a) the least pairing found was a cell of #24 with 7II 8I; b) plants ##27 and 33 were the two most ♂-fertile of these five.									

* T = translocations identified, U = laggard univalents per cell and BF = bridges with fragments; pairs of univalents at M1 and U are given as frequencies per cell.

comparable with those in crosses within *M. acuminata* (Chapter 2). In the five plants studied of the cross of IR 53 with *M. schizocarpa*, however, they were strangely inconsistent and often rather low; this deficiency was also reflected in the larger numbers of pairs unsynapsed. Again, the Selangor accession showed itself to be not the best representative of its species! Exceptionally, the frequencies per cell of univalents at A_1 are also shown and for these last hybrids particularly, they sometimes showed an even higher frequency than at M_1, logically only a sampling difference.

Table 5.3 deals with the M_1 stage in hybrids with the two *M. acuminata* parents of the **N1** translocation class, Calcutta 4 or IR 124 and "Siam" or IR 403. While two plants of the former were relatively deficient in multivalents, the two crosses gave closely similar high average numbers otherwise. On the whole, quadrivalents were much more common than trivalents; closed quadrivalent rings were few and seemed to be more numerous in the Siam cross. The two crosses were again alike in their frequencies of arms synapsed other than in multivalents.

The other cross involving ssp. *siamea* very evidently was heterozygous for three translocations (Table 5.4), although the third was hidden in one of the five plants analysed, the one converting a ring of four into a theoretical ring of six. Perhaps in concordance, the aberrant plant also had the lowest mean number of arms paired in potential bivalents, which were otherwise again high. As noted in Chapter 2, the other four plants provided the only evidence on hand that identified IR 476 as belonging to the **N2** group.

First anaphases in crosses with **N1** and **N2** *M. acuminata* strains, as shown in Table 5.5, displayed the errors expected of such material. They tended to high frequencies of

Table 5.3. First meiotic metaphases in pollen mother cells of F_1 hybrids with *M. schizocarpa* of forms of *M. acuminata* having the N1 translocation structure.

Subspecies & IR	Plant	Cells	Translocations*					Groups		x 2I	"Xta"
			3	2	1	0	Mean	IV	III	**	per II
burmannica IR 124:											
	# 7	20		4	10	6!	0.90	11	7	0.30	1.79
	#16	20		10	10	0!	1.50	22	8	0.25	1.72
	#17	20		11	8	1	1.50	23	7	0.40	1.64
	sum 11	206	0	81	102	23	1.28	180	84	0.21	1.68
	cf SH 153 ***	20						19	3	0.05	1.58
Note: only 15 of the 180 quadrivalents were closed rings.											
siamea IR 403:											
	# 3	20		8	9	3	1.25	14	11	0.20	1.69
	#51	20		11	8	1	1.50	22	8	0.20	1.58
	sum 5	100	0	41	53	6	1.35	91	44	0.19	1.69
Note: only nine of the 91 quadrivalents were closed rings.											

* Cells in which these numbers were identified.

** Pairs of univalents as frequencies per cell.

*** Meiosis studied in Trinidad, all the others in Jamaica.

Table 5.4. First meiotic metaphase in pollen mother cells of F_1 hybrids of ssp. *siamea* Thai (IR 476 - N2 structure) with *M. schizocarpa*.

Plant	Cells	Translocations *					Groups					x 2I	"Xta"
		3	2	1	0	Mean	VI	V+I	2III	IV	III+I	**	per II
# 1	19	4	11	3	1	1.95	2	3	1	18	7	0.26	1.59
# 7	16	**0**	4	12	0	1.25	0	0	0	13	7	0.38	1.47
# 9	20	5	8	7	0	1.90	2	5	1	15	7	0.25	1.64
#12	20	3	11	5	1	1.80	4	1	0	21	5	0.20	1.61
#13	13	2	5	5	1	1.62	1	2	0	9	6	0.23	1.61
sum 5	88	14	39	32	3	1.52	9	11	2	76	32	0.26	1.59

Notes:

a) no hexavalent was a closed ring and only one was an alternating chain;
b) nine out of the 76 quadrivalents were closed rings.

* Cells in which these numbers were identified.
** Pairs of univalents as frequencies per cell.

Table 5.5. First meiotic anaphases in pollen mother cells of F_1 hybrids of *M. acuminata* ssp. *burmannica* and *siamea* with *M. schizocarpa*.

Plants studied	Cells	11-11	12-10 *	Univalents **					BF ***	Other B
				1	2	3	4	mean cell		
♀ ssp *burmannica* Calcutta 4 (IR 124):										
11	283	187	13	59	14	3	5	0.41	5	4
Note: sizes of fragments with the bridges were not noted.										
♀ ssp *siamea* Siam (IR 403):										
5	177	122	10	31	9	1	1	0.32	3	3
Note: fragments with bridges were all small.										
♀ ssp *siamea* Thai (IR 476):										
5	217	137	22	52	4	0	1	0.29	5	2
Note: one fragment was large and four small.										

* A heading of convenience to include various numerical discrepancies.
** Cells in which these numbers were identified.
*** Bridges with fragments.

univalents not reaching the poles and to lack of numerical balance between poles, the latter no doubt partly resulting from an irregular alignment of multivalents.

Male and female fertilities of the F_1 progenies

As estimated from anther bulk contents, male fertilities were distinctly variable within the larger hybrid families (Table 5.6) and, inexplicably, between different samplings from the same genotypes. Of the three sets with **ST** strains, only that with ssp.

Table 5.6. Estimates of anther content of F_1 plants, as percentages of the expected content of a fully fertile standard.

Crop	Contents found in sequence of plant numbers
♀ ssp *banksii* Madang (IR 448):	
	1 2 3
2nd	25 35 25
"	**60**
♀ ssp *microcarpa* Sarawak (IR 303):	
	1 2 3 5 6 7 8 10 12 13 15
1st	50 75 75 35 25 60 25 80+
2nd	25 60 **100** 35 **15** 25 50 **15** **100** 25 30
♀ ssp *malaccensis* Selangor (IR 53):	
	2 5 6 8 9 11 19 24 27 28 29 31 33
1st	15 30 5 5 **0** 15 15 50 **80** 60 10 50
2nd	**50** 30 25 15 15 **50** **100** **10** **40**
♀ ssp *burmannica* Calcutta 4 (IR 124):	
	2 3 4 5 6 7 8 9 10 11 12 14 15 16 17 18
1st	15 <10 10 15 <10 15 0 20 15 10 <10
2nd	0 <10 0 10 15 <10 <10 0 <10 15 10 10 20 15 <10 <10
"	0 10 20 0 <10 <10
	19 20 23 24 25 26
1st	25 0 0 0 15
2nd	15 <10 15 15
♀ ssp *siamea* Siam (IR 403):	
	3 8 12 15 21 27 28 36 39 41 45 50 51 54 56 57
1st	**50** **<10** 25 25 **50** 30 30 25 25 **10** **50** **10** 30 15 20
2nd	40 **0** **10** 20 **50** 40 25 25 40 15 25
	60 65 67
1st	**10** **10** 15
2nd	25 30 40
♀ ssp *siamea* Thai (IR 476):	
	Of nineteen estimates made from fourteen plants in two crops, none exceeded 10% and some verged on nil.

malaccensis from Selangor included nearly male-sterile plants as well as highly fertile ones. Translocation hybridity clearly intervened as expected in the **N1** and **N2** crosses, but some of those with ssp. *siamea* IR 403 appeared to be quite surprisingly fertile, with up to 50% of the content of a "full" anther.

An additional feature of studies on these hybrids was that some few tests were done on pollen potency, that is, on comparative seed sets in fruits of the *M. acuminata* parent

when pollinated alternatively by F_1 hybrids or by AA sources. As Table 5.7 indicates, setting was sharply reduced when the pollen came from either of two sparingly male-fertile F_1's of IR 124 x *M. schizocarpa*, but was still of a sufficiently high order to permit the production of backcross populations by this route.

There were general tests of female fertility in the hybrids. These again included assessments of pollen function in that self pollination was compared with pollination by either the *M. acuminata* parent or by a plant taxonomically allied to this (Table 5.8). Much the highest level of female fertility was found in the hybrids of ssp. *microcarpa*. It was fair in the combination with ssp. *banksii* and rather poor in the others. It quite evidently varied between plants within a family. Seed germination was sometimes inefficient, but without any certainty now existing of the uniformity of the greenhouse environment. The selfs were apparently not inferior in viability to the backcrosses.

Table 5.7. Relative yields of hard, black seeds (G) and others (B), in seven hands/blocks of randomised four-fruit plots of Calcutta 4 with pollen from the same, from ssp. *microcarpa* (Borneo IR 291) and from two F_1 hybrids of Calcutta 4 x *M. schizocarpa*.

Block	♂ IR 124		♂ IR 291		♂ 8192-5		♂ 8192-6	
	G	B	G	B	G	B	G	B
1	204	45	144	98	6	5	77	26
2	225	59	162	106	19	10	29	10
3	204	74	165	83	93	36	44	18
4	172	75	105	96	36	30	15	16
5	325	64	291	127	44	10	75	11
6	220	97	248	129	15	14	13	13
7	297	72	178	164	105	59	103	43
Sums	1647	486	1293	803	318	164	356	137
Means	235	69	185	115	45	23	51	20
Per fruit	59	17	46	29	11	6	13	5

F_2's and backcrosses in the field

This stage was handicapped by a shortage of adequate field space, having had to face strong competition from AA hybrids and tetraploids in the mainstream of the genetic improvement programme. The area allocated in fact was very uneven in quality of growing conditions, so prohibiting more than approximate conclusions as to relative plant vigour. Regrettably, records of assorted plant characters are not on hand, a deficiency also reported in the case of hybrids in the same area derived from IR 124 x *M. laterita* (Chapter 4). In the present instance, the lowest numbers of plants were derived from the F_1's with ssp. *banksii* and the greatest numbers from the ssp. *microcarpa* hybrids; an old field plan gives only some indication of basic performance, as summarised in Table 5.9 for these last only. Plants "without record" were in the least favourable locations.

Table 5.8. Means of hard, black seeds per fruit (G/F) and germinations from F_1 hybrids of *M. acuminata* x *M. schizocarpa*, either self-pollinated or back-crossed to the female parent (or to a related form indicated as R). Values are given for all plants pollinated and for the most female-fertile.

Parents & plant(s)	Self-pollinated *				x AA parent			
	F	G/F	Sown	Germ.	F	G/F	Sown	Germ.
ssp *banksii* x *M. schizocarpa*:								
sum three	402	15.7	200	6	240	29.6	200	14
					245R	31.8	none	
ssp *microcarpa* x *M. schizocarpa*:								
sum twelve	1250	22.9	1851	492	1967R	51.4	1700	281
- 5	139	40.3	200	25	176R	72.9	200	8
ssp *malaccensis* x *M. schizocarpa*:								
sum nine	976	1.7	892	142	962	13.9	1197	247
- 27	149	3.5	505	100	117	22.8	300	70
ssp *burmannica* x *M. schizocarpa*:								
sum twelve	1939	1.8	1445	339	2005	15.1	2024	266
- 5	190	2.9	295	43	189	20.6	200	64
ssp *siamea* (IR 403) x *M. schizocarpa*:								
sum fourteen	768	2.5	642	314	871	12.3	none	
-21	89	4.1	200	112	none			
Note: backcross pollen was often omitted in error.								
ssp *siamea* (IR 476) x *M. schizocarpa*:								
sum twelve	1166	1.4	709	165	1251	13.8	1000	204
-9	32	4.9	100	14	34	22.8	100	21

* Pollen from a sib had to be used sometimes instead of the same F_1.

Table 5.9. F_2 and backcross plants of ssp. *microcarpa* x *M. schizocarpa*: incidence of deaths, disease and polyploids; vigour of healthy surviving plants on a scale from 1 - feeble to 5 - excellent.

Plant class	Numbers planted	Without record	Died	*Foc*	3x	5x	Vigour 2x plants				
							1	2	3	4	5
F_2	96	13	25	5	1	1	5	22	14	8	2
Backcross	146	16	12	9	1	0	7	19	19	35	28

Foc once more intervened commonly in F_2 progenies and sometimes in backcrosses. The triploid recorded in the table was the only one identified among over four hundred established plants, as compared with ten confirmed or probable pentaploids. Backcrosses were as expected better on average than F_2's, but the latter included an appreciable number of either moderately or highly vigorous plants. At least a proportion was male-fertile in either type of plant.

Male sterility was encountered in a few plants, both F_2's and backcrosses, derived from ssp. *burmannica* Calcutta 4 x *M. schizocarpa*. In attempts at meiotic analysis only one plant gave any reliable first metaphase data, without revealing any obvious cause of total pollen failure.

Taxonomic implications

Even with the limited direct surviving evidence for the second generation in the field, it seems very likely that no critical isolation barrier exists between *M. schizocarpa* and *M. acuminata* ssp. *banksii*. While Argent (1976) concluded that the natural hybrids "were not observed to intergrade" with either parent, he also thought that some forms of the latter "suggested a limited introgression". It would be surprising if this did not in fact occur, particularly as a result of the fertilization of F_1's by the parent species. The hermaphrodite basal flowers of the latter might be an effective barrier in the other direction. Is it merely coincidence that some New Guinea strains of ssp. *banksii* (but not IR 448 or its hybrids) have also been found to be very highly susceptible when exposed to *Foc*?

However, the subspecies of *M. acuminata* found to be evidently the most compatible, in crosses with *M. schizocarpa*, was not the sympatric ssp. *banksii*. It was ssp. *microcarpa*, unless it is a mistake to regard one single accession as representative of a subspecies, as much as it clearly is in the case of the entire species.

Our overall phylogenetic chart has become more complex yet with the conclusions of this chapter. Referring again to the one proposed by Simmonds (1962): if the important criteria are taken to be the degree of chromosome homology, including differentiation by translocations and inversions, and the general vigour and fertility of diverse hybrids between the species, then *M. schizocarpa* is closer to *M. acuminata* than is *M. flaviflora* or either "half" of section Rhodochlamys, as defined in Chapter 4. It may be asked whether any two-dimensional chart will ever suffice to convey the relationships between the species!

CHAPTER 6

Three more species of section *Musa*

The heading is not meant to imply that the three species to be discussed have much in common. Rather, this is a space for tidying up on some 22-chromosome species about which there is limited definitive cytogenetic information, namely, *M. basjoo*, *M. itinerans* and *M. ochracea*.

For simplicity, the first two can be treated together. Simmonds (1962) pointed out that they "cross (albeit with difficulty) and give slightly fertile hybrids that show remarkably high chromosome pairing". As he further stated, these interspecific hybrids were only vigorous with *M. basjoo* as female parent. The relative affinity of the species was despite their marked divergence in plant habit and general appearance. The degree of dispersal achieved by vegetative offshoots of *M. itinerans* has to be seen to be well believed; it is of an order distinctly greater than that shown by *M. laterita*.

Few viable hybrids were ever achieved between either species and others of the sections *Musa* or Rhodochlamys, but Simmonds does make a possibly important point that diploid backcross plants were recovered from backcrossing of *M. balbisiana* x *M. basjoo*. Here we can do no more than present greater detail on first meiotic divisions in relevant species hybrids, including some results not known to the earlier author.

M. ochracea, the most recently described species to enter this treatise, was received as seeds in Trinidad in 1959 from India, shortly before the final departure of Simmonds from Trinidad and from active banana research. He passed them to the author with the comment that the seeds could not be of any banana species as they were much too small. This was a point on which we readily agreed. They were smaller yet than those of *M. halabanensis* (Meijer, 1961), which this last had sent us a year or two sooner. However, it was certain that they ought to be sown as a measure of confirmation of their irrelevance and, in the impending transfer of all banana genetic research to Jamaica, that is where the accession went to germinate and be planted out.

The outcome of this was a new and strange addition to section *Musa*, for which there has not been established up to now any exact natural distribution within India. Its laterally developed and transversely corrugated petiole bases, the four-rowed ovules in each loculus of the ovary and the very long-stalked, obovate fruits are among the other unusual characters that go along with the ochreous yellow aspect of the vegetative parts, many of them well illustrated by the Kew artist who assisted with the publication (Shepherd, 1964).

In the fields at Bodles it displayed one unfortunate characteristic, that it would not set seeds with any foreign pollen, for reasons never elucidated. Perhaps germination or tube growth from such pollen was in some way inhibited. For this reason, our knowledge of its place in the scheme of section *Musa* has so far had to depend on only two interspecific crosses obtained with its pollen.

Plant materials

M. basjoo: The accession IR 78A in the Trinidad/Jamaica register came from the Liukiu islands of Southern Japan, being therefore the most northerly in distribution of *Musa* species, although a strain of *M. balbisiana* is also known from there (sometimes wrongly referred to as "*M. liukiuensis*"). Reflecting the subtropical habitat of *M. basjoo*, there is a thriving plant of it in a garden near to the author's present home in Cascais; the only other banana genotype seen to survive well outdoors in the town is the ABB cultivar Bluggoe.

M. itinerans: Apparently identical plants in Trinidad came from two lots of seeds, Tagwin 3 and Tagwin 4 from Burma (IR 183 and IR 184); the latter was most used in crosses but they have not been differentiated in the results reported here.

M. ochracea: IR 558 received in Trinidad but sown in Jamaica.

M. acuminata:

- ssp. *malaccensis*: IR 53 from Selangor;
- ssp. *burmannica*: IR 124 or Calcutta 4, from India;
- ssp. *microcarpa*: IR 291 or Borneo, from Sabah.

M. balbisiana: only IR 100 or Ceylon, from the now Sri Lanka;

M. flaviflora: IR 209 or Mariani for hybrids produced in Trinidad; IR 241 for those with *M. ochracea*, studied in Jamaica.

M. velutina: IR 212 in Trinidad.

Results

Meiosis in hybrids of *M. basjoo* and of *M. itinerans*

Summarised data are presented in Table 6.1 where, in the first place, the remarkably good chromosome pairing at M_1 in the hybrid between the above species is further emphasised. However, so far as could be judged from the old notes and drawings inherited by this author, ring bivalents were few. For other hybrids listed we must be more struck by the inconsistencies in chromosome pairing between different combinations, even between reciprocals, than by the general levels displayed.

The lowest levels of homology were apparently in the crosses with the Calcutta 4 accession of *M. acuminata*, where the expected level of heterozygosity for translocations was not readily revealed. Only in one sample out of four were cells seen with two multivalents, one with IV + III and another with 2III. Bridges with fragments were seen in most plants but, except as could be forecast for hybrids with *M. flaviflora* (Chapter 4), these were only rather frequent in one case, and inexplicably so.

Smears of the hybrid SH 103, *M. itinerans* x *M. velutina*, included a number of cells with a strange bivalent, where a possible "repeat" segment was seen as an attached or included addition to the pair or even as a detached fragment (Fig. 6.1).

Table 6.1. Meiosis in some interspecific hybrids involving *M. basjoo* and *M. itinerans*. D and S indicate data of Dodds and/or Simmonds; others were plants studied by the author in Trinidad.

		First metaphases *				First anaphases *			
SH	**Cross**	**Cells**	**T**	**x 2I**	**"Xta" per II**	**Cells**	**BF**	**2BF**	**Other B**
57	*basjoo* x *itinerans*	50**DS**	0	0	(few)	100**DS**	1	0	0
23	Calcutta 4 x *basjoo*	39**DS**	**1**	2.64	?	-			
	"	22	**1**	2.72	0.96	47	1	0	0
36	BB Ceylon x *basjoo*	25**D**	1	2.40	±1.1				
	"	21**S**	1	2.00	1.06	50**DS**	1	0	0
85	*basjoo* x BB Ceylon	20	1	1.10	1.49	50	0	0	2
122	*flaviflora* x *basjoo*	20	0	0.40	1.33	50	11	0	3
42	Selangor x *itinerans*	20**S**	0	1.90	0.89	50**S**	1	0	0
121	*itinerans* x Selangor	20	0	0.85	1.49	50	1	0	1
119	*itinerans* x Calcutta 4	21	**1**	3.00	?	50	7	0	1
120	Calcutta 4 x *itinerans*	20	**2**	2.40	0.91	50	3	0	0
156	*itinerans* x BB Ceylon	20	1	1.10	1.51	50	0	0	0
130	*flaviflora* x *itinerans*	20	0	1.25	1.16	50	15	0	0
103	*itinerans* x *velutina*	20	0	0.20	1.59	42	0	0	2

* T = translocations identified and BF = bridges with fragments; pairs of univalents at M_1 are given as frequencies per cell.

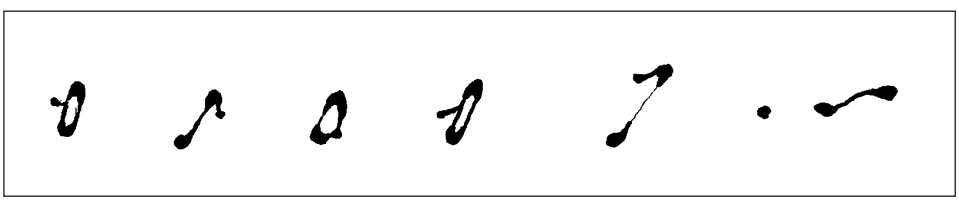


Figure 6.1. Some strange bivalents in SH 103 (*M. itinerans* x *M. velutina*).

These are each from a different cell and may reflect a repeated segment. Approximate scale x1500.

Hybrids of *M. ochracea*

ssp. *microcarpa* Borneo x *M. ochracea*

The ten F_1 plants were in a line ending on a sand bank. All were healthy but vigour varied according to position in the row, from luxuriant and densely stooling to merely fair individuals. Plant height was about 2.0-2.5 m in the first crop, then increasing to 4 m or more in ratoons. Plant colours were pale, only slightly ochreous and both dark markings and wax were weakly evident. Petiole wings were little developed and not markedly wrinkled. Peduncle pubescence segregated once more from dense to glabrous (confer

Table 6.2. Meiosis and male fertility in hybrids of *M. ochracea*.

Plant	First metaphase *			First anaphase *				Pollen
	Cells	x 2I	Arm pairs per II	Cells	U	BF	Other B	
female ssp. *microcarpa* IR 291:								
2	40	1.05	1.32	12	18	5	none	traces
7	20	0.95	1.33	42	45	17	3	
				+ 13 chaotic **				25
8	20	1.00	1.26	42	63	17	4	
				+ 6 chaotic **				15
10	20	0.45	1.48	50	28	18	5	25
female *M. flaviflora* IR 241:								
2	20	0	1.67	24	0	10	0	75
3	20	0.05	1.57	19	0	3	2	75
5	20	0.05	1.54	21	0	9	2	70
8	20	0	1.59	50	4	25	4	70

* T = translocations identified and BF = bridges with fragments; pairs of univalents at M1 are given as frequencies per cell. ** These were cells with defective spindle formation (see text).

Chapter 5). Ovules were two-rowed in the loculi; only scarce seeds were set. Pollen production was low and variable, as shown in Table 6.2.

This table principally analyses first meiotic divisions in four hybrid genotypes. Unsynapsed pairs were frequent at metaphase and, correspondingly, frequencies of arms with chiasmata were rather low. At anaphase, bridges with fragments were present in about 40% of cells, but never more than one per cell. From the latter observation, it appears that a single large inversion was responsible. Unusually, one was in fact a double bridge with two fragments as a consequence of a four-strand double crossover, this further emphasising the high crossover frequency between the affected segments.

In plants numbers 7 and 8, particularly, there were many irregular spindles, these affecting all phases of the first division. Some of them were abnormally wide and others were elongated with an indefinite equator. In two instances among 200 cells two distinct ones were found. Varying numbers of bivalents might be excluded or misaligned in relation to the poles. Plant 10 was much the most normal in this as in pairing characteristics.

M. flaviflora x *M. ochracea*

In a better location than the preceding cross, the ten F_1 plants suffered only from an attack of *Foc*, which eliminated three from study in just over a year from planting. The others were very vigorous and densely stooling. Height at emergence was about 1.5 m in first crop and 2.5 m in ratoons. Plants were slender and yellowish-green but not ochreous; petiole wings were developed but not wrinkled. Leaves were noted as distinctly mottled. Peduncles were glabrous, ovules were irregularly two-rowed and the plump, well-set fruits long-stalked.

Male fertility ranged apparently up to 100%, but counts of sound grains in the four plants analysed in detail did not exceed 75%. Meiosis in these four is also analysed in Table 6.2 and was very strikingly more regular than that in the other cross above. Pairing failures were quite rare, chiasmata were more numerous. Once more, however, there was the high frequency of 40% of anaphase cells with one bridge and fragment, evidently from a single large heterozygous inversion.

In Chapter 4 it was implied from the incidence of such bridges that *M. flaviflora* differed structurally from *M. acuminata* by two inverted segments. Now we have *M. ochracea* differing from both of these others by one! The provisional conclusion is that the new entity has one in common with the first-mentioned species, or less likely one involving different break points in the same almost homologous chromosome arms.

The phylogenetic position

No more can here be said about the relationships of *M. basjoo* and of *M. itinerans*. As we have stressed in accounts of hybrids of other species of this section and of Rhodochlamys, crosses with a range of subspecies and forms of *M. acuminata* and of accessions of *M. balbisiana* might have resulted in more solid information.

In the case of *M. ochracea*, it is all the more a pity that no study could have been made of a wider range of hybrids of the new accession, including those with Rhodochlamys forms, and that plants of the second generation have not been examined. Perhaps someone will undertake both tasks in the future. For the moment it has to be assumed that the place of *M. ochracea* is that of a branch of a common divergent line with *M. flaviflora*, close but not very close to *M. acuminata*.

CHAPTER 7

Australimusa and Callimusa

In Chapters 2-6 relationships have been analysed between diverse *Musa* species in two sections with chromosome number 2n = 22, but two other sections with 2n = 20 have long been named as Australimusa and Callimusa (Cheesman, 1947). The taxonomic discrimination between these latter to some extent mirrors that between sections Musa and Rhodochlamys in that Australimusa comprises tall to very tall plants and Callimusa species are of relatively short stature. However, the latter has species with horizontal inflorescences as well as with erect ones and the seeds have quite distinctive forms. The sectional distributions are largely different, although overlapping. Callimusa species have been described from Thailand and Indo-China through Malaya and Indonesia as far east as Kalamantan and Sulawesi (Simmonds, 1960a, 1962); Australimusa extends east from Sabah and the Philippines to the Solomon Islands. Numbers of truly distinct species are not very certain in either case; Argent (1976) has added to the diversity of names in Australimusa, a section that includes current or former economic plants. These are the edible-fruited "*M. fehi*" and the fibre producer *M. textilis*.

Simmonds (1962, p.66) was unable to report any cytogenetic information on hybrids with 2n = 20, although he commented that Australimusa species appeared to be "little differentiated reproductively". In fact, the writer's study of this material was already well advanced in Trinidad at the end of the 1950's, to be complemented soon after in Jamaica. On the aspect of intersectional hybrids between species with 22 and 20 chromosomes, Simmonds (*loc. cit.*) again offered little except for citing the work of Bernardo (1957) in the Philippines. Further cytological data is now put on record on some such hybrids, the interpretation of which conflicts with the Philippine one.

In addition to the four principal sections, at least two other *Musa* species do not conform; these are *M. ingens* with 2n = 14 and *M. beccarii* with 2n = 18 (Shepherd, 1959). Seeds of the former germinated at ICTA, hence the chromosome count, but plants were inviable in the climate there. The second species was maintained in Trinidad for some time and the very strange 20-chromosome hybrid *M. balbisiana* x *M. beccarii* was produced, to be raised and studied in Jamaica. For convenience, this cross will be mentioned alongside hybrids between the two principal basic chromosome numbers.

Plant materials and ease of crossing

Trinidad/Jamaica accessions that enter into these studies are listed in Table 7.1 and, for the species with 2n = 20, these follow an approximately geographical sequence of supposed areas of origin.

Table 7.1. Plant materials involved in this chapter.

Denomination	Coding*	IR	Provenance
Section Australimusa:			
M. textilis:			
St Vincent (wild?)	StV	71	St Vincent Botanic Garden
cv Libuton	Lib	339	Philippines
cv Tangongon	Tan	340	Philippines
Kundasan (wild)	—-	499	Sabah
M. lolodensis	lol	247	Halmaheira
M. angustigemma	ang	194	Papua New Guinea
"	"	395	Papua New Guinea
M. peekelii	pkl	229	New Ireland
M. maclayi	mac	201	Buka island, Bougainville
" (?)	—-	585	Bougainvile
"*M. fehi*" 'lnk'	—-	504	Jamaica!
Section Callimusa:			
M. coccinea	coc	142	Trinidad Botanic Garden
M. violascens	vio	108	Malayan peninsular
M. borneensis	—-	118	Sarawak (Sulawesi)
"	bor	273	S.E. Borneo (Kalamantan)
Other sections:			
2n = 22:			
M. acuminata ssp. *burmannica*			
Calcutta 4	C 4	124	Calcutta Botanic Garden
Calcutta 6	C 6	132	Calcutta Botanic Garden
M. balbisiana	bal	100	Sri Lanka
M. ornata	orn	1	Trinidad Botanic Garden
2n = 18:			
M. beccarii	bec	503	Sabah

* To facilitate subsequent identification of hybrids in other tables.

In Trinidad conditions, most species and hybrids with this chromosome number were much affected by seasonal droughts. Some, including *M. borneensis* and *M. coccinea* are believed additionally to have been mildly but chronically infected by *Fusarium oxysporum* f. sp. *cubense*. Also, the efficiency of seed setting was erratic, even in the better periods of the year. These problems, allied to the great stature of many Australimusa plants tended automatically to restrict the production of hybrids. Nevertheless, to generalise, *M. violascens* crossed as freely and yielded as vigorous hybrids with Australimusa species as these did among themselves.

Hybrids of *M. coccinea* were the most difficult to achieve in either direction. As female parent, some fifty flowers pollinated with Australimusa forms yielded only six mature seeds which failed to germinate. About forty flowers with pollen of *M. violascens* gave 18 good seeds and one of these germinated; the plant died in the nursery. The cross

with *M. borneensis* was more successful and several F_1 plants were at one time raised; unfortunately, none survived for the present study. Crosses with *M. coccinea* as male parent were easier but such plants as resulted were weaklings to some degree. Only one ever flowered, of *M. violascens* x *M. coccinea*, but no data on it have survived. In this light it is all the more remarkable that a relatively vigorous plant resulted from *M.balbisiana* x *M. coccinea*.

M. borneensis was only a little more successful as a parent in crosses and fewer attempts were made, for want of vigour in either of the two accessions. Seeds resulted from the use of pollen of the species in most combinations tried and at least some relatively vigorous hybrids were raised. The two reported below came from the later of the two accessions.

Crosses between species with 20 and 22 chromosomes invariably failed to yield seeds with the former as female parent; yet surprisingly strong plants were sometimes obtained in the reverse direction and also in the cross mentioned earlier of 22 x 18 chromosomes.

Meiotic data

Accessions of Australimusa and Callimusa

Simmonds and Dodds (1949) disclosed data on three species in each of the sections. From their Table 3, the least normal was *M. peekelii* which had quite a distinct frequency of pairing failures. Their data have been repeated for comparison, in a slightly modified form, in Table 7.2, together with a note of Simmonds on *M. maclayi*, omitted from the 1949 publication. Also added are two repeat analyses by this author of Australimusa species and five of later accessions within this same section.

The earlier authors reported strong tensions between bivalents at first metaphase which they concluded to be typical of 20-chromosome species. Yet this phenomenon occurred occasionally and irregularly in one only of the newer sets of smears of accessions (cv Libuton). It was not a "characteristic" of this type of material, either as accessions or hybrids. Bivalents in *M. textilis* from Kundasan were later noted by contrast as unusually compact.

Dodds' original record for the St Vincent accession and the author's for cv Tangongon revealed a tendency to persistence of a nucleolar remnant up to the first metaphase and anaphase, as otherwise noted at such a late stage only in *M. flaviflora* (Simmonds and Dodds, 1947). There was no recorded suggestion in these new examples, however, that the remnant might have impeded meiotic events. Unexpectedly, apparent inversion bridges were seen in two accessions of *M. textilis*, more conspicuously in cv Libuton.

Among the later accessions, IR 585 from Bougainville has been identified with some doubt as *M. maclayi* in Table 7.1. This is because the few plants raised displayed appreciable variability in robustness of plant, angle of inflorescence and other characteristics. The substantial frequency of inversion-type bridges at first anaphase in plant number 6 also suggests natural hybridisation with another Australimusa form. This

Table 7.2. First meiotic divisions in accessions with 2n = 20.

Accession	First metaphases				First anaphases			
	Cells	IV/III	x 2I	"Xta" per II	Cells	BF	2BF	Other B
M. textilis:								
St. Vincent	20*	0	1	?	100*	0	0	0
cv Libuton	20	0	0	1.74	25	4	0	0
cv Tangongon	50	0	1	1.86	50	0	0	5
Kundasan	27	0	0	1.93	30	1	0	2
M. lolodensis	20*	0	0	?	101*	0	0	4
	+ 20	0	0	1.78				
M. peekelii	20*	0	**26**	?	81*	0	0	4
M. maclayi (201)	21**	0	0	1.92	50*	0	0	5
	+ 20	0	1	1.80	30	0	0	0
M. maclayi (585)								
plant -1	50	0	1	1.71	50	1	0	6
plant -3	9	0	1	(1.5)	28	0	0	3+
plant -6	2	0	0	-	48	**10**	0	8
"*M. fehi*" 'Ink'	20	**5**	7	1.59	50	1	0	6
M. coccinea	20*	0	0	(high)	20*	0	0	0
M. violascens	5*	0	0	(few?)	34*	0	0	3
M. borneensis (118)	20*	0	0	(few?)	29*	0	0	0

* After Simmonds and Dodds, 1949. ** Unpublished record of Simmonds.

same plant manifested 20% of bad grains in a pollen analysis presented in another section.

The clone of "*M. fehi*" also showed evidence of hybridity in the presence of five translocation quadrivalents at first metaphase; one of them was a closed ring. Another ring of IV was present in an exceptional late prophase cell examined (Figs. 7.1 A & B). Pollen was also deficient (see later). These facts tend to substantiate Simmonds' suggestion (1962, p.150) that more than one species may have been involved in the evolution of these edible banana clones.

Hybrids within Australimusa

Among the five species treated, all but one of the ten possible pairings were raised and studied cytologically, in a total of eleven combinations. The deficient case was that of *M. angustigemma* x *M. maclayi*, in either direction. First meiotic data from the eleven hybrids are shown in Table 7.3, in a sequence that lists those of *M. textilis*, then of *M. lolodensis*, *M. angustigemma* and *M. maclayi*, that is to say, once more listing these parents from west to east in relation to their localities of collection.

Pairing failures were again most evident in some hybrids of *M. peekelii* but were not present in all, suggesting that the behaviour of the parent material was more an

Figure 7.1. Translocations at first meiotic divisions of Australimusa.

A: late prophase with ring of IV and nucleolar remnant (cross-hatched); B: M_1 with alternate chain of IV; C: M_1 with adjacent chain of IV perhaps off the spindle equator; D: A_1 with a "bivalent" segregating from a III or IV; E & F: further M_1's with an alternate ring and an adjacent chain of IV, respectively. Approximate scales x1500 (A) to x2000. Plant sources: A & B—"*M. fehi*" (IR 504); C & D—*M. textilis* St Vincent x *M. angustigemma* (SH 148); E & F - *M. maclayi* x *M. lolodensis* (SH 160).

Table 7.3. First meiotic divisions in hybrids between Australimusa forms.

SH	Cross*	First metaphases				First anaphases			
		Cells	IV/III	x 2I	"Xta" per II	Cells	BF	2BF	Other B
142	StV x lol	40	0	0	1.78	50	6	0	5
113	lol x StV	20	0	1	1.65	32	11	1	2 +
148	StV x ang	30	2	0	1.83	50	0	0	8
144	StV x pkl	20	0	0	1.74	28	0	0	0
161	Tan x mac	20	2	0	1.64	50	0	0	6
157	lol x ang	50	0	2	1.80	20	5	0	2
141	lol x pkl	20	0	6	1.48	45	5	0	7
117	pkl x lol	20	0	3	1.68	50	24	1	12
160	mac x lol	20	2	2	1.64	50	10	0	5
146	pkl x ang	5	0	0	-	50	1	0	7
147	pkl x mac	20	0	0	1.86	50	0	0	12

* Accessions abbreviated as in Table 7.1.

irrelevant aberration than any indication of hybridity. Data on pollen production in the next section reinforce this view. The lowest value for arm pairing in bivalents was also found in such a hybrid, *M. lolodensis* x *M. peekelii* (SH 141), but these generally appeared to be at least as numerous as in hybrids within *M. acuminata* (Chapter 2) and in frequencies rarely equalled in interspecific hybrids within 2n = 22, reviewed in other earlier chapters.

There was evidence of structural hybridity, again as also found within *M. acuminata*, in the forms of translocation and inversion. The former never accounted for more than a very small number of cells at first metaphase and could not be consistently attributed to hybrids of any one of the parent species; at least five of the six cells recorded were of quadrivalents (Fig. 7.1 C, E & F). Bridges with fragments on the other hand were conspicuous in all hybrids involving *M. lolodensis*; two independent ones were seen in two cells among a total of 247 first anaphases. Moreover, two quite different fragment sizes were noted, the large one approaching the size of a chromosome, the other quite small (Fig. 7.2 C). This size difference was a further indication of hybridity for at least two relatively inverted segments, one of them sub-median and one sub-terminal.

Otherwise, rare cells suggested errors of metaphase alignment (Fig. 7.2 A). There were also occasional instances of mistiming of anaphase in the form of precocious or retarded separation of bivalents (Fig. 7.2 B & D). In the former case the co-orientation and morphology of the parts, with well-marked "tails", left no doubt as to their earlier association.

A triploid Australimusa hybrid

Among the few F_1 plants from which SH 113 was selected was one that was quite distinctive from the others, more robust and closer phenotypically to the female parent *M. lolodensis*. It was found to be triploid and presumed to be the result of an unreduced egg cell.

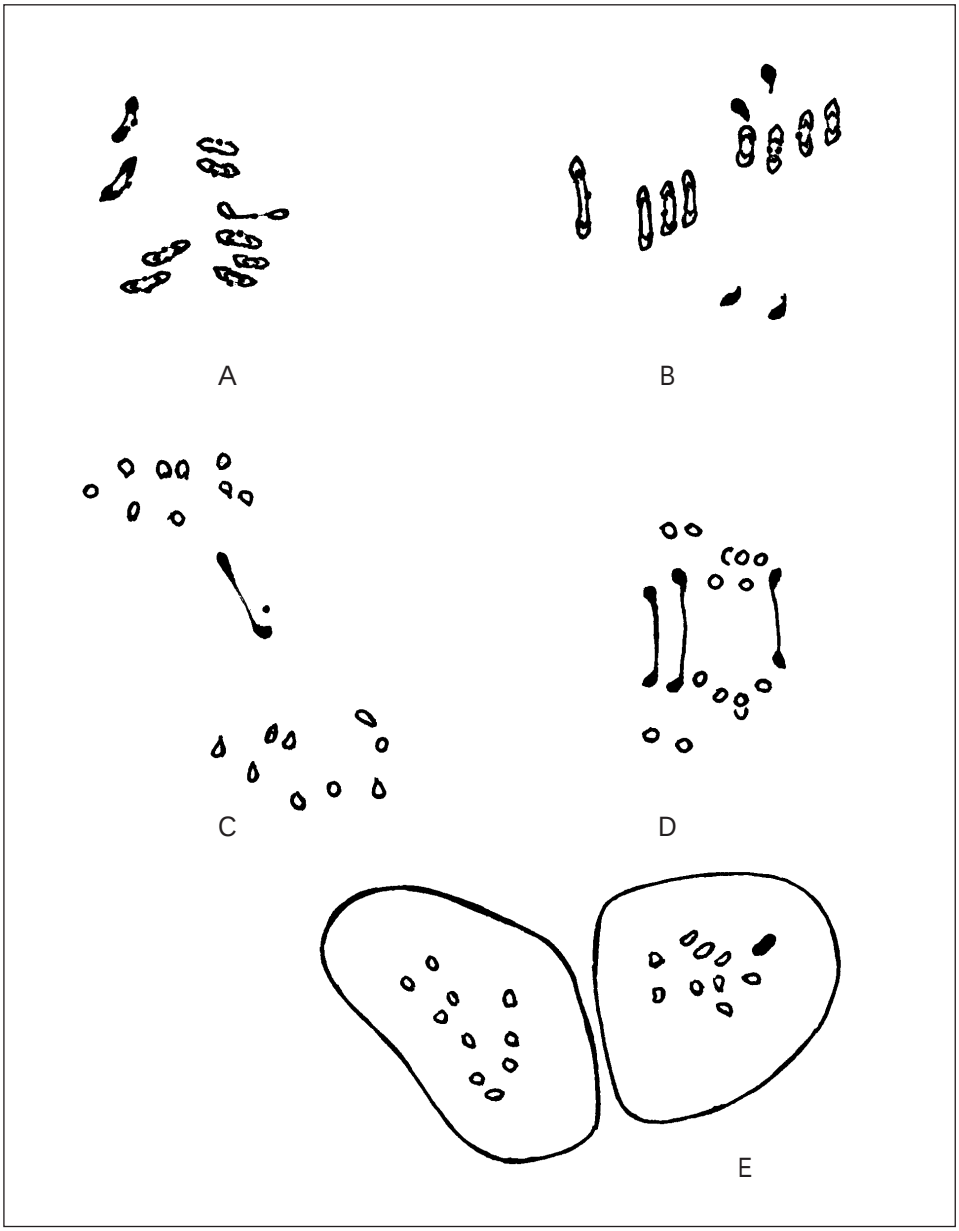


Figure 7.2. Diverse anomalies in first meiotic divisions of hybrids between *Musa* species with 2n = 20 chromosomes.

A: two bivalents possibly misaligned at M_1 in *M. peekelii* x *M. maclayi* (SH 147); B: precocious separation of two pairs in the same hybrid; C: inversion bridge with the small fragment at A1 in *M. maclayi* x *M. lolodensis* (SH 160); D: three bridges from laggard pairs at A_1, again in SH 147); E: an M_2 cell pair in *M. violascens* x *M. borneensis* (SH 140) with the typical very large dyad. Approximate scales x1200 (E) to x 2000.

Trivalent frequency was very high in the plant's pollen mother cells; 30 first metaphases averaged 8.87III 1.17II and 1.07I. The discrepancy between the last two means came from a single cell, in which a tenuous connection seemed to exist between two chromosomes which may have had only faint mutual homology. The frequency of trivalents was surely appropriate to an autotriploid and higher yet than usually found in cultivars of the AAA group (Chapter 9). Chiasma arrangements were not discernible for all trivalents. Of 245 classified, 201 had only two apparent chiasmata, including seven Y-shaped with both involving a common arm. 43 were "frying-pans" and one very exceptionally was a "ring", presumably resulting from crossing over within an inversion. The mean for arms paired per cell lay between 21 and 22, compared with 16.5 for the diploid SH 113 (Table 7.3).

43 first anaphase cells analysed had a mean of 0.67 laggard univalents per cell, with a maximum of three; the majority were scattered in the cytoplasm away from the spindle. 11 cells contained a bridge with fragment and four an uninterpretable fragment without the presence of a bridge.

A Callimusa cross and intersectional hybrids

By comparison with Australimusa, the one available hybrid between Callimusa parent species demonstrated a quite low frequency of arm pairing (Table 7.4), of an order yet lower than the norm for crosses of *M. violascens* with Australimusa types and below that found in *M. lolodensis* x *M. borneensis*. No translocation was revealed nor any well-marked inversion in the former hybrid, but it seemed that the *M. borneensis* parent had one chromosome of its haploid set distinctly larger than those of other species studied with 2n = 20. This exceptional chromosome in the hybrids rarely formed a ring bivalent with its presumed counterpart and was often unpaired. It was conspicuous too at first anaphase and at second metaphase (Fig. 7.2 E).

Crosses of *M. violascens* with Australimusa also showed lower arm pairing means than hybrids within Australimusa, as were summarised in Table 7.3. Lowest values were found in the combinations of *M. violascens* with *M. peekelii* but then the latter had otherwise shown a variable tendency towards failures of pairing, even as an accession (Table 7.2 and discussed above). The two sections were not seen to be differentiated by any translocation but bridges with fragments were conspicuously numerous at first anaphase in certain hybrids of *M. violascens*. That these included the reciprocal crosses with *M. lolodensis* shows that these two species do not possess this structural modification in common. Otherwise, the occurrences were not explicable on a consistent basis.

Hybrids between different basic chromosome numbers

In the relatively chaotic presentation of chromosomes at the first meiotic division in such hybrids, whether paired or not, the first essential was to make a correct discrimination between metaphase and anaphase. Segregants from bivalents at anaphase often have visible "tails", as pointed out in relation to precocious separation in Australimusa hybrids, while unpaired chromosomes do not; they are of rounded outline. On this consideration

Table 7.4. First meiotic divisions in hybrids within Callimusa or between Australimusa and Callimusa.

SH	Cross*	First metaphases				First anaphases			
		Cells	IV/III	x 2I	"Xta" per II	Cells	BF	2BF	Other B
Callimusa x Callimusa:									
140	vio x bor	30	0	26	1.10	32	1	0	0
Intersectional:									
143	StV x vio	20	0	11	1.44	50	13	0	5
114	vio x lol	20	0	28	1.27	50	20	1	3
135	lol x vio	20	0	17	1.44	21	7	3	3
145	vio x ang	20	0	10	1.41	50	0	1?	10
		+ 20	0	10	-	+ 25	4	0	6
115	vio x pkl	20	0	17	1.14	50	0	0	6
118	pkl x vio	20	0	**46**	0.98	50	1	0	3
116	lol x bor	20	0	8	1.48	2	1	-	-

* Accessions abbreviated as in Table 7.1.

and on a general tendency in *Musa*, for unpaired chromosomes on the spindle at anaphase to go through a precocious "second division", both Dodds (unpublished) and the author have differed from the account of Bernardo (1957). The bivalents and higher groupings at M_1 were usually if not always stretched, but not to be confounded with bridges. Unusually, and to confirm the interpretation now adopted, it was possible to examine some cells at the diakinesis stage in two hybrids. One of these with two bivalents in plant SH 29 is illustrated by a drawing (Fig. 7.3 A) and there exists a photograph of another with no bivalent at all in plant SH 39 (Fig. 7.4 A). Another photograph (Fig. 7.4 B) shows four bivalents at first metaphase in plant SH 39, a cell not in the sampling tabled, while further drawings show a first metaphase with two pairs and an anaphase apparently without bivalents at all, both from plant SH 149 (Figs. 7.3 B & C). In the latter instance ten univalents had congressed on the spindle equator and were dividing.

The data of Table 7.5 on first metaphases therefore include incontestable pairs of chromosomes and higher associations. They were very few on average in all six plants analysed and chromosomes with both arms synapsed were rare indeed, so that the frequency of arms paired could be taken as equal to that of chiasmata formed.

Other irregularities were found in SH 39 (Calcutta 6 x St Vincent). A number of pollen mother cells had giant nuclei, having evidently become polyploid without visible evidence of preparation for meiosis or of restitution (see Chapter 8). One metaphase cell had two distinct spindles, one perpendicular to the other. At anaphase, despite the context of low chiasma frequency, two cells showed a bridge and fragment and one cell two bridges with fragments.

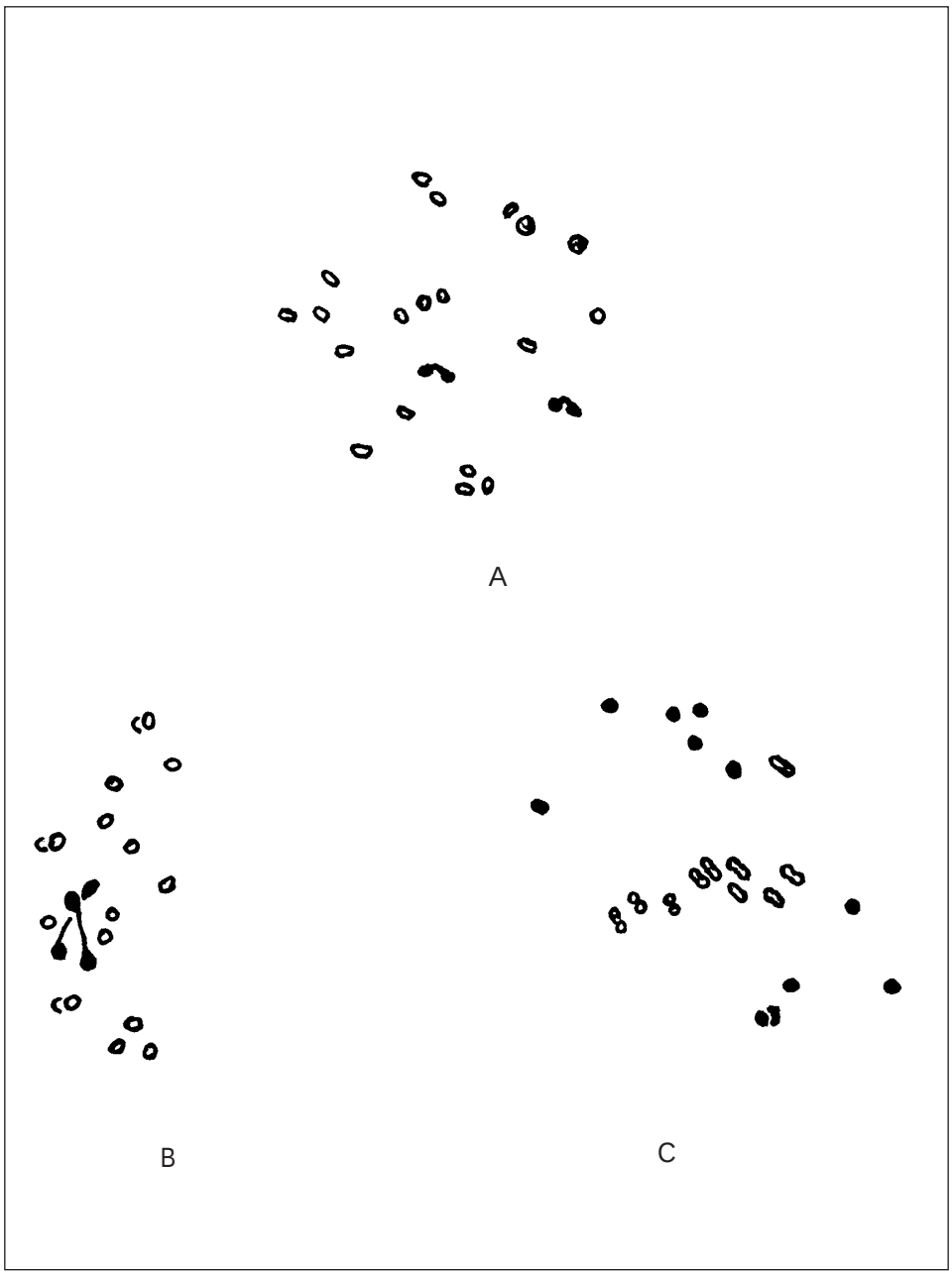
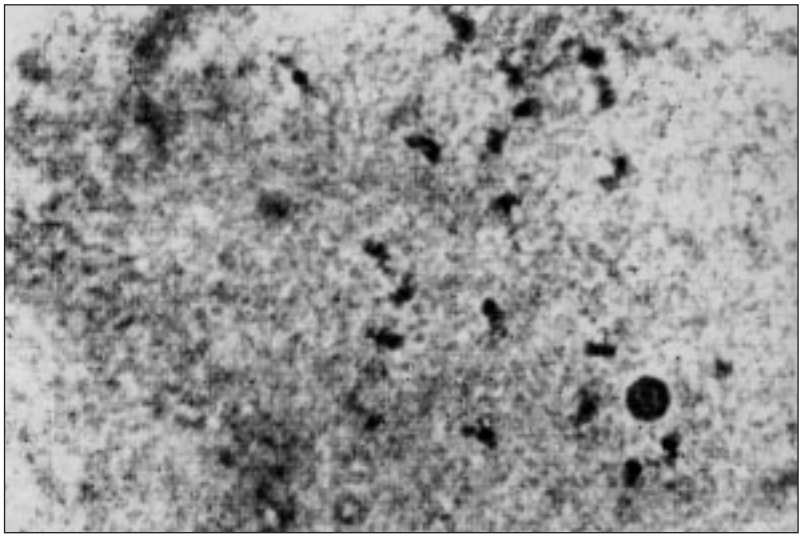
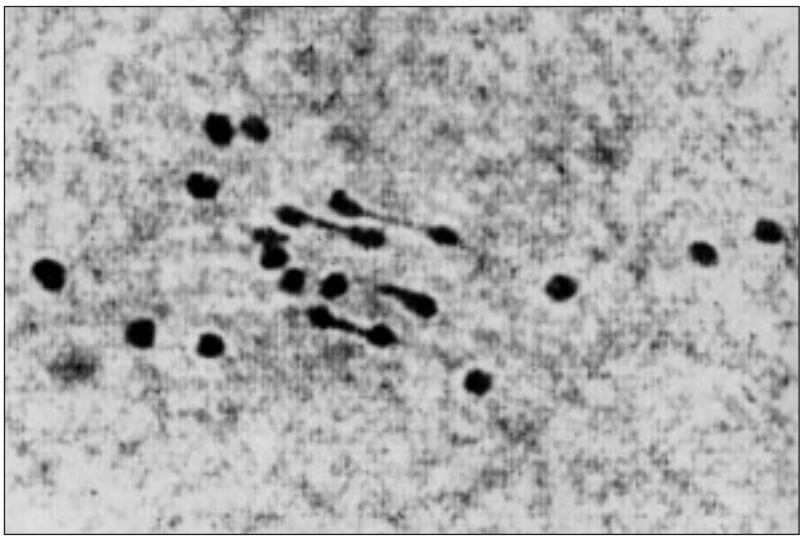


Figure 7.3. Stages of the first meiotic division in 21-chromosome hybrids.

A: diakinesis in *M. ornata* x *M. violascens* (SH 29) with two bivalents only, plus two small nucleolar remnants; B: M_1 in *M. balbisiana* x *M. coccinea* (SH 149) with two bivalents only; C: A_1 in SH 149 apparently without any bivalent, but with ten univalents misdividing on the equator. Approximate scales x1250 (A) to x 2200.



A



B

Figure 7.4. Photomicrographs of meiosis in *M. acuminata* C6 x *M. textilis* St Vincent (SH 39).

A: diakinesis with 21 univalents and nucleolar remnant; B: M_1 with four bivalents and thirteen univalents. Both x 1600.

Table 7.5. First meiotic metaphases in hybrids between species of unlike chromosome number.

Cross*	Cells	Highest Level of Association	Frequencies per cell			
			IV/III	II	I	Xta
2n = 21 (11 + 10):						
C 4 x vio (SH 24)**	54	IV + II	0.06	0.69	19.44	0.81
orn x vio (SH 29)	100	2 II	0	0.40	20.20	0.40
C 6 x StV (SH 39)	100	III + 3II	0.01	1.12	18.73	1.24
C 4 x bor (SH 139)	20	2 II	0	0.85	19.30	0.85
bal x coc (SH 149)	50	4 II	0	0.90	19.20	1.00
2n = 20 (11 + 9):						
bal x bec (8209B)	40	3 II	0	0.90	18.20	1.02

* Accessions abbreviated as in Table 1. ** Unpublished record of Dodds.

F_1 fertility

Not surprisingly, all the plants of the last subsection were totally male sterile. On the female side, 495 flowers pollinated of three hybrids yielded not even an empty seed.

Pollen fertility and grain sizes in hybrids of 20-chromosome species

For most plants studied estimates were made of sound pollen, based on 1,000 grains in each instance, and diameters of 100 sound grains were measured, as in other chapters in units of about 4 µ. The detailed and extensive results are given in Appendix 7.1.

One surprise among the accessions was the sample of universally sound grains of relatively uniform size from *M. peekelii*. Dodds (unpublished) had noted only 73% of sound grains in a sample from this plant, more consonant with its pairing failures and an indication that, even in the parent material, this was not a constant feature. Also conspicuous was the variation in grain size from *M. textilis* to *M. maclayi*, from one end of the geographical range of Australimusa to the other. Three plants of Australimusa had size means with higher than normal standard errors. Of these, hybridity was already probable in IR 585 and in "*M. fehi*", but the case of *M. textilis* cv Tangongon could arouse speculation on the origin of such cultivars.

By and large, hybrids between Australimusa accessions were highly male-fertile, despite the known presence in some of chromosome stuctural heterozygosity. The poorest was again a combination of *M. peekelii*. Pollen grain sizes tended to be intermediate between those of the parents, with somewhat diffuse modes contributing to an increase in standard errors.

The four hybrids studied of combinations of Australimusa with Callimusa, the latter as *M. violascens* in each case, were very much less male-fertile; SH 114 (*M. violascens* x *lolodensis*) had no pollen at all and three others gave estimates not exceeding 20% sound. These also exhibited broad ranges of grain size, such that the discrimination between haploid and probably diploid was difficult. Only in SH 118

(*M. peekelii* x *M. violascens*) was there no observed overlapping. The presence of giant grains was recorded despite the absence of any obvious restitution mechanism at meiotic stages.

Female fertility and progenies of hybrids

Eight hybrids were pollinated by their parent species, four within section Australimusa and four of *M. violascens* with Australimusa. As was the case in crosses between parent species, it was impossible to make an accurate assessment of relative female fertility in the field environment of ICTA. Even with this reservation, however, the *M. violascens* hybrids were clearly infertile. None yielded more than one good seed per fruit; 112 pollinated flowers of SH 145 (*M. violascens* x *M. angustigemma*) yielded no seeds at all. Among the four hybrids tested within Australimusa SH 144 (*M. textilis* St Vincent x *M. peekelii*) was apparently the most fertile, often giving more than 100 good seeds per fruit. None of the other three was conspicuously infertile.

Germination rates of seeds from the latter group ranged from 4% to 78% but presumably differing because of environmental hazards, since they varied as much between inflorescences as between crosses. Chromosome counts were made from some 150 seedlings, including fourteen from SH 118 (*M. peekelii* x *M. violascens*). All were diploid.

Backcross progenies were not raised in Trinidad but plants from a number of seed lots, derived by pollination of Australimusa hybrids, were germinated and raised in New Britain. As reported in correspondence, these second generation plants were little if at all inferior to the parent species and F_1's in survival and vigour.

Taxonomy and isolation in review

The two "sections" analysed in this chapter are very clearly quite isolated from one another, but equally obviously they are of very different taxonomic status within their boundaries. Admittedly, the sampling of Callimusa has been a narrow one, in relation to the nine or possibly ten species listed by Simmonds (1960a). Also, the data remain incomplete for the few accessions compared, but we have the evidence of at least three long divergent evolutionary lines of which one, represented by *M. violascens*, may have closer affinities than the others to Australimusa.

By contrast, this latter is very plainly a young group in its evolution, as hinted by Simmonds (1962). The survey in this case, discounting "*M. fehi*", has covered virtually the whole of the known range and hybrids between "species" have been invariably very vigorous and have been either moderately or abundantly fertile. Their fertility may be limited solely by the small amount of structural hybridity found. Moreover, one spontaneous triploid hybrid encountered had the meiotic behaviour appropriate to an autotriploid, with a trivalent frequency higher than is commonly found in autotriploid *M. acuminata* (Chapter 9).

The degree of divergence in Australimusa is in fact comparable with that within the wide-ranging *M. acuminata* (Simmonds, 1956, 1962). In both, the greater part of the

variability is between discrete geographical isolates. In the latter species these are ranked as subspecies; their degree of chromosome structural change is greater and their morphological differentiation is also more marked. The physical contrasts between the aspects of ssp. *burmannica*, ssp. *malaccensis* and ssp. *banksii* are perhaps the most striking examples.

What then is the proper taxonomic status of "Australimusa"? For practical convenience, this may not be vitally important, so long as it is clearly recognised that in their breeding behaviour the distinct forms behave as a single species. In this light, by precedence, they could all be described as *M. textilis* Née. The nature of the territory in which they are dispersed confers many spatial barriers, of seas, mountains or other unfavourable environments. However, there must exist situations even if temporary where the named "species" might interbreed naturally. Argent (1976) states, from ample field experience, that hybrid swarms are not so common as might have been foreseen, but our IR 585 appears to have been such an example.

The reference above to a cline of increasing pollen grain size from west to east is matched, fortuitously, by one of seed size. Those of *M. textilis sensu strictu* are relatively small, smooth and rounded, while size and irregularity of form increase steadily from west to east.

Finally it has to be stressed that a major gulf exists between species with different basic chromosome numbers. Whatever speculation has been or might be made on the phylogeny of the genus must be precarious or unprofitable.

CHAPTER 8

Abnormal spore development

The present theme is a departure from the preceding ones, where structural hybridity and deficiencies in chromosome homology in diploids have been treated in relation to their effects on hybrid vigour and fertility. There have been rare instances of spindle abnormalities in first meiotic divisions and, additionally, some hybrids have generated small numbers of diploid pollen grains, presumably resulting from restitution at meiosis (Simmonds, 1962). Other than in these instances, the chromosome events so far portrayed in this work have been within the normal course of meiosis as has long been observed in the genus *Musa*.

As pointed out by sundry authors, this is characterised by a well-marked interphase between the two divisions, during which the chromosomes lose their condensed form and a transverse wall is formed between the two daughter cells. The second division occurs perhaps twelve hours or more after the first, as far as may be guessed from the contents of succeeding bud clusters and with allowance for some lack of synchronisation of events, both within and between buds of the same cluster. When both divisions are completed in standard cases, the four very juvenile spores are still very thin-walled and more or less irregular in shape, within the outer covering of the pollen mother cell wall.

In addition to the consequences of chromosomal errors, Simmonds (*loc. cit.*, p.52 and Fig. III.6) referred to instances of "meiotic breakdown", which he held to contribute significantly to (interspecific) isolation. These included chromosome division by endomitosis and also failures of wall formation, either before or after the second meiotic division, aspects suggested as possible sources of diploid or tetraploid spores.

There is a renewed occurrence of spindle irregularities in the plants now to be reported, as well as of chromosome loss, but the principal new data refer to deficient wall formation and its consequences. It must be stressed that these bizarre and striking instances were **not** in species crosses; they were in some backcross hybrids within *M. acuminata* ssp. *burmannica*, namely from the F_1 Long Tavoy IR 187 x Calcutta 4 IR 124 (SH 111). The plants were raised in Trinidad, in an aborted attempt to follow segregations of the N2 translocation and resultant male fertility in progenies. No meiotic analysis was in fact performed but occasional plants were unexpectedly deficient in pollen, with only half or less of the standard anther content.

Studies of the exceptional plants related to meiotic events, to forms and sizes of mature pollen grains, forms and nuclear contents of immature ones and to ploidy of some progenies. For immature grains, age is identified by bract number, counting inward from the most mature and unopened. In this particular material, it has to be emphasised that two or more bracts may be lifted per day, so that days from maturity were appreciably less than the bract number.

Results

Meiosis

Plant 4439A- 7: Only one cell was recorded at first metaphase and this had a tripolar spindle (Fig. 8.1 A). Three pollen mother cells at first anaphase had a normal appearance except that one segregated 12-10 between poles. Second divisions were classified in fifty cells, mostly at metaphase but some at early anaphase:

– 5 had interphase walls well formed; four were probably normal but one had two units excluded in each half;

– 11 had walls partially formed; no chromosomes were excluded; one had the two spindles close to but not aligned with the partial wall;

– 34 were wall-less but 29 of these presented apparently normal division of chromosomes; two had the pair of spindles either adjacent (Fig. 8.1 B) or partly fused, one had three distinct spindles with 11 + 5 + 6 units (Fig. 8.1 C), two had one or two units excluded.

Plant 4552B- 9: Approximately one thousand pollen mother cells were examined at second metaphase or anaphase, between six smears of different flower buds. Interphase walls were absent or incomplete in variable numbers but not in high frequency. One case was seen of a restitution diploid plate. An unrecorded but appreciable number had untidy spindles with longitudinal scattering of chromosomes and a few dyads had the two spindles in the same half of the double cell.

Plant 4552B-12: Fifty second division pairs were classified for presence or absence of an interphase wall, numbers of spindles, excluded chromosome units and degree of longitudinal dispersal on the spindles, with the following summarised results:

– walls—none was totally developed; two were nearly so and ten partly;

– spindles—one case was of full restitution on a single plate with a 22-22 segregation; seven cells had three independent ones (Fig. 8.1 D) and another had one which was tripolar;

– exclusions—these numbered from one to thirteen in 31 cells, with a mean of 2.8 in 49 non-restituted cells;

– longitudinal dispersion—of 74 spindle equators seen in side view and classified, 12 were tidy, 41 diffuse and 21 intermediate; only three pairs were considered normal in both halves, except for wall deficiency.

Mature pollen

Samples were compared from eleven plants of the cross 4552B, of which four seemed to be almost entirely haploid lots. Only one uncommonly large grain was seen and no irregular ones. These latter categories also occurred only rarely in five others. Estimates of good grains were usually above 95%, except for one plant with only 84% sound. In the remaining two plants, numbers 9 and 12, pollen was extremely variable in size. These samples also included a proportion of abnormal forms, noted for convenience as egg-shaped, ellipsoid or “Siamese twins” (Figure 8.2 A-C).

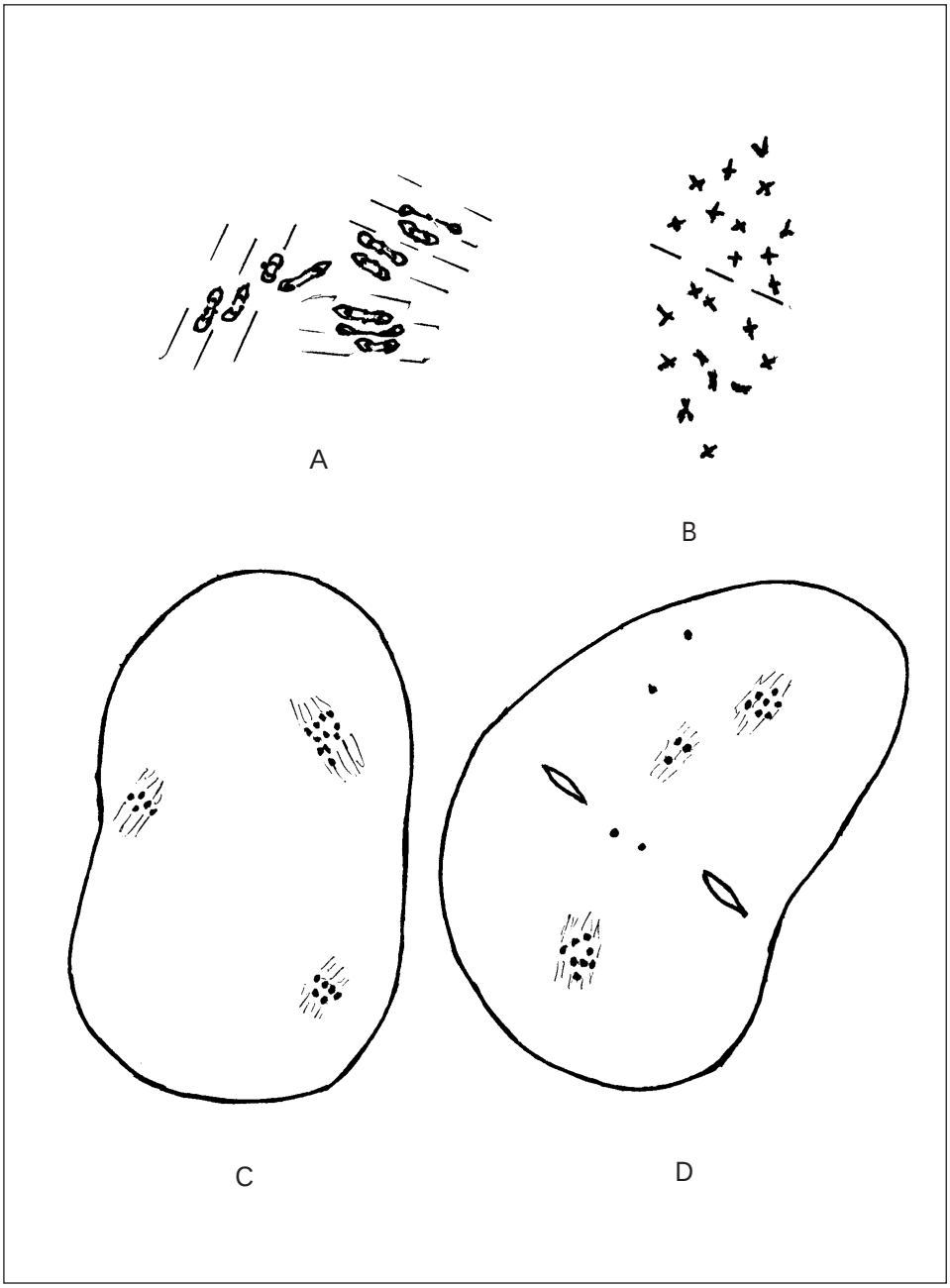


Figure 8.1. Meiosis in plants 4439A-7 (A-C) and 4552B-12 (D).

A: M_1 with tripolar spindle (x1950); B: wall-less M_2 with joint or parallel spindles (x2350); C: wall-less M_2 with three spindles containing 11 + 5 + 6 units (x1100); D: part-walled M_2 with three spindles, containing 8 + 3 + 7 units, as well as four excluded (x1100).

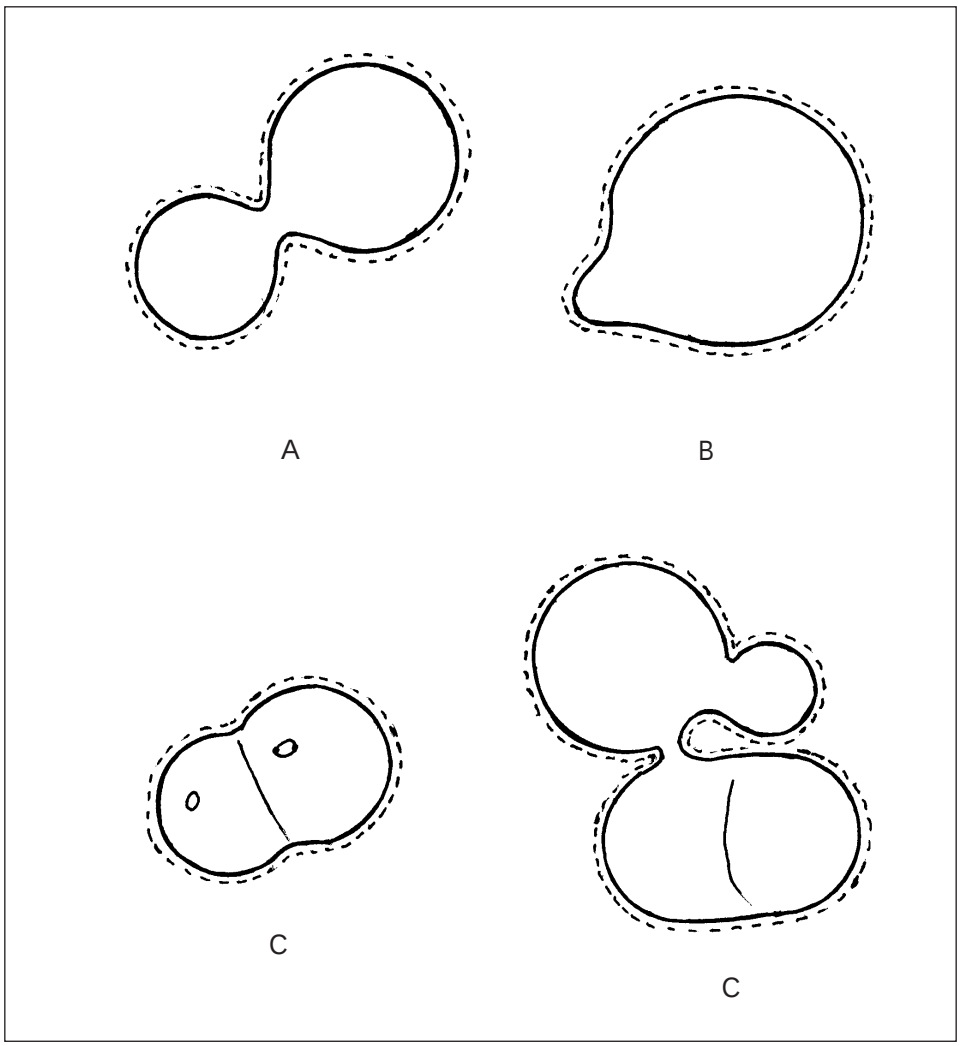


Figure 8.2. Abnormal forms in mature pollen of plants 4552B-12 (A-C) and 4552B-8 (D).

A: unequal Siamese; B: ± ovoid; C: ellipsoid and unhealthy, with unequal nuclei visible; D: diverse "quadruplets". All x270; internal membranes shown by fine lines, outer walls by broken ones.

Appendix 8.1 shows the relative diameters of round grains of four normal plants and these two abnormal ones. Values for the former were typical of haploid *Musa* pollen, of the order of 100-120µ. Both abnormal plants showed three distinct modes. In 4552B- 9, these were at about 28.5, 36 and 45.5 units which convert to cubic ratios of 1.0: 2.0: 4.1. Modes in 4552B-12 were at about 30.5, 37 and 46 units, with cubic ratios of 1.0: 1.8: 3.4. If the latter were rather less than the expectation, the conclusion is unavoidable that these two lots represented mainly haploid, diploid and tetraploid pollen.

Grains of abnormal form, principally Siamese pairs, were measured in plants 9, 12 and 16 (Appendix 8.2). The degree of constriction between the component parts was quite variable. Two pairs were noted as being without a separating wall; much more common was an incomplete wall with a protoplasmic anastomosis or even two of these in a single case. Only in few instances were the members of a pair of nearly equal size. Some were very small "buds" and additional buds were also registered with diameters of 40-60µ. Figure 8.2 D shows an exceptional case from the almost normal plant 4552B-8 with "quadruplets".

Immature pollen

Very probably, although not certainly from the record, immature anthers were treated as for root tips at the time, following the Tjio and Levan (1950) fixation and maceration-staining stages. Outer walls were generally removed in the treatment. The material so treated was chiefly from plant 4552B-9 for anthers from a series of bracts, with the following results.

Bract 4: the great majority were 2-nucleate and normal in form, but some were ovoid or budded. There were some Siamese twins with equal halves, with or without partial walls, which had a nuclear apparatus in one half only (Figure 8.3). Other exceptional cases, several of them illustrated in this same figure, included:

– **1-nucleate:** partly rounded or in elongated, "vegetative" form;

– **2-nucleate:** both rounded, both elongated or one of each, with or without the vegetative one budded off;

– **3-nucleate:** tending to be unequal in size and varied in form;

– **4-nucleate:** apparently of equal size but sometimes undifferentiated in form.

Bracts 10-13: already at this age, the first mitotic division of normal pollen nuclei should have taken place. Consequently, the numbers of nuclei noted in abnormal forms might or might not have owed something to such a division, which was still evidently in progress in some instances (Figure 8.4, top line). As in the older samples above, nuclei seen ranged from one to four per complex form, again sometimes strikingly unequal in size. Among the more curious examples were several with the only nucleus, or with one out of two or more, lying on the interface between two joined grains. This nucleus was commonly misshapen or variably stretched between the halves and, in the extreme case (Figure 8.4, fourth line), was linking two otherwise separated grains.

Abnormal spore formation in general

It is not in dispute that diverse irregularities have been observed in the regulation of the meiotic process of species hybrids. New examples have been revealed such as those of Chapter 7, of diploid pollen grains in 20-chromosome inter-sectional hybrids. One Siamese twin was also seen in a pollen sample of the accession of "*M. fehi*". However, the newer data accumulated suggest that these errors are relatively uncommon in hybrids, so that they cannot now be held to be a typical or significant occurrence for any pairing

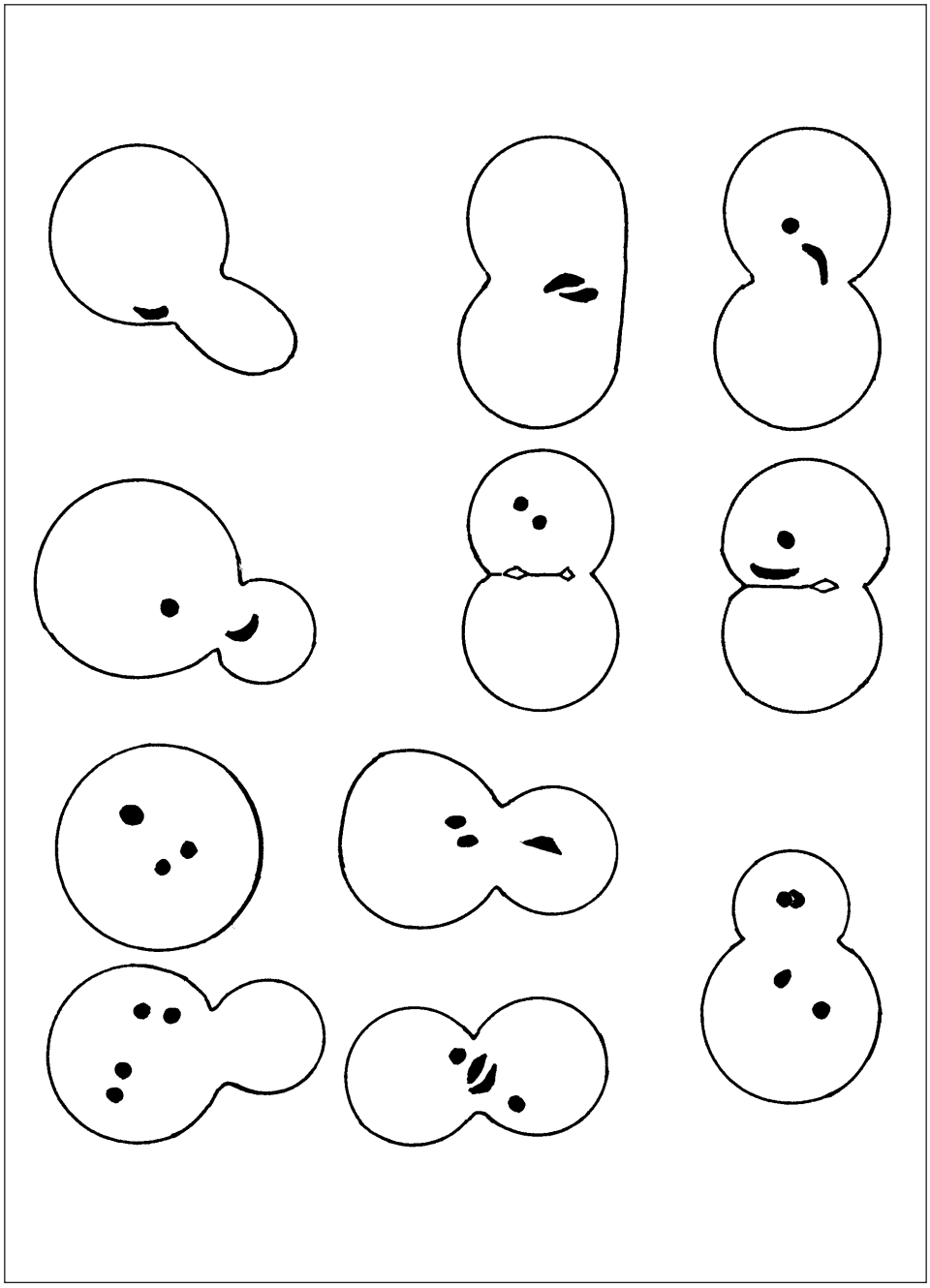


Figure 8.3. Immature pollen grains from bract 4 of plant 4552B-9.

Diagrams illustrating variation from one to four nuclei per unit, of rounded or elongated form, as well as in nuclear size and distribution.

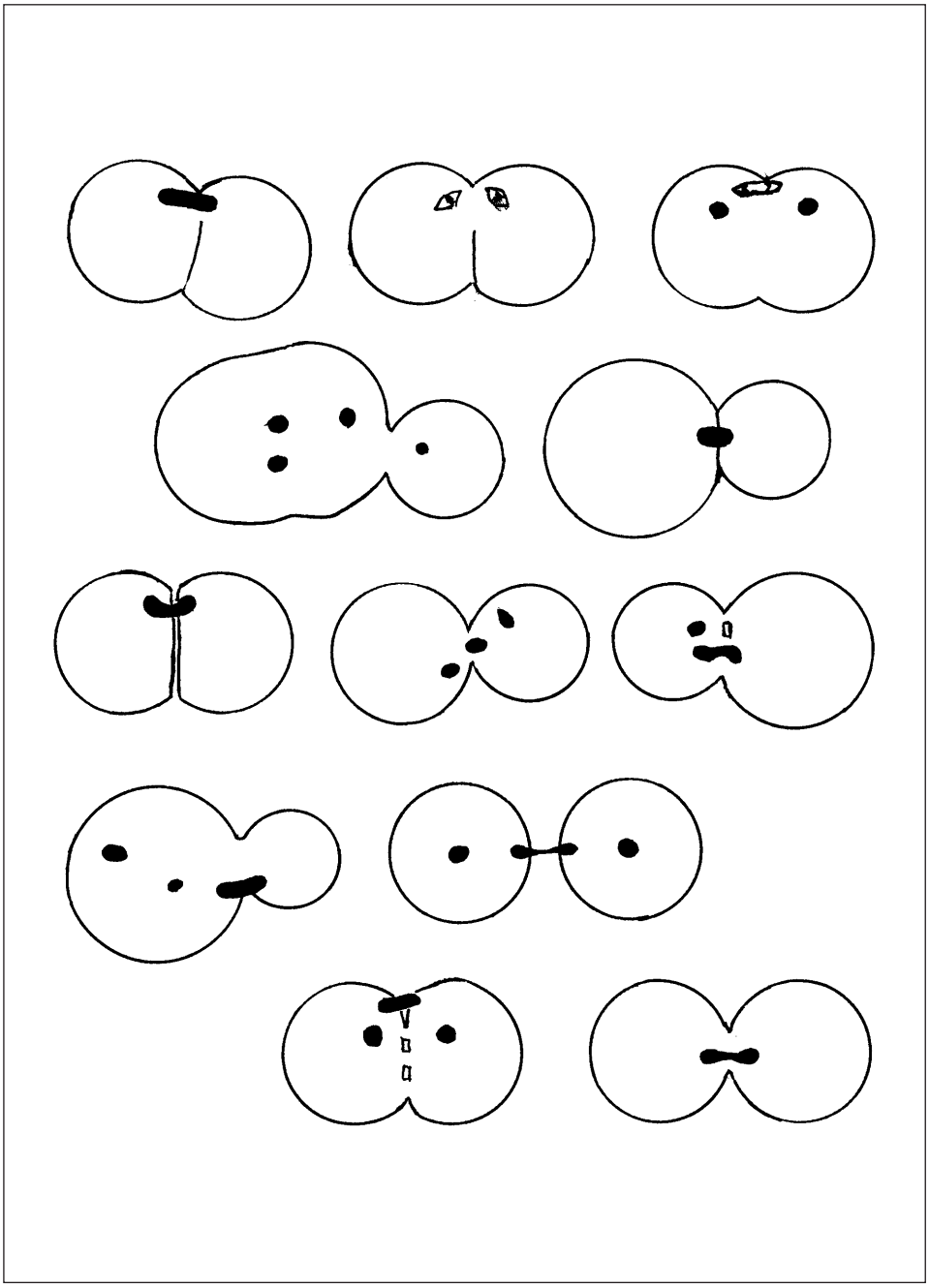


Figure 8.4. Immature pollen grains from bracts 10 to 13 of plant 4552B-9.

Diagrams illustrating variation in numbers of nuclei, of differing sizes, but these more or less rounded except where they are stretched between Siamese pairs or in the process of division.

of two more or less allied species. Furthermore, as is shown above, they may occur even in hybrids within a subspecies.

In a wider survey, the most interesting error is probably the substitution of meiosis in a pollen mother cell by the formation through endomitosis of a giant nucleus, without any recognisable condensation of the chromosomes (Simmonds, 1962, citing Dodds and Pittendrigh, 1946, Dodds and Simmonds, 1946). These nuclei are presumably tetraploid in diploid mother plants, Perhaps in these cases "meiotic breakdown" is not the best designation when what is encountered is "meiotic avoidance". The phenomenon was encountered by the above authors in hybrids of *M. balbisiana* with *M. laterita* and in the AA group cv Lilin; this writer has seen such giants in conspicuous numbers also in the hybrid Calcutta 4 x *M. basjoo* although they apparently did not occur in an earlier study of the same plant by K.S. Dodds.

A range of lesser examples is perhaps lost from the surviving record but, parenthetically, one other pertinent instance was an attempt by the author in Brasilia to study meiotic behaviour in pollen mother cells of the AAB cv Prata (Pome). This was thwarted by the majority of these having formed giant nuclei, presumed in this plant to be hexaploid. No events seen in pollen mother cells have suggested a half-way stage, of the formation of pollen with the maternal chromosome number without the intervention of meiosis. However, these could quite possibly be overlooked on the assumption that the relevant pollen mother cells were not yet ready for meiosis.

On the female side, the formation of functional polyploid megaspores in diploids is not wholly or necessarily restricted to interspecific hybrids. A known exception is the AA group cultivar Jari Buaya which, on pollination in Brazil, has been found to be very prone to the production of pentaploid offspring.

In this area we are still handicapped by the paucity of knowledge of events in megaspore mother cells. To what extent do these parallel those in pollen generation? How indeed do certain, regrettably few, triploid ovaries seem to contain triploid embryo sacs, rather than hexaploid, haploid or aneuploid ones (Chapter 10)? On this aspect, speculation without cytological or histological evidence does not suffice.

Of this last kind of data, what the author has seen in both AA and AAB ovaries was of a later stage, where many megaspores were arrested without any mitotic nuclear division, and **without any semblance of meiotic remnants at their micropylar ends**. The first was a hybrid in Jamaica of ssp. *banksii* x cv Paka, one of a family of plants which were known, on haploid pollination, to yield a proportion of triploid hybrids mixed with the expected diploids. Nuclei in these arrested megaspores were very probably diploid from their relative size. The second source of observations was in cv Prata Anã.

CHAPTER 9

Meiosis in triploids and tetraploids

Some early reports for chromosome associations in triploids were summarised by Simmonds (1962, p.110), with the conclusion that: "the data available suggest that pairing in triploid bananas is generally low and so variable with genotype and environment as to be useless for comparative purposes". On the aspect of environment this writer certainly agrees and has indicated such apparent differences in meiotic results in diploids in Chapters 2 and 3. But genuine comparative data on triploids already did exist in the author's own then unpublished studies, effected solely at ICTA and during 1953 except for a few plants added to the record in 1954. This information now forms the sole basis for the present disclosure on this ploidy level.

On meiosis in tetraploids nothing was known prior to the 1950's. Efforts from 1952 to 1954 in Trinidad proved them to be very difficult material, this because of the extreme congestion of figures on the first metaphase plates. For this reason, few cells could be safely interpreted at this stage. However, these gave some indication of the essential features.

Dessauw (1987) tabulated these same Trinidad results on both ploidy levels, with the author's prior permission and from a summary already in existence. He did not discuss them but, in the case of tetraploids, tabulated some extra data which are of value in confirming the general conclusions. In no case did he report on first anaphases.

Triploid plant materials

The purpose in listing the triploid cultivars here, since they are in the same sequence in Tables 9.1 to 9.3, is to identify them where necessary with other familiar or even unfamiliar names and provenances. This is most relevant for some of the ICTA accessions of Asian plants already in the Caribbean since the beginning of the century. Many merely echo Simmonds (1959) for convenience of reference.

AAA group cultivars

– Gros Michel needs no clarification;
– Cavendish subgroup representatives are Robusta and Chinese, the latter a Trinidad name for Dwarf Cavendish;
– Red is also universally known;
– "Orotava" was at ICTA an unidentified accession from the Canary Islands; it was collected in East Java by the Brazilian mission of 1985 as Kayu and appeared subsequently to be a blunt-fruited variant of Sri, from the same Indonesian source;

Table 9.1. First meiotic metaphases in triploid AAA group bananas.

Clone	Cells	Associations:					Frequencies:				Arm pairs
		12	11	10	9	<9	IV	III	II	I	
Gros Michel	30		18	6	4	2	0.10	4.17	5.87	8.37	15.9
Cavendish subgroup:											
Robusta	30	8	10	12	-	-	0.20	5.13	5.53	5.73	18.9
Chinese	30	2	15	10	2	1	0.07	5.00	5.43	6.87	17.4
Red	30	9	20	1	-	-	0.17	7.17	3.93	2.97	22.2
"Orotava"	30	2	27	1	-	-	0.77	6.97	3.30	2.43	22.2
"Rio"	30	3	24	3	-	-	0.13	7.20	3.67	3.53	20.8
Marathuva V.	30	5	22	3	-	-	0.17	6.60	4.30	3.93	22.1
Palimbang	30	2	24	4	-	-	0.20	7.27	3.47	3.47	22.0
Rajah	30	-	27	3	-	-	0	7.87	3.03	3.33	22.2
E. African subgroup:											
Lujugira	30	3	27	-	-	-	0.27	8.23	2.60	2.03	23.4
Lwekilo	30	1	28	1	-	-	0.23	8.87	1.90	1.67	23.5
Mitahato	30	1	29	-	-	-	0.10	8.47	2.47	2.27	23.3
Mutika	30	4	26	-	-	-	0.10	8.53	2.50	2.00	24.4
Ndoyo	20	4	15	1	-	-	0.10	8.50	2.55	2.00	23.5
	140	13	125	2	-	-	0.16	8.52	2.36	1.99	23.6
Kitarasa	22	3	18	1	-	-	0.27	6.36	4.45	3.91	21.6
Makifui	30	3	25	2	-	-	0.33	6.93	3.73	3.40	22.4
Synthesised hybrids:											
AA (wild) x IC 2 (AAAA):											
3225A-2	30	1	28	1	-	-	0.17	7.00	3.83	3.67	22.3
3293A-1	30	one cell with 13									
		5	21	3	-	-	0.37	5.77	5.00	4.23	20.8
3308 -3	24	4	18	2	-	-	0.12	6.71	4.25	3.88	22.6
AA (with spontaneous doubling) x AA: *											
J349-7	30	1	26	3	-	-	0.23	7.53	3.17	3.13	23.3
4216-18	30	3	26	1	-	-	0.20	8.50	2.37	1.97	23.8

* Respectively, a selfing of a rare diploid hybrid of 'Gros Michel' ♀ and an unexpected plant from a cross of Selangor x 'Paka'.

– "Rio" similarly arrived at ICTA without a name from Rio de Janeiro; from the author's defective memory of the plant it could have been Leite ("Milk") of Brazil, although results differed in relation to its female fertility (Chapter 10);
– Marathuva Vali from Sri Lanka seems to be a very uncommon clone, although once recognised by the author in 1962, by its quite yellow male flowers, in the then Guaruma collection of the United Fruit Company;

Table 9.2. First meiotic metaphases in triploid AAB group bananas.

Clone	Cells	Associations:					Frequencies:				Arm pairs
		12	11	10	9	<9	IV	III	II	I	
Mysore	30	4	24	2	-	-	0.17	5.27	5.63	5.27	20.5
King	30	4	15	11	-	-	0.10	3.83	6.83	7.43	19.6
Celat	14	2	10	2	-	-	0.07	4.86	6.07	6.00	20.4
Pome	**7**	**-**	**-**	**4**	**3**	**-**	0	2.4	7.1	11.4	**13.1**
Silk	18	-	17	1	-	-	0.22	5.89	4.83	5.50	21.5
N. Padaththi	29	2	7	8	4	8	0.03	2.69	6.79	11.21	**14.0**
Rajapuri	24	1	5	9	9	-	0.29	3.67	5.96	8.92	16.1
Grindy	24	-	14	10	-	-	0	4.96	5.67	6.79	20.6
Plantains:											
French Pl.	30	-	29	1	-	-	0.23	5.80	4.93	4.80	24.1
Mporo Moka	26	3	23	-	-	-	0.08	4.42	6.58	6.08	
							and one V = 0.04				22.0
Nyakahe	30	1	29	-	-	-	0.13	5.23	5.67	5.43	23.3
	86	4	81	1	-	-	0.15	5.19	5.69	5.41	23.2
Synthesised hybrids:											
AA x AABB:*											
3277C	30	3	23	4	-	-	0.43	6.60	3.90	3.50	
							and one V = 0.03				22.5
3606C-1	30	5	17	8	-	-	0.03	5.97	4.90	5.17	21.0
AB (Java IR 294A) x AA 'Lilin':											
3302B	27	3	11	9	4	-	0.11	4.44	5.93	7.37	19.5

* The tetraploid parents were two different hybrids of 'Awak Legor'.

– Palimbang, of provenance unremembered, is an AAA readily distinguishable from others of the Malayan-Indonesian region by its very rapid bunch maturity;
– "Rajah", again of unknown origin, is not to be confused with the AAB cultivar (Pisang) Raja; the clone included under this name here had some similarity to Gros Michel (Cheesman and Dodds, 1942);
– the "East African" subgroup clones included in the study were of the boiling or beer highland bananas, first identified as the "Lujugira-Mutika group" (Shepherd, 1957); Lwekilo is a black-stemmed mutant of Lujugira, Mitahato is perhaps equal to Mutika and Ndoyo is a semi-dwarf;
– Kitarasa and Makifui are other distinctive East African varieties.

AAB group cultivars

– Mysore is the Poovan etcetera of India;

Table 9.3. First meiotic metaphases in triploid ABB group bananas.

Clone	Cells	Associations:					Frequencies:				Arm pairs
		12	11	10	9	<9	IV	III	II	I	
Awak subgroup:											
Awak Legor	30	2	26	2	-	-	0.20	6.33	4.47	4.27	23.2
Nyeupe	30	5	25	0	-	-	0.23	5.83	5.10	4.37	22.6
	60	7	51	2	-	-	0.22	6.09	4.78	4.32	22.9
Peyan	30	1	22	7	-	-	0.20	5.67	4.93	5.33	23.1
Bluggoe subgroup:											
Bluggoe	30	0	30	0	-	-	0.03	3.70	7.27	7.23	21.0
P. Bontha											
Bathees	30	0	30	0	-	-	0	3.70	7.30	7.30	20.2
Ney Mannan	25	2	28	0	-	-	0.04	4.04	7.00	6.72	21.0
	85	2	88	0	-	-	0.02	3.80	7.20	7.11	20.7
Synthesised hybrids:											
BB Ceylon x IC 57 (AABB):*											
3484 -1	30	0	29	1	-	-	0.03	6.23	4.70	4.77	23.1
3484 -3	30	1	29	0	-	-	0.23	5.77	5.03	4.70	24.1
	60	1	58	1	-	-	0.13	6.00	4.87	4.73	23.6

* IC 57 was one of the 'Awak Legor' hybrids mentioned in Table 9.2.

– King is a West Indian name for a cultivar perhaps best known as (Pisang) Kelat, although it has other names in Asia;
– Celat is another West Indian accession and still a mystery otherwise;
– Pome is the well-known type of India, Brazil, Australia etcetera;
– Silk also is common and equal to Rastali or language variants of Apple;
– Nendra Padaththi and Rajapuri are the Indian cultivars, but the author has never agreed with Simmonds that one is a mutant of the other;
– Grindy from Grenada was later identified with the (P.) Raja or Radja of Asia;
– in the Plantain subgroup, there are a Trinidad French, an East African French as Mporo Moka and an East African False Horn as Nyakahe.

ABB group cultivars

– Awak Legor is the highly female-fertile form of Awak (Namwa) long present in the ICTA collection; Nyeupe from East Africa was indistinguishable in appearance but nearly seed-sterile, like recent accessions of the type from Asia and from Africa (via Martinique) to Brazil;
– Peyan from India is a plant morphologically similar to the above and very different from the next;
– the Bluggoe subgroup here includes the blunt-fruited (Pacha) Bontha Bathees and, less convincingly, the shorter-statured Ney Mannan.

Synthesised hybrids

A number of these were available for reference, either derived from diploid x tetraploid crosses or directly as a result of restitution cells in diploid-diploid combinations. These and the origins of the few tetraploids are identified as necessary in tables and text.

First meiotic metaphases in triploids

AAA cultivars

Quadrivalents were identified in varying frequencies in all clones examined except "Rajah" (Table 9.1). They were especially frequent in "Orotava", to the extent of two in the same cell in six out of thirty cases. Two were also seen in one cell only of the samples of Red and Marathuva Vali. While of positive interest in diploids, this indicator of translocation is more of a complication in triploids of any group, since there is no way of discriminating between a IV that includes three more or less homologous chromosomes and one that links two pairs. The sometimes high numbers of cells with a total of twelve, or as many as thirteen associations of two or more does suggest the latter case as very possible.

It appeared further that synaptic failures could at least sometimes reduce this number of recorded associations, since there is a well-marked deficiency in Gros Michel and Chinese. In the former, one extreme cell was limited to 3II 27I!

The most important observation is that the frequencies of both multivalents and total arms paired were still quite variable between the majority of cultivars where ansynapsis was not conspicuous. In extreme cases, such as that of Robusta, these were scarcely what would be expected of autotriploids. The highest numbers found were in the East African subgroup and a similar range of means was found among five new AAA hybrids.

AAB cultivars

Among the clones analysed (Table 9.2), the pollen mother cells processed of Silk were exceptional in that thirteen of the eighteen clear cells encountered at this stage had 34 chromosomes instead of 33. Nevertheless, all cells but one had eleven associations.

Another special case was that of Pome, where the metaphase associations were densely congregated and tense, so that few cells could be interpreted. Those recorded tended to have some degree of asynapsis and quite few multivalents. The handling problem was once more encountered in a new attempt to study meiosis in the synonymous Prata in Brazil. On this occasion, there were also many "cryptic restitution" pollen mother cells which did not enter into meiosis (Chapter 8). Partial asynapsis was evident too in King, Nendra Padaththi, Rajapuri and Grindy.

Quadrivalents were again found in a majority of clones, but never two in the same cell except in the hybrid 3277C; the plantain Mporo Moka showed one association of five. On average and as expected, multivalents were fewer than in AAA. Higher values were found in the two AA x AABB plants, but rather low ones in the cross of the spontaneous

AB accession Java 294A with cv Lilin. Arms paired were once more quite variable in numbers between plants, as many in the plantains as in any AAA.

ABB cultivars

The few accessions studied of this group fell into merely three types (Table 9.3). All but 'Peyan' demonstrated high mean frequencies of total associations; all had numbers of arms paired comparable with those in AAA. The Bluggoe subgroup differed consistently from the other cultivar clones and the two hybrids. These clones displayed rare quadrivalents, less frequent multivalents generally and rather fewer arms paired.

The three groups in summary

As already made clear in detail, there was no marked differentiation in meiotic association between auto- and allotriploids. The alternative genomic combinations were all capable, in some instances at least, of yielding high numbers of multivalents.

Additionally, the types of triploids were noted, as accurately as possible, and a discrimination was made between ring and rod bivalents (Table 9.4). Two curious conclusions have resulted, that both frying pan trivalents and ring bivalents seem to have increased in relative frequency with the increase in B genomes. This is in contrast with the low chiasma frequencies manifest in AB F_1 hybrids (Chapter 3) but no explanation can be offered. The frying pan, like the "Y", is a product of two chiasmata involving the same chromosome arm. Is it the alien chromosome that tends to tack itself on terminally in AAB or ABB, or one from the pair of the same species?

Whichever is the case, the reduced affinity between A and B genome chromosomes, as found in diploid AB hybrids, is not always more conspicuous in the competition for partners at the triploid level. On the other hand, it has been argued in Chapter 3, from the aspects of diploid hybrids of ABB x AA, that there is definitely much elimination of A chromosomes in the haploid products of meiosis in the female parent. This might however be at a later stage than meiosis itself, manifested in non-vigour of the introgressed spores. Even clearer cases arise in AAB Pome/Prata or Prata Anã x AA. Diploid products of the crosses are normally indisputably AA in type, after the certain elimination of B chromosomes in haploid megaspores of the female parent.

First meiotic anaphases in triploids

On the whole these were understandably chaotic in terms of numbers of univalents present and no record has been kept of these. This fact and the random polar acquisition of two units or one from each trivalent should clearly have minimised potential fertility, apart from the segregation of interspecific allele differences. Bridges of two types were noted however and these are listed in Table 9.5. In all three groups, there were very few clones that did not present bridges with fragments. They were least evident in ABB, most common in "Orotava" (AAA) and King (AAB). Two were found at least once in the same cells of eight distinct genotypes.

Table 9.4. Forms of trivalents (III) and bivalents (II) found in first metaphase cells of the diverse genomic groups of triploids.*

Clone	III	V	L	Y	FP	II	O	R
AAA group:								
Gros Michel	125	77	43	1	4	176	38	138
Cavendish subgroup	304	190	95	5	14	329	97	232
Red	215	151	41	3	20	118	84	34
"Orotava"	209	130	40	5	34	99	47	52
"Rio"	216	105	66	3	42	110	27	83
Marathuva Vali	198	120	43	2	33	129	89	40
Palimbang	218	157	27	4	30	104	69	35
Rajah	236	136	41	5	54	91	48	43
E. African subgroup	1191	456	353	42	340	335	175	160
Kitarasa	140	60	39	3	38	98	39	59
Makifui	208	99	49	2	58	112	54	58
synthesised hybrids	977	487	213	11	266	516	280	233
Totals	4237	2168	1050	86	933	2217	1047	1170
%		51	25	2	22		47	53
AAB group:								
Mysore	158	102	19	0	37	169	79	90
King	115	68	18	2	27	205	116	89
Celat	68	32	14	0	22	85	39	46
Pome	17	7	10	0	0	50	8	42
Silk	106	44	37	0	25	87	51	36
Nendra Padaththi	78	40	30	1	7	192	40	152
Rajapuri	88	28	26	6	28	143	19	124
Grindy	119	66	21	0	32	134	83	51
Plantain subgroup	446	155	69	5	217	489	353	136
synthesised hybrids	497	295	63	1	138	424	224	200
Totals	1692	837	307	15	533	1978	1012	966
%		49	18	1	32		51	49
ABB group:								
Awak subgroup	365	132	91	4	138	287	178	109
Peyan	170	84	24	2	60	148	125	23
Bluggoe subgroup	323	125	69	9	120	612	379	233
synthesised hybrids	360	133	59	6	162	292	220	72
Totals	1218	474	243	21	480	1339	902	437
%		39	20	2	39		67	33

* III: V = alternate, L - ± linear, Y = Y-form with one double chiasma, FP = frying pan with one double chiasma and one other; II: O = ring, R = rod.

Table 9.5. Bridges* in first meiotic anaphases of triploids.

Clone	Cells	2BF	1BF	B	Clone	Cells	2BF	1BF	B
AAA group:					**AAB group:**				
Gros Michel	50	-	4	5	Mysore	30	2	5	1
Cavendish subgroup					King	50	4	**26**	7
	53	1	9	7	Celat	50	1	10	7
Red	50	1	15	6	Pome	40	-	4	1
"Orotava"	50	2	20	2	Silk	50	-	-	1
"Rio"	50	1	15	4	N. Padaththi	50	-	5	14
Marathuva Vali	17	-	-	4	Rajapuri	19	-	-	11
Palimbang	50	1	17	10	Grindy	50	-	11	12
Rajah	50	-	2	-	Plantain subgroup				
East African subgroup						44	-	2	3
	235	-	13	30					
Kitarasa	50	-	4	4	**ABB group:**				
Makifui	50	-	5	5	Awak subgroup	100	-	2	24
					Peyan	50	-	4	3
					Bluggoe subgroup	140	-	3	7

* BF = bridge with a fragment; B = bridge without one.

Meiosis in tetraploids

Among the few cells at first metaphase the only AAAB examined, resulting from a cross of Pome, behaved differently from either the AAAA representative or the three AABB hybrids examined (Table 9.6). This was shown in its relatively high frequencies of trivalents and univalents in relation to bivalents. The other two classes were similar in revealing quite low numbers of multivalents, of which the AABB hybrids had unexpectedly more rather than less. Despite the genomic differentiation between two sets each of A and B, the amphidiploids AABB came no nearer to being functional diploids in pairing than did the autotetraploid AAAA.

The data of Dessauw (1987) came from one allotetraploid AABB, in contrast with colchicine-induced autopolyploid lines of ssp. *malaccensis* Selangor (AAAA) and of two accessions of *M. balbisiana* (BBBB). In contrast with the results newly published here, he did find an increase in numbers of quadrivalents in the genetically more uniform colchicine products and his AABB plant had an average of 18.56 II per cell. It has to be concluded that studies are needed of a wider range of tetraploids of different origins, despite the technical problems, but that a major disparity in behaviour between autotetraploid and amphidiploid is unlikely to be revealed.

First anaphases in the writer's plants were more amenable to analysis (Table 9.7). They varied from a low incidence (AAAA) to a rather high one (AAAB) of excluded univalents and the almost inevitable bridges with fragments, although these were found in only three of the four genotypes recorded for this feature.

Table 9.6. First meiotic metaphases in some tetraploid hybrids.

Nº & 3x parent	Cells	Frequencies:				Excess of pairs I:					
		IV	III	II	I	-1	0	1	2	3	Mean
AAAA:											
IC 43 (Rajah)	8	0.4	1.8	17.2	2.8	-	6	1	-	1	0.5
AAAB:											
IC 62 (Pome)	9	0.6	5.0	9.6	7.7	-	3	-	6	-	1.3
AABB:											
IC 24 (Mysore)	2	-	2.5	17.0	2.5	-	2	-	-	-	-
IC 57 (Awak Legor)	25	0.7	2.3	15.0	4.2	1	9	7	6	2	1.0
IC 61 (Awak Legor)	13	1.0	2.2	15.0	3.3	-	8	3	2	-	0.5
Totals	38	0.8	2.3	15.0	3.9	1	17	10	8	2	0.8

Table 9.7. First meiotic anaphases in some tetraploid hybrids.

Nº & 3x parent	Cells	Univalents:				Bridges:*		
		0	1-3	4 +	Mean	2BF	1BF	B
AAAA:								
IC 43 (Rajah)	30	12	18	-	1.07	not recorded		
AAAB:								
IC 62 (Pome)	50	-	18	32	4.32	2	15	1
AABB:								
IC 24 (Mysore)	33	15	15	3	1.33	-	5	2
IC 57 (Awak Legor)	50	10	29	11	2.32	-	-	8
IC 61 (Awak Legor)	50	14	33	3	1.46	2	5	2

* BF = bridge with a fragment; B = bridge without one.

CHAPTER 10

Polyploids and aneuploids

Some of the plant materials included in this penultimate chapter are of spontaneous origin, others are the products of hybridisation. The majority of both kinds shares a common characteristic, that they are without any evolutionary future, with or without human intervention. However, a review of their fertilities and breeding behaviours, also of their vigour in some cases, should help in establishing what are the likely limitations of their improvement by conventional means, independently of the quality of diploid germplasm available. This assessment has to assume that environment did not have a major impact on the results obtained. The purpose here is to give as broad an account as is possible, successively, of triploids, of tetraploids, of higher euploids and of aneuploids.

Triploids

Male fertility

When reference is made to the irregular meiosis that is found at this ploidy level (Chapter 9), it is at least a little surprising that they are not always male-sterile. Breeders in Honduras have taken practical advantage of the fact that the medium statured to tall members of the AAA Cavendish subgroup produce a small amount of functional haploid pollen and they have also noted haploid pollen production in 'Laknau' (AAB). Observations at the CNPMF in Brazil have confirmed both instances, the latter initially as 'Kune' from Papua New Guinea. Two AAA accessions from the same country, 'Dodoga' and 'Walebo', closely similar to one another and probably related by mutation, also display appreciable amounts of pollen which has been used in generating diploid offspring. Other examples in AAB are the 'Kelat' type, long noted by the author, and the 'São Domingos' of Brazil, which almost certainly is found under other names in northern South America, such as 'Maritu' in Colombia. This could be the 'Iholena' of Hawaii according to Simmonds (1959). On meiotic behaviour 'Bluggoe' and its relatives seem to be among the more likely triploids to yield some haploid microspores, but the writer has never seen pollen in any ABB clone, even in those shown below to produce large numbers of haploid or near-haploid embryo sacs.

Female fertility in general

Partial female fertility in triploids has long been known to be widespread (Cheesman, 1932; Cheesman and Dodds, 1942, Shepherd, 1960a), but merits a new scrutiny to include the much broader spectrum of cultivars now studied. Before embarking on this, however, an initial observation is that a common restrictive element in this fertility is apparently

not dependent on ploidy level. An important barrier in diploids too, apart from meiotic and post-meiotic abnormalities, was thought to be the failure of penetration of pollen tubes as far as the ovules (Chapter 2). With respect to cultivars of both ploidy levels, Shepherd (1960a) recorded instances of morphological or physiological immaturity of the stigma or style and others of growth inhibition of pollen tubes within the style. In all clones then studied there was a relative, and sometimes dramatic, decline in seed numbers from the apex of the ovary towards the base, this despite at least one case where embryo sac frequencies were known to be similar. The bias was sometimes reduced by precocious pollination. A possibly relevant observation has been the premature decay of the nectary in the ovary apex. These aspects were again emphasised by Shepherd, *et al.* (1987b), as a justification for research on fertilisation *in vitro*.

In triploids there is the additional expectation that, at least where an attempt at meiosis is the norm, there will be a pronounced deficiency of balanced and functional megaspores and consequently of mature embryo sacs. The experimental verification of events in ovules has long been a difficult and time-consuming process, relying on sectioning of wax-embedded ovary segments. Consequently, the author knows of few results on triploids. Shepherd (1954) found as many as about 10% of 'Gros Michel' ovules with morphologically mature embryo sacs at the time of flower receptivity. Simmonds (1960b) reported their absence in samples of 'Mysore' and 'Bluggoe' at up to five days after flower receptivity; in practice, many pollinated bunches of both are indeed seedless. Sacs were frequent in 'Awak Legor' even before the stage of flower opening. Apparently, the flowers used in Simmonds' research had not been pollinated but they were for some more recent and yet unpublished data secured in Brazil. Structurally mature stages ranged from extremely rare in two Cavendish subgroup clones to surprisingly more than 50% of ovules in the French plantain 'Terrinha'. In these last studies, no pollen tube was identified within any ovule of any clone up to three days after normal pollination.

It is with such results in mind that the actual seed and hybrid production recorded for diverse cultivars must be surveyed. Much the greater part of the results here refer to crosses with *M. acuminata*. In the older literature cited it was found that crosses of AAA with *M. balbisiana* were rarely successful and that those of AAB with BB were very much less profitable than those with AA. Such attempted hybridisations have therefore been omitted here.

To avoid much repetition, the sources of information used in the detailed data are frequently identified as T1 (Cheesman, 1932), T2 (Cheesman and Dodds, 1942), T3 (Shepherd, 1960a), all from Trinidad, and B for new information from the CNPMF in Brazil. In relation to these last results there is the added analysis available of the quality of supposedly "good" seeds, insofar as they were processed by the culture of embryos in the biotechnology laboratory.

Some general comments on two hybrid classifications are necessary. At first, the thick-leaved dwarf plant type arising from crosses of triploids with diploids tended to be regarded at least for convenience as a heptaploid (T1, T2); some counts were achieved of cells with 73 to 77 chromosomes. However, the writer has noted appreciable variation in

intensity of dwarfing in Jamaica and in Brazil. Precise chromosome counts have only occasionally been possible, and logically from the least unvigorous examples. They have included approximate **hexaploid** counts, but apparently here too there has been some tendency to chromosome loss. By good fortune, the writer was once able to view some embryo sacs of 'Ney Mannan' (ABB) in hand sections of ovaries some time after pollination. In one of these, perhaps relevantly, there was a clear indication of early endosperm stages without the presence of a pollen tube, although another sac was already fertilised.

At the other extreme there have been plantlets in culture tubes of a like appearance to 6x or 7x, but so much reduced in leaf and root development as to suggest even higher ploidy. For this chapter the vaguer denomination of high polyploid (HP) has been commonly employed for all dwarf plants.

The aneuploid fraction in the hybrid classifications of Brazil, with chromosome numbers from 23 to 32, was in an appreciable number of plants judged by appearance rather than by counting, as was also the practise in some backcross families of Chapter 3. Counts of 23 or 24 were often but not always associated with narrower and coarser leaves than those of the exact diploids; plants with this aspect but without vigorous roots were at times assigned without counting to the 23 to 32 class. Without doubt, this same classification must have been underestimated repeatedly by the exclusion of the least vigorous offspring, either in culture tubes or in the greenhouse.

AAA x AA

Relevant tables are 10.1 and 10.2 (seed yields), 10.3 (quality of embryos and germinations in culture) and 10.8 (chromosome numbers verified).

The Gros Michel subgroup was the classic case of a tetraploid producer (T1). Factors affecting the seed-bearing of 'Gros Michel' itself have been shown to include localities within Jamaica and other influences external and internal to the plant (Shepherd, 1954 and 1960b). The semi-dwarf mutant 'Highgate' was less seedy in Jamaica, but compensated by yielding a higher ratio of tetraploids to HP's in its progenies. Diploid hybrids also occurred occasionally in crosses of the tall parent and were characteristic of the semi-dwarf at one Jamaican location.

The Cavendish subgroup has long been noted for its nearly total female sterility. Two seeds were recorded among 460 bunches of the taller member clones in Trinidad and neither germinated (T2). A tetraploid hybrid now exists in the FHIA programme in Honduras but at what expense of effort is not known to the writer, whose further attempt (B) yielded a wholly negative result.

Much of the work at the CNPMF consisted of a survey of fertility patterns in accessions from Papua New Guinea. None of the AAA clones tested on any worthwhile scale was totally seedless, but the yields of these, as of the generality of triploid parents, depended very much on the type of pollen employed. That of Calcutta 4 (code 03) was usually much more successful than that of any parthenocarpic diploid, whether AA cultivar accession or hybrid and whether apparently highly male-fertile or not. Shepherd

Table 10.1. Seed yields from crosses at the CNPMF, Brazil, between triploids of the AAA group, from a single source, and various AA diploids.

Cultivars and equivalents	Source	Pollen*	Fruits	Seeds**		
				"Good"	Bad	G/100F
Ambey	Papua	03	598	15	11	2.5
	New Guinea	PC	231	2	0	0.9
Bagul & Lakem	"	03	916	22	27	2.4
		PC	435	0	0	0
Dengree	"	all	597	0	5	0
Dodoga + Walebo***	"	03	1542	703	215	45.6
		PC	2226	68	10	3.1
Kapoosnarootoo	"	all	413	25	5	6.1
Kuru Peck	"	03	329	10	9	3.0
		PC	284	0	0	0
Markatooa	"	03	1203	205	33	17.0
		PC	557	7	1	1.3
Morong & Torp	"	03	1512	313	28	20.7
		PC	599	16	1	2.7
Muga	"	03	253	1	0	0.4
		PC	478	0	0	0
Nombum	"	03	363	149	34	41.0
		PC	274	2	0	0.7
Pagatow	"	03	945	167	44	17.7
		PC	762	9	0	1.2
Siminarook	"	03	1178	393	169	33.4
		PC	801	31	14	3.9
Wasolay	"	03	716	152	156	21.2
Who-Gu	"	WT	1217	5	1	0.4
		PC	249	2	0	0.8

* Codings used are: 03 = Calcutta 4, 41 = Calcutta 4 x Madang, WT = wild- type generally and PC = parthenocarpic (AA cultivars and hybrids).
** "Good" seeds were judged on appearance and hardness, not on quality.
*** Dodoga and Walebo are closely similar but not synonyms.

et al (1987a) offered evidence of markedly different pollen tube growth rates between diploid sources as a possible contributory cause.

Many but not all of the "good" seeds were processed by the extraction and culture of embryos. In nearly all cases, a substantial or quite high proportion was without an embryo and only 'Siminarook' displayed a moderately satisfactory rate of germinations. Even so, many plants were weaklings. Only 'Ambey' and 'Wasolay', of the fourteen distinct types listed, provided any exactly tetraploid hybrid and the great majority of the 49 seedlings classified, of all accessions, was composed of aneuploids with chromosome numbers between 23 and 32.

Table 10.2. Seed yields from crosses at the CNPMF, Brazil, between triploids of the AAA group, from diverse sources, and AA diploids.

Cultivars and equivalents	Source	Pollen*	Fruits	Seeds**		
				"Good"	Bad	G/100F
Caru Roxa/Verde	Brazil	WT	2133	1183	3721	55.5
(Red/Green Red)	& Asia	PC	867	18	103	2.1
Kayu & Sri	Java	WT	2396	97	120	4.0
("Orotava")	& France	PC	658	4	39	0.6
Leite	Brazil	WT	715	4	0	0.6
		PC	1631	0	0	0
Nam	Thailand	WT	266	56	11	21.1
		PC	477	38	31	8.0
Ouro Mel	Brazil	all	694	1	0	0.1
São Tomé	Brazil	03	2215	85	249	3.8
(Lujugira)		PC	1886	3	0	0.2
Yangambi Km 5	France &	03	1134	51	24	4.5
(Khom & Khai	Thailand	41	1689	48	48	2.8
Thong Ruong)		PC	660	2	10	0.3
Amritsagar	Hawaii	all	668	0	0	0
Bakar	Java	all	482	0	0	0
Cavendish subgroup	Brazil	all	7802	0	0	0
Sapon	Java	all	1235	0	0	0

* Codings for male parents are as for Table 10.1.
** "Good" seeds were judged on appearance and hardness, not on quality.

Among clones from other sources, the 'Red'/'Green Red' forms and the various "Orotava"-type accessions, 'Kayu' and 'Sri', showed similar orders of fertility between T2, T3 and B. Seed quality was poor to only fair and germination rates were low in Brazil as in Trinidad. The 'Red' clones in both countries yielded plants with a range of chromosome numbers only from 22 to 33. The few hybrids classified in this table of 'Kayu' or 'Sri' (B) differed from the earlier T2 results for "Orotava" in that they included counts of less than 33 and no tetraploid.

The Brazilian record for 'São Tomé'/'Lujugira' differs from the T3 one, where no seeds at all were found in the East African highland clone, but it mirrors lost data from attempts on the subgroup in Jamaica, where some seeds were obtained but not viable ones. The single hybrid transplanted in Brazil was a weakling and soon died. "Rio" (T2 and T3) was somewhat seedy when crossed with AA and solely tetraploid hybrids were identified (T2). By contrast 'Leite' (B) was nearly ♀ sterile and the only plantlet secured was a high polyploid.

In Trinidad 'Marathuva vali' and "Rajah" yielded seeds in adequate numbers and at least one tetraploid hybrid each (T2). The seeds of the former germinated better and gave a wide range of chromosome counts, from 23 to 35. 'Palimbang' (T3) yielded only

Table 10.3. Quality of seeds of AAA group cultivars, as processed in the embryo culture facility of the CNPMF, and numbers germinated.

Cultivars		Classifications of seeds*					Totals**		
		N+	N-	A+	A-	S±	Σ	G	T
From Papua New Guinea:									
Ambey		9	0	2	4	1	16	3	3
Bagul & Lakem		6	1	6	2	7	22	9	7
Dodoga & Walebo		130 + 1?	28	107	68	140	474	21	4
	(%)	(28)	(6)	(23)	(14)	(30)		(4)	
Kapoosnarootoo		9	0	3	2	8	22	x2	2
Kuru Peck		3	0	0	0	6	9	0	
Markatooa		30	3	40	10	83	166	0	
Morong & Torp		56	4	48	31	91	230	2	2
	(%)	(24)	(2)	(21)	(13)	(40)		(1)	
Nombum		34	23	9	17	53	136	9	6
Pagatow		49	7	26	29	56	167	11	3
Siminarook		103 + 5?	1	43	11	131	294	69	35
	(%)	(37)	(-)	(12)	(16)	(45)		(23)	
Wasolay		11	16	20	34	95	176	3	2
From other sources:									
Caru Roxa/Verde		128	6	63	80	227	504	45	31
	(%)	(25)	(1)	(12)	(16)	(45)		(9)	
Kayu & Sri		17	0	23	14	10	64	21	11
Leite		3	-	1	-	-	4	1	HP
Nam		35	4	30	1	18	88	22	19
São Tomé		30	9	37	8	45	129	**5**	**1**
Yangambi Km 5		46	1	24	10	20	101	34	20

* The first classification is of embryos as N = normal, A = abnormal or reduced and S = absent; subsequently endosperm is rated as + for normal and - for reduced or absent.
** Σ = total seeds processed, G = germinated and T = transplants; HP = evident high polyploid discarded without transplanting.

bad seeds. In Brazil, the other seemingly "indigenous AAA" 'Ouro Mel' was virtually sterile; the only seed failed to germinate. Three other introduced clones, 'Amritsagar', 'Bakar' and 'Sapon' gave no seed at all.

Seeds were obtained in significant numbers from 'Nam' and 'Yangambi Km5'. These were fairly readily germinable but not one of 26 plants classified had a count of 33 or more chromosomes.

To summarise the analysis of this group, only a few clones have been found to yield tetraploid hybrids and those with difficulty. As additional euploids, some few newly triploid hybrids and a number of exact diploids have resulted, these of unknown usefulness.

AAB x AA

The tables here are 10.4 (seed yields), 10.5 (seed quality and germination) and again 10.8 (chromosome numbers).

'Mysore' and 'Pome' have been long known to have a tetraploid hybrid production capacity, whereas 'Silk' does not (T1 and T2). These cases, including variants of the Pome subgroup, have been thoroughly re-examined and confirmed in Brazil (Shepherd *et al*, 1987a and b, 1994). In practical terms, useful female fertility in 'Mysore' has

Table 10.4. Seed yields from crosses at the CNPMF, Brazil, between triploids of the AAB group and AA diploids.

Cultivar and equivalents	Source	Pollen*	Fruits	Seeds**		
				"Good"	Bad	G/100F
Garoto	Papua New	03	316	63	16	19.9
	Guinea	PC	297	1	2	0.3
Kabai	"	41	212	4	0	1.9
Komtar	"	03	280	30	3	10.7
		PC	110	1	0	0.9
Kune	"	see Laknau below				
Tomnam	"	WT	812	36	31	4.4
		PC	351	48	14	13.7
Umpako	"	WT	391	0	46	0
		PC	188	0	11	0
Warik	"	WT	375	2	1	0.5
		PC	369	3	0	0.8
Adimoo	"	all	914	0	0	0
Mbei	"	all	497	0	0	0
Saney/NBB20	"	all	2543	0	0	0
"Java"	Brazil	all	1513	10	17	0.7
(Thong Ruong/Raja)	& Asia	PC	1260	20	8	1.6
Kelat etc.	France	WT	1193	16	17	1.3
	& Asia	PC	725	3	24	0.4
Laknau/Kune	France	03	670	198	34	29.6
	& PNG	41	256	39	3	15.2
		PC	607	18	2	3.0
Moenang	Thailand	WT	283	34	9	12.0
		PC	199	4	4	2.0
São Domingos	Brazil	WT	861	9	7	1.0
Eslesno	Hawaii	all	620	0	0	0
Padath (N. Padaththi)	Brazil	all	6252	0	0	0
Pulut (Seribu)	Asia	all	2469	0	0	0
Tip Kham	Thailand	all	1865	0	0	0
Walha (Rajapuri)	Hawaii	all	2168	0	0	0

* Codings used are as in Tables 10.1 and 10.2.

** "Good" seeds were judged on appearance and hardness, not on quality.

Table 10.5. Quality of seeds of AAB group cultivars, as processed in the embryo culture facility of the CNPMF, and numbers germinated.

Cultivars	Classifications of seeds*					Totals**		
	N+	N-	A+	A-	S±	Σ	G	T
From Papua New Guinea:								
Garoto	4	5	5	8	20	42	0	
Kabai	1	3	-	-	-	4	4	HP
Komtar	0	0	2	9	20	31	0	
Kune - see Laknau below								
Tomnam	19	0	35	5	24	83	5	3
Warik	0	0	2	2	1	5	1	HP
From other sources:								
"Java"/Raja	11	2	8	8	**0**	29	18	HP
Kelat etc	6+1?	0	6	3	3	19	9	8 +HP
Laknau/Kune	12	0	35	4	14	65	27	12 +3HP
Moenang	11	0	11	2	14	38	16	2
São Domingos	0	0	0	1	8	9	0	

* Ratings of embryo and endosperm as in Table 10.3.
**Σ = total seeds processed, G = germinated and T = transplants; HP = evident high polyploids discarded without transplanting.

appeared to be limited and strongly seasonal there. That of 'Prata' and its relatives has been emphatic, with production of some diploids as well as many tetraploids and HP's. As an exception within this Pome subgroup, 'Nadan' was again found to be female-sterile. The 'Prata Anã' of Brazil, whose alliance with the Pome subgroup remains doubtful, yields relatively few but quite viable seeds, again with a range from diploid to HP. The last paper cited also raises genetic improvement hopes through the 'Silk'-like 'Yangambi Nº 2' introduced from France, which was shown to be another efficient enough source of tetraploid hybrids.

No special comment is needed either on female fertility in the Plantain subgroup, where certain clones of the French class have been found, in Honduras, Nigeria and the Cameroons, to produce seeds and a useful spectrum of hybrids (Rowe and Rosales, 1994; Ortiz and Vuylsteke, 1994; Jenny *et al*, 1994). The Brazilian 'Terrinha' has also given tetraploid and diploid hybrids.

Once more, the newest data have a strong representation of genotypes known only from Papua New Guinea. Few produced more than occasional "good" seeds at the CNPMF and three appeared to be quite sterile. Except in the sparsely fertile 'Kabai' embryo quality and germination were poor. Chromosome counts were made from 'Tomnam' alone and two out of three plants were tetraploids.

Other accessions listed in the tables include 'Raja' and 'Kelat', on which there exist some earlier data. The former as 'Grindy' (T3) and the latter as Type 13 (T2) were just as sparingly seed productive; we now have the information that the few seeds from both

tend to be viable, although neither has yet yielded a tetraploid hybrid (B). High polyploids have been common to both and 'Kelat' has generated triploids and aneuploids. 'Kune', from PNG, was found to be a possible tetraploid hybrid source at the CNPMF before its synonymy with 'Laknau' was recognised. The nature of the latter's hybrids had already been established in Honduras.

The sterility of 'Nendra Padaththi' and 'Rajapuri' (B) confirms the earlier record (T3) and three other CNPMF accessions failed to yield seeds.

In summary, the AAB group has shown itself to be more amenable than AAA to yield tetraploid "copies" in crosses with *M. acuminata*, although many types are excluded from this outlet on the available evidence. Exact diploids have been recovered from the Pome subgroup, from 'Prata Anã' and from French Plantains in particular. Those seen in the field by the author have shown no discernible suggestion of inheritance of B genes, an observation that seems to have been made at IITA also.

ABB x AA and x BB

Details are to be found in tables 10.6 (seed yields), 10.7 (quality and germination) and 10.9 (hybrid classes).

Table 10.6. Seed yields from crosses at the CNPMF, Brazil, between triploids of the ABB group and AA diploids.

Cultivars and equivalents	Source	Pollen*	Fruits	Seeds**		
				"Good"	Bad	G/100F
Awak subgroup:						
Four tall forms	France &	41	843	9	2	1.1
	Thailand	PC	12074	196	42	1.6
Namwa Khom	Thailand	all	5118	0	0	0
Bluggoe subgroup:						
Six tall forms	Brazil &	WT	4803	4371	2060	91.0
	C. America	PC	8343	964	453	11.6
Dwarf mutants	Equador &	WT	302	3	1	1.0
	France	PC	1841	7	0	0.4
Others:						
Champa Madras	France	WT	210	292	180	139
		PC	468	455	145	97
Ice Cream/Abu Perak	Hawaii &	WT	468	618	483	132
	France	PC	3680	1324	828	36
Kepok Bung	Java	all	945	276	173	29
Pelipita	C. America	WT	231	584	87	253
		PC	1612	361	41	22
Saba	C. America	41	673	2	18	0.3
	& France	PC	2079	0	6	0
Tip	Thailand	all	1329	66	25	5.0

* Codings for male parents are as for Table 10.1.

** "Good" seeds were judged on appearance and hardness, not on quality.

Table 10.7. Quality of seeds of ABB group cultivars, as processed in the embryo culture facility of the CNPMF, and numbers germinated.

Cultivars	Classifications of seeds*					Totals**			
	N+	N-	A+	A-	S±	Σ	G	T	+HP
Awak subgroup:									
Four tall forms	33	0	62	12	82:	189	21	7	6
(%)	(17)	(33)	(6)	(43)		(11)			
Bluggoe subgroup:									
Six tall forms:									
- processed 1983-85	174	6	390	126	191:	887	164	98	14
(%)	(20)	(1)	(44)	(14)	(22)	(18)			
- processed 1986-90	123	9	157	29	108:	426	131	56	3
(%)	(29)	(2)	(37)	(7)	(25)	(31)			
Dwarf mutants	2	0	5	0	2:	9	3	1	0
Others:									
Champa Madras	206	0	223	7	113:	549	177	72	2
(%)	(38)		(41)	(1)	(21)	(32)			
Ice Cream/Abu Perak	271	34	241	145	153:	844	218	62	14
(%)	(32)	(4)	(29)	(17)	(18)	(26)			
Kepok Bung	90	3	57	46	80:	276	69	36	4
(%)	(33)	(1)	(21)	(17)	(29)	(25)			
Pelipita	130	0	146	11	137:	424	71	3	5
(%)	(31)		(34)	(3)	(32)	(17)			
Saba	0	2	0	0	0:	2	1	1	0
Tip	28	0	21	1	11:	61	14	7	0

* Ratings of embryo and endosperm as in Table 10.3.

** Σ = total seeds processed, G = germinated and T = transplanted; HP = evident high polyploids discarded without transplanting.

Within the Awak subgroup, 'Awak Legor' in Trinidad was quite highly seed-productive in crosses with either wild species as male parent (T2 and T3). Germination rates were poor to fair and the plants obtained were vigorous tetraploids mixed with HP's. 'Nyeupe', an accession from East Africa, was much less fertile and the few hybrids recorded were all tetraploids (T3). Both of these accessions were regarded as **exact** triploids (Chapter 9), but all four tall accessions and the semi-dwarf 'Namwa Khom' pollinated in Brazil have been found to have 34 chromosomes rather than 33 in the great majority of their root tip cells (Shepherd and dos Santos, 1996; Shepherd and da Silva, 1996). The tall forms have been very sparsely seed-fertile and the dwarf totally sterile, lamentably. In the former case seeds without embryos were common and germination rates were poor. Other than HP's, there was a plant with less than 33 chromosomes, one hyper-triploid and two hyper-tetraploids; none was normal in appearance or vigorous.

Table 10.8. Chromosome numbers in Brazil of hybrids of AAA and AAB with AA diploid parents.

Cultivar	Total classified	Plants with chromosome numbers: 22	22 etc	23-32	33	44	HP*	Others
Female AAA group:								
Ambey	2	-	-	1	-	1	-	
Bagul/Lakem	5	2	-	3	-	-	-	
Dodoga & Walebo	3	1	-	2	-	-	-	
Morong/Torp	1	-	-	1	-	-	-	
Nombum	4	-	-	4	-	-	-	
Pagatow	2	-	-	1	-	-	-	1x46
Siminarook	30	5	1	23	1	-	-	
Wasolay	2	1	-	-	-	1	-	
Caru/Red etc.	12	2	1	6	3	-	-	
- confer Trinidad*	29	8	-	19	2	-	-	
Kayu & Sri	8	1	-	5	1	-	-	
Nam	14	-	-	14	-	-	-	
Yangambi Km 5	12	2	-	10	-	-	-	
Female AAB group:								
Tomnam	3	-	-	1	-	2	-	
Kelat	7	-	-	2	3	-	1	1x35
The triploid plants had an unbalanced appearance.								
Laknau/Kune	33	-	-	3	1	12	17	
Moenang	1	-	-	-	-	-	-	1x46

* HP = high polyploids, usually discarded on their typical aspects.
** Joint results for comparison of Cheesman and Dodds (1942) and Shepherd (1960).

The limits of the Bluggoe subgroup have not yet been fully or positively defined. Very probable mutant variations are plant stature, including a dwarf, the strongly developed blunt fruit tips of 'Monthan'/'Bontha' compared with the normal 'Nalla Bontha' = 'Bluggoe' and also the **bathees** mutants where the female phase of the inflorescence is prolonged by several small-fruited hands. The slightly shorter-statured 'Ney Mannan' was thought by the writer in Trinidad to belong because of its identity of structure and coloration of the male flowers; it had more hands of slightly smaller fruits. Superficial comparisons in Brazil point to the possible inclusion of 'Champa Madras', while 'Ice Cream' or 'Abu Perak' presented a different male flower colouring.

'Bontha Bathees' in Trinidad gave only 2.0 "good" seeds with A pollen (T3); no record survives of their germination nor of the nature of any hybrids. More extremely, 'Monthan' appeared to be female-sterile at the CNPMF, where no seeds were found in 766 fruits from seven pollinated bunches. Two probably identical Brazilian accessions of the dwarf mutant have also been found to be almost sterile; the only hybrid recovered was too weak to yield a chromosome count.

Table 10.9. Chromosome numbers in Brazil (B) and in Trinidad (T)* of hybrids of ABB with AA and BB diploids.

Cultivars	Total classified	Plants with chromosome numbers: 22	22 etc	23-32	33	44	HP**	Others
Awak subgroup (tall):								
- x AA (B)	11		-	-	1?	34-35	2	7
The "tetraploids" had 45-46 chromosomes.								
Awak Legor (T)	52		-	-	-	-	31	21
Nyeupe (T)	4		-	-	-	-	4	-
- both x BB (T)	28		-	-	-	-	27	1
Bluggoe subgroup:								
- tall x AA (B)	570	39	-	434	19	16	57	5
"Others" comprised: 35 40 42 46 and 55.								
- tall x BB (B)	23	0	-	5	0	2	16	
- tall x AA (T)	46	27	-	16	0	3	**0**	
- tall x BB (T)	5	0	-	0	3	2	0	
Others:								
Champa Madras (B)	61	8	-	48	1	2	2	
Ice Cream (B)	50	7	1	35	0	3	4	
The mixed plant had counts from 20 to 22!								
Kepok Bung (B)	13	0	(1)	7	1	0	4	
The mixed plant had counts from 19 to 25!								
Saba (B)	1	-	-	(23)	-	-	-	
Tip (B)	6	0	-	4	1	1	0	

* Joint results of Cheesman and Dodds (1942) and Shepherd (1960).

** HP = high polyploids, usually discarded on their typical aspects.

All the other forms mentioned above have been found to be moderately to abundantly seed-fertile with pollen from AA, much less so when tested with that from BB (T2, T3, B). Germination capacity was often poor in greenhouse conditions but could be much higher in embryo cultures, although surely in part because of the frequent survival of relatively unbalanced segregates. These accessions behaved similarly in terms of the relative numbers of different hybrid types (Table 10.9), such that crosses with AA included a very great predominance of aneuploid plants with numbers from 23 to 32 and mostly with 23 or 24. Exact diploids have not been uncommon and both triploids and tetraploids occurred in small numbers. Seeds from 'Bluggoe' itself x BB (T3 and B) or from 'Ney Mannan' x BB (T3) yielded greater proportions of polyploids than hybrids with AA. One ABBB hybrid from the 'Bluggoe' type in Brazil was a vigorous clone of some interest.

Based on observation of what is a considerable sample of plants, the task of separation of types could be much simplified in practice, since the small minority of normal-looking products should include all the useful euploids and only these would need to be confirmed by chromosome counts. As mentioned previously, diploids as a

class have thinner and broader leaves than aneuploids. Triploids and tetraploids tend to have broader leaves than either and these are progressively slightly thicker to the touch. These rules of thumb are equally applicable to other hybridisations and leaf thickness is a tolerably safe discriminant between 3x and 5x.

In Brazil, the dissimilar parent 'Kepok Bung' was again highly productive of moderately viable seeds. Few plants were vigorous in the greenhouse at the CNPMF but aneuploids between diploid and triploid levels were the common component. 'Pelipita' also yielded many seeds and hybrids, but those surviving in the greenhouse came solely from a natural sowing and not from the culture laboratory. The few certain counts resulting included a diploid and a tetraploid as well as six plants confirming that the typical hybrid, unaccountably, was an aberrant tetraploid with variable counts in the range from 41 to 46. These had a generally reduced root system. Two identical accessions of 'Saba' and also 'Tip' gave few seeds. As table 10.9 shows, the only count from the former was of 23 chromosomes, while 'Tip' provided a triploid and a tetraploid.

To summarise for ABB then, there is undoubtedly a greater overall capacity than in the other triploid genomic groups to generate euploid hybrids, whether at 2x, 3x or 4x levels. In the first class it must be noted that it was evidently the B genome that was passed on (Chapter 3). The only triploid hybrid that was critically examined was an ABB again (T3), but clearly derived from fertilisation of an AB embryo sac. Among tetraploids, both AABB and ABBB genotypes are possible, depending on the pollen applied. The latter of course has been confirmed naturally in 'Tiparot' (Simmonds and Shepherd, 1955).

Tetraploids

In Chapter 9 it was pointed out that tetraploids, in an admittedly small sample of different genomic constitutions, were not very regular in their meiotic associations. Against the theoretical expectation, the frequency of bivalents in AABB plants was no greater than that in the one AAAA studied, but much greater than in the solitary AAAB plant. It is only in the two latter classes that the writer has any experience of pollen production and it has indeed seemed to be substantially greater in 'Gros Michel' or 'Highgate' hybrids, in Trinidad or in Jamaica, than in those obtained from AAB clones in Brazil.

For male fertility, the case of AAAA was treated long ago by Cheesman (1932), who found that "seed was freely set and freely germinated" in crosses of diploid parents with two 'Gros Michel' tetraploids. Chromosome counts of fourteen plants indicated only triploids, as expected. In the same paper, Cheesman reported two similar plants from a reciprocal cross of 4x with 2x. On the other hand, he concluded that the tetraploid IC 1, although having abundant pollen, "exhibits a high degree of self-incompatibility".

Secondary triploids have been raised in some numbers in the Honduras programme, but the author does not know how many nor in what direction the crosses were made. Attempts at 2x female with 4x male in Jamaica foundered on the very numerous diploid hybrids recovered, despite morphological and some cytological confirmation that the

pollen applied was indeed diploid. The development of seeds without fertilisation had to be suspected.

Among tetraploid 'Highgate' products, female fertility with applied haploid pollen varied considerably, but was sometimes substantial, as for instance in that named (informally) as 'Buccaneer', giving yields of triploids which duly segregated for the simplex dwarfing dominant. Triploids also resulted in Trinidad from pollinations of 'Tiparot' (Shepherd, 1960a), but in that case unexpectedly mixed with pentaploids.

The early knowledge, that at least some seeds were produced from selfings or crossings of tetraploids, may have contributed to an objection raised to tetraploids as cultivars, that they should even be capable of setting some (undesirable) seeds in a plantation. Natural pollinators are widely enough distributed. This possibility was also tested by hand-pollination of a range of 'Highgate' hybrids in the Jamaican improvement programme. Seeds proved to be absent in many and quite rare in the others.

Details have been lost of an examination of pollen tube growth in four combinations in Jamaica: haploid and diploid pollen in diploid and tetraploid styles. Diploid pollen from different tetraploids germinated badly and the tubes were much retarded in both ploidies of style, so that the few found were growing at not more than about half the pace of the haploids in other styles of the same hand. Strangely, the haploid tubes grew conspicuously faster in the tetraploid styles than in the diploid ones, a contrast between approximately 4 mm and 3 mm per hour. On the facts available, the greater peril with a planting of tetraploids is to have male-fertile diploids in the near vicinity. For the tetraploids in isolation, at all events, the early removal of the male inflorescence was found in Jamaica to be a sound agronomic practice.

Higher euploids

In general, as has been widely enough agreed for *Musa*, further additions of haploid chromosome sets above the tetraploid level result in progressive diminutions in plant vigour (Simmonds, 1962). This is expressed in a steep decline in rate of growth, with tendencies to fewer, shorter and relatively malformed roots and to a narrowing, thickening and arching of the leaf laminae.

The highest ploidy level confirmed cytologically has been in two more or less octoploid hybrids between triploids and tetraploids, the former as the donors of hexaploid embryo sacs (Cheesman and Dodds, 1942). Even the one with a count reported of 88 precisely did not live long. Approximate heptaploids also have never survived for field observation. Cheesman (1932) showed a photograph of one kept alive for four years in a greenhouse; the writer has only maintained one for a few months, in a more or less static condition. Hexaploids have occasionally reached the field, three of them at the CNPMF which were hybrids respectively of 'Prata', of the 'Terrinha' plantain and of 'Bluggoe'. Vigour varied from poor to fair; not one of the three ever flowered. A few hexaploids have also been met in Brazil as spontaneous products of stem tip meristem cultures of 'Mysore' (Shepherd *et al*, 1997). These similarly manifested slow growth with reduced and droopy leaves.

Pentaploids have resulted from diploid x diploid crosses in many instances related in earlier chapters of this book, also rarely from triploid x diploid (B), triploid x tetraploid (T2) or tetraploid x diploid, as mentioned above for 'Tiparot'. The young plants were coarse and droopy-leaved but not always unvigorous. One particular example from 'Bluggoe was seen to produce an inflorescence in the CNPMF fields and, incredibly, it yielded some seeds and had conspicuous pollen. A sample of the latter seemed to have both haploid and diploid grains, on the basis of their diameters. The plant succumbed because of a failure to produce viable suckers.

Aneuploids

It was stated in the introduction to the section above on triploids that banana hybrids with 23 or 24 chromosomes could often be recognised by their general aspect, but the converse was also true that often they could not. Much would have depended on the individual chromosome that was surplus to the balanced diploid, in terms of its degree of internal genetic balance. As was shown in Chapter 3, certain trisomics (approximating to either 12A + 11B or to 11A + 12B) were not only vigorous but female-fertile. It was also true, as noted earlier in this chapter, that by no means all normal-looking and vigorous plants were euploids. The level of deception tended to increase with chromosome number within the range from about 25 to 31 or 32. Only in the early days of the Brazilian programme was there field space to spare to look at some of these and at plants with higher irregular numbers. No suggestion is made that these might be useful plants but their performance was sometimes surprisingly normal. For convenience they will be noted here by triploid sources and in rising order of numbers. A few plants at near tetraploid level are also briefly described.

Hybrids of 'Prata' or 'Prata Anã (AAB)

24 or "23-24": all three fairly vigorous at first; only one persistent;
25: the only example was quite vigorous and flowered freely;
30±: vigorous and persistent but prone to petiole breakage;
32: shot first bunch but weak afterwards;
36: inviable;
4x±: always weak and died out without flowering;
45-46: inviable;
46: fair but with only a single follower; prone to leaf breakage;
48±: short, unvigorous plant with narrow leaves.

Hybrids of 'Bluggoe' (ABB)

22 to 25: despite the inconstancy registered, this was a vigorous plant with fair bunches and quite female-fertile;
24 or 23-24: six plants generally vigorous and sometimes seedy;

25: five plants included one inviable, one weak and three vigorous;
26 or 25-26: three plants all more or less inviable;
27 or 27-28: four plants included one inviable and the others rather weak;
one was conspicuous for its "*Ravenala*" phyllotaxy;
28±: one vigorous;
29: two plants, inviable or poor;
30: the only plant was very slow-growing;
31: five plants ranging from very poor to quite vigorous, the two last with a triploid aspect;
40 and 42: one plant of each count soon died in the field.

CHAPTER 11

The banana gene pool

Priorities for components

Among the author's not so distant memories is one dating from somewhere in the middle 1970's, about twenty years before the time of writing this. He was asked in correspondence for an opinion on the definition of banana germplasm. The answer, not presently available for verification, was very probably passed on to the then International Bureau for Plant Genetic Resources. At the time the IBPGR was in the process of considering the inclusion of bananas in its ambit of priority crops for the conservation of genetic resources.

Possibly no very reliable statistics or estimates exist to show the relative contributions of diploids, triploids and tetraploids to the world's production of edible bananas. With certainty, however, an overwhelming part comes from triploids. It is these last, consequently, that are in the greatest need of either genetic modification or substitution, because of disease problems in particular.

Yet an important point, in the writer's estimate of that former time, was that the triploid cultivars are of very limited long-term genetic value and this only inasmuch as they are capable of yielding useful sexual offspring. Tetraploids would constitute an important outlet, but now it may be said that they are not necessarily the only means of access to useful genes or gene combinations in triploids. The alternatives were analysed in the preceding chapter for a wide spectrum of cultivars, and also in Chapter 3 for one instance. The conclusion was inevitable that only a minority of triploids seem to have in fact any useable sexual capacity. In cases where the common product may indeed be a tetraploid, many of the parents are themselves quite poor producers. They would offer little in terms of heritable yield characteristics, in a cross where they would make about a 75% contribution to the genetic potential for food productivity of the hybrids. For disease resistance the major genetic resources lie in the diploids, as practical banana breeders have long known.

More recently, the International Network for the Improvement of Banana and Plantain (INIBAP) has promoted the development of a now large international bank for the *in vitro* conservation of banana genetic resources. Despite this enormous and praiseworthy effort, it may be feared that the proportional constitution of this collection is unbalanced - that there is in fact a relative excess of triploid genotypes. Some of them are apparently repeated in greater or lesser degree under a diversity of names. Even if it may be argued that these are not necessarily exact synonyms, then there exists only an insignificantly small possibility of unknown but genetically valuable variants. There seems to be a need still for a very comprehensive field gene bank, even at much expense

in the short term, to avoid the more expensive longer term conservation of virtual duplicates in vitro.

As has been suggested again in Chapter 2, there is also much variability in fertility among diploid cultivars, and therefore also in their provisional utility in the production of new hybrids. Nevertheless, they clearly merit a relatively strong representation in any conservation bank, at least until the potential value of each clone is better understood.

All the above leads to an urgent appeal. On the whole perspective, where this book might have a critical impact on genetic conservation policy is in its emphasis on the vast possible worth of wild collections, of not one or two species but of many interrelated ones. The only restriction that has been indicated with anything like certainty is that, for the genetic improvement by hybridisation of the important edible bananas, species with haploid chromosome numbers of ten or nine are irrelevant (Chapter 7). But several with 2n = 22 could jointly provide a much expanded gene reservoir, as revealed in other chapters and summarised in Fig. 11.1. Some of the long-described species need further study; others long or newly named have not been examined at all for their capacity to

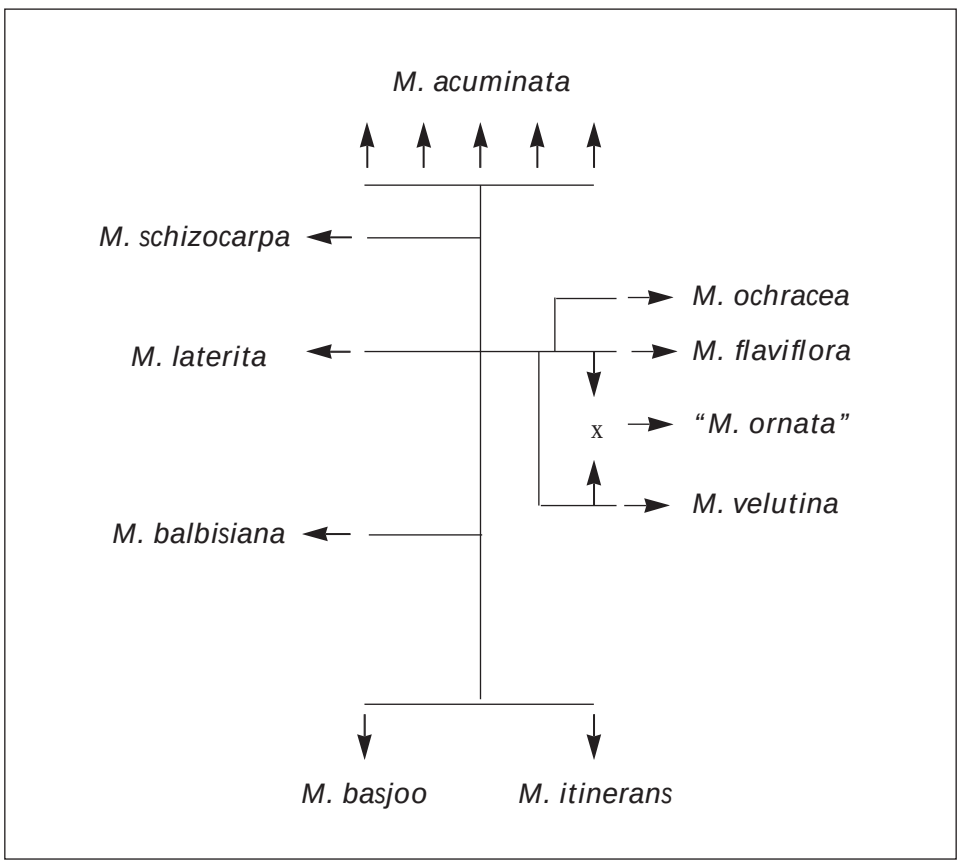


Figure 11.1. Relative reproductive isolation between *Musa* species with 2n = 22 chromosomes, as studied in this book.

make fertile intercrosses with **any** *M. acuminata* strain. Such crosses might be made either directly or, if necessary, by way of intermediate hybrids with "bridge" forms. In both cases, a backcross programme would be vital to the practical utilisation of the initial hybrids.

For such a purpose it is clear again that a working field gene bank would be an imperative, with adequate space and perceptive scientists, including a competent cytologist, to produce and study F1 hybrids and later generations. It could save much valuable time in the future, if a central *in vitro* bank were already able to offer partly fertile "AX" hybrids, where X is a species other than *M. acuminata*, or later even modified "AA_x" backcross stocks, for testing against new disease problems. Perhaps two or more sites would be needed, in close proximity to one another, to allow for different ecological preferences of the base material.

The genetic structure of wild populations

Wherever new collecting of wild *Musa* is contemplated, as well as in the analysis of the material already dispersed among existing gene banks, the degree of genetic variability present within each population or isolate has to be a factor for consideration. Doubtless the **ex** IBPGR (now IPGRI) had established models from other crops for collection procedures, to suit different genetic situations. The purpose now is to summarise the basic information that might point to an appropriate mode for wild bananas. It has to introduce some new data, since published factual information is scarce or bordering on non-existent.

Once more, Simmonds (1962, p.32-34 and 44-47, respectively) provides a starting point with his general account on the ecology and on the breeding system, to which further comment may be added. He concluded that "the plants are intermediate between the two extremes of inbreeding and outbreeding". The first important factor is of course that wild forms with few exceptions have unisexual flowers, with female and male phases successively. Obviously, an inflorescence of this type cannot possibly fertilise itself, but inbreeding is not excluded where the same clump bears two or more inflorescences. Natural pollinators are numerous and miscellaneous, although bats may predominate in Asia as visitors to these strong-smelling and commonly night-opening flowers. **What is not on record, to the writer's knowledge, is an evaluation of the likely frequency of pollinations within the clump in the wild**. This is an area where careful and detailed studies of wild populations, from their origin to their extinction, would be of great value. The life span of an individual population, as a first colonist in the typical newly opened habitat, is an important factor. Here, grass competition in particular may not only reduce vegetative propagation to an insignificant role (Simmonds, 1962, p.33), **but may greatly limit the number of plants per clump that achieve mature seeds**.

M. balbisiana is a striking exception of competitiveness among the species treated in this book. It can survive or even overcome the incursion of grasses in more open habitats.

Simmonds, as cited, emphasised that the breeding system of bananas is flexible; they can tolerate an occasional generation of close inbreeding. This latter property has been

well reinforced by the satisfactory outcome of seed accessions distributed from other established collections. Simmonds further suggested that "sib-pollination must be not infrequent". This would obviously depend on the sources of the seeds that must so promptly germinate in newly opened and sunny spaces. Are they a broad sampling of earlier populations or sometimes quite a narrow representation, of seeds deposited by only a few animal agents of dispersal and from few maternal bunches? Only in the latter case might sib-crossing make a substantial contribution towards a reduction in variability, reinforcing the random element inherent in small and ephemeral populations. Probably, different populations would vary among themselves in the breadth of their genetic base and therefore in their manifest diversity of characters.

At the other extreme Simmonds (1962, p.63-65) refers to natural interspecific hybrids, either growing in the wild or among seed accessions received in Trinidad, including IR 294A of Chapter 3. Examples are cited below of seed accessions within *M. acuminata* which have displayed variability between plants and others of intraspecific F_1 families with striking genetic diversity. A number of instances in interspecific crosses have received attention in earlier chapters and merit an additional brief review.

A theoretically important difference between accession siblings and hybrid lots is that the former are more likely to conceal recessive genes in their diploid makeup, as latent diversity. In hybrids, **haploid** chromosome sets from each parent are isolated; there is no certainty that dominance and gene interaction effects follow the same patterns in hybrids as in the parents. Strange segregations have been clearly noted by the writer in backcross populations of interspecific hybrids (Chapter 4).

Variation between plants raised from seed accessions

The Trinidad collection, later transferred to Jamaica, was relatively rich in wild accessions that had been received as seeds, some from populations in the wild but others from Botanic Garden specimens. The latter might have been grown in close proximity to other fertile forms. In theory, therefore, they were less reliable for purity of type and only one is mentioned in this section. Because of limitations of space, the initial planting of a new form in Trinidad was usually as a row of five seedlings from which one was selected for clonal propagation. It may be suspected that the five were often judged superficially, on similarity of general type, and that no very detailed examination was made of variations between plants from the same seed batch. Yet three instances are well remembered of conspicuous variability:

- IR 187: this was a seed collection from the wild in the Tavoy district of Myanmar, as it is now; the plant retained later became known internationally as "Long" Tavoy, **only** because it had distinctly longer fruits than the other four;
- IR 269: the collection from Langet in Central Malaya segregated in Trinidad for plants with or without bract anthocyanin; the presence of anthocyanin was found to be determined by a simple dominant gene;
- IR 296: this one from Pahang in Central Malaya again segregated for fruit length and the plant selected for retention had the longest ones.

At the CNPMF, a more detailed characterisation was made by the author on ten different seedlings of IR 205, originally received in Trinidad from the Buitenzorg (Bogor) Botanic Garden, but now introduced as seeds from Jamaica. The plants had a common general aspect but segregations noted were:

- peduncle pubescence: from very coarse and dense to glabrous;
- length/breadth ratio of male bracts: 1.4 to 1.8;
- colour of inner bract face: pale to moderately intense;
- red ring at ovary apex of male flower: present in six, absent in four;
- stigma colour in male flowers: orange in six, yellowish in four;
- purple anther pigment: from very intense to pale;
- in two plants the bract inner pigment only faded slightly at the base.

The five seedling row was also adopted for two later seed collections of *M. acuminata* raised in Bahia, both of ssp. *malaccensis*. That from a remnant roadside population in Perak segregated both for numbers of hands and for size of fruits, while the male bracts of one plant were broader than the norm and slightly imbricate. The other collection was from Jambi in Sumatra and plants again varied in hand numbers and fruit size.

Variations between plants within F_1 hybrid families

Hybrids between subspecies of *M. acuminata* were raised as part of the diploid breeding programmes in Jamaica and in Brazil. In the former case, the two instances remembered involved the accessions IR 430 or 433 of ssp. *banksii* from Samoa, crossed with ssp. *malaccensis* IR 296 from Pahang and with ssp. *burmannica* IR 124 (Calcutta 4). The former parents had hermaphrodite flowers rather than female ones in the basal hands and were therefore much more likely than most strains to be inbred. Yet it was possible to select individual hybrids from both crosses that showed superiority in fruit numbers or fruit size, the principal characters sought.

The Brazilian crosses included all the possible combinations between single accessions representing each of five different subspecies, as well as one or two other combinations. One of the subspecies was again a relatively inbred *banksii*, but this time IR 448 from Madang in Papua New Guinea. Some twenty to twenty-five plants were normally raised of each pairing. In every case, selection was possible for bunch form or size and for fruit length, sometimes also for relative vigour. One of the more dramatic was the cross of Calcutta 4 with Pahang, where two individual recombinants stood out for their neat, pendulous bunches and above average fruit length.

Of the parents, the Madang accession of ssp. *banksii* was itself slightly susceptible to yellow Sigatoka, more evidently so in the cool, wet season from July to October. All of its diploid hybrids with other wild accessions, except that with Calcutta 4, segregated for relative resistance or susceptibility. The greatest tendency to susceptibility was in its crosses with two forms of ssp. *malaccensis*, IR 296 from Pahang and, to a lesser extent, II-357 from the Honduras (now FHIA) collection. Plants varied from moderately to highly resistant in families with ssp. *microcarpa* IR 291 from Borneo, and ssp. *Zebrina* IR 205 from Buitenzorg (Shepherd, 1990b).

Another possible segregation for disease resistance occurred in Jamaica, in the F_1 family of Calcutta 4 (IR 124) with the presumably inbred *M. schizocarpa*. Plants not obviously attacked by Fusarium wilt were vigorous enough to be presumed resistant, in conditions where pathogen inoculum was general (Chapter 5). Clonal *Fusarium* resistance tests at the CNPMF, of tetraploid hybrids of Prata/Pome types with Calcutta 4, also varied appreciably in their disease reactions (Shepherd *et al.*, 1994). But in the case of the diploid interspecific hybrids there was no possible recourse to any hypothesis other than that Calcutta 4 was segregating for resistance to the disease, to which it was very unlikely to have been exposed in its natural habitat.

In general, the sets of F_1 plants from "AA x SS" showed variability in both quantitative and qualitative features and they were markedly uneven within crosses for pollen and seed production (Chapter 5). Female fertility was again among the characteristics that varied markedly in *M. balbisiana* Butuhan x *M. acuminata* ssp. *burmannica* Calcutta 4 (Chapter 3) and in ssp. *microcarpa* Borneo x *M. ornata* (Chapter 4).

Collection criteria

From the above discussion there is no lack of evidence for heterozygosity in wild bananas. The breeding system must be close to the one that Simmonds (1962) outlined. Ideally then, a wild collection would be a composite one, based if possible on more than one population in each well defined district, certainly on several plants within each population.

One set of problems must be of how to relate this to the practical aspects, of which the writer's experience must be substantially less than that of several other botanists. However, they presumably include difficulty of access to wild bananas growing on uneven terrain, not facilitating the removal of vegetative samples, and a scarcity of fruit bunches sufficiently mature to be ripened and to yield viable seeds. Once a collection is achieved on a broader genetic base, a later set of problems then arises of how to maintain such a diverse stock in an easily accessible form.

Unfortunately, there seems to be no simple answer without a very secure method for seed or embryo preservation. Simmonds (1962, p.34) stated that dry seeds could be safely stored for several years over calcium chloride, but that has not often been the author's experience. At the CNPMF, on the other hand, seeds of *M. balbisiana* Butuhan maintained an extremely high germination capacity for two years when dried and kept in a domestic refrigerator. The experiment was then discontinued. For rapid and good germination of pre-dried seeds, the author has found it to be very important to imbibe them for some days before either sowing or embryo extraction.

APPENDIX - Chapter 3
M. balbisiana and *M. acuminata*

Appendix 3.1: Some characteristics of AB x AA plants that flowered in the field in Brazil.

Plant	General vigour	Months to flower	Height (m)	Circum. height	Suckers at 1st flower	Stalk l/d (cm)	Angle **	Hands-fruits	Fruit length (cm)	Fertility	
										seeds	pollen est. %
0703.xx:											
Plants with 2n=22:											
01-15	good	10	1.7	0.22	7	17/38	45	6- 86	6	none	none
		+ 9	1.8	0.17		11/29	45	4- 64		none	none
-31	good	12	1.6	0.15	2	16/24	15	5- 44	4	none	none
		+ 6	1.8	0.14		13/24	30	3- 22		few	<10
-33	good	12	1.9	0.21	6	16/24	0	6- 71	4	none	20!
		+ 6	no records								
		+10	2.4	0.16							
-39	good	12	1.3	0.22	2	15/30	0	5- 56	4	none	15
		+ 8	no records								
		(+ 8)	1.6	0.18		13/29	15	4- 48		few	20!
		(+ 1)	1.3	0.17		11/28		3- 22		none	20!
03-06	good	12	1.8	0.21	5	20/34	15	8-131	5	few	10
		+ 7	1.9	0.16			15	6- 96		many	10
-08	SLOW,	16!	1.4	0.24	2	10/20	-75	4- 29	3	none	?
	streaky	+11	1.7	0.18		**0**/26	-30	3- 25		none	15
	leaves	(+ 3)	1.8	0.18		8/33	30	2- 15			
-13	good	13	1.8	0.21	5	23/42	0	6- 99	6	none	<10
		+ 6	2.1	0.20		30/44	0	6-102		none	none
-18	SLOW	20!	1.7	0.17	3	8/27	-45	3- 30	3	none	none
04-10	good	13	1.8	0.23	4	20/32	0	5- 50	4	none	none
		+ 5	2.2	0.21			15	3- 36		few	none
05-05	poor	11	1.4	0.27!	5	16/44	15	5- 80	4	none	some
		+ 7	1.9	0.22		16/42	30	5-?		none	20!
09-02	good	15	2.6!	0.20	3	16/40	-15	10-161!	4	few	some
		(+13)	2.9	0.17		22/49	0	7-105		none	10
20-04	good	12	1.7	0.24	5	24/37	15	5- 57	5	none	15
		+ 8	1.9	0.18			30	4- 50		some	30!
20-13	SLOW, abnormal	17	0.9!	0.17	3	7/15	-75!	2- 14	4	none	some
21-05	SLOW,	17	1,7	0,19	1	8/28	15	6-108	2!	few	10

Appendix 3.1: (continued)

Plant	General vigour	Months to flower	Height (m)	Circum. height	Suckers at 1st flower	Stalk l/d (cm)	Angle **	Hands-fruits	Fruit length (cm)	Fertility seeds	Fertility pollen est. %
0703.xx:											
Plants with 2n=20-22:											
01-02	good	11	1.6	0.19	3	9/39	0	5- 75	5	none	none
		+10	no records								
-21	fair	12	1.3	0.25!	1!	24/32	45	6- 77	5	none	none
		+11	no records								
0703.12:											
Plants with 2n=22:											
06-11	SLOW,	19!	1.7	0.22	2	16/25	0	4- 43	3	?	none
	improved	+ 4!	2.3	0.20	(8!)	29/46	30	6-113		few	none
-14	fair					record lost except for male fertility (15%)					
15-15	good	16	1.8	0.20	3	14/24	-75	5- 50	4	few	some
		+ 6	2.6	0.17		30/32	-15	5- 55		none	10
-21	fair	13	1.5	0.19	1!	20/27	-15	5- 39	6	few	±none
		+ 7	2.1	0.17			-30	5- 57		some	±none
	This genotype was susceptible to yellow Sigatoka!										
-23	good	14	2.2	0.19	3	13/34	-75	5- 70	5	none	none
		+ 9	3.0	0.20			-60	7-?			
17-01	fair	14	1.6	0.18	1!	16/26	0	5- 61	5	none	20!
		+ 7	2.2	0.19		40/44	30	7-117		few	20!
20-13	good	11	1.2	0.27	4	12/36	30	5- 65	**9!**	many	10
		+ 8	1.8	0.21				4- 61		few	5
		(+ 2)	2.0	0.20		14/45	30	5- 75		many	10
-15	good	15	1.7	0.23	3	16/33	-15	6- 86	3	few	none
		+ 6	2.4	0.19		12/45	15	5- 84		few	5
-30	good	8!	**0.8**	0.18	2	16/19	-30	3- 26	3	none	none
		+ 3!	1.3	0.18		27/30	0	4- 41		some	none
		(+ 2)	1.5	0.19		32/30	15	4- 53			
Plant with 2n=16-22:											
20-27	fair	11	1.4	0.21	2	17/30	15	5- 54	6	none	<10
BL03-07.12:											
Plants with 2n=22:											
-61	fair	13	1.2	0.27	3	16/26	-15	6- 75	4	some	none
		+ 8								none	10
		(+ 4)					-30	6- 86			
-98	good	12	1.6	0.22	6	44/42	0	4- 59	9	none	none
Plants with 2n=22&23											
-50	good, but the apical bud probably died:										
		(17)	2.3	0.18	(5)	20/25	-45	5- 71	3	few	some
	&	(18)	2.3	0.16			-45	5			
		(+ 3)						5- 69		none	<10

Appendix 3.1: (continued)

Plant	General vigour	Months to flower	Height (m)	Circum. height	Suckers at 1st flower	Stalk l/d (cm)	Angle **	Hands-fruits	Fruit length (cm)	Fertility seeds	Fertility pollen est. %
BL03-B19.xx:											
Plants with 2n=22:											
-10	fair	15	1.6	0.20	2	13/29	-15	5- 41	4	?	some
		+								none	none
-17	v. good	?-first shooting apparently missed									
		(23)	3.1	0.20	++	**65/50**	30	9-?		?	none
FC03-42.xx:											
Plant with 2n=22:											
-06	good	9	2.4	0.21	7	30/42	-45	8-151!		some	5
		+ 4	1.7	0.18		24/37	0	5- 80		some	5
FC03-93.xx:											
Plant with 2n=22&23:											
-22	fair	10	1.8	0.18	3	24/37	0	7- **56**	5	few	none
		+ 6	2.1	0.17		38/36	15	7?- undeveloped			none
		bis	2.1	?		22/32	0	6- undeveloped			none

* Index of relative girth based on circumference at 30cm from ground level; extreme values of <0.16 and >0.22 in the first crop were especially slender or robust plants, respectively.
** Estimated as degrees positive below horizontal and negative above.

APPENDIX - Chapter 4
Rhodochlamys and *M. flaviflora*; their hybrids with AA and BB

Appendix 4.1: Phenotype scores for assorted characters in backcrosses of species hybrids of *M. flaviflora* (Frequencies of unexpected phenotypes are in bold)

Character	Score frequencies					Total plants	Mean score	Score frequencies					Total plants	Mean score
	1	2	3	4	5			1	2	3	4	5		
SH 104 *M. flaviflora* Mariani (= M = 5) x *M. velutina* (= V = 1):														
	x Mariani							**x *M. velutina***						
Stature : V = ≤1.0m, M = ≥ 2.0m, F_1 score = 3:														
	-	**2**	14	20	18	54	4.0	-	7	15	**5**	-	27	2.9
Plant colour : V with red flush, M wholly green, F_1 faintly flushed = 3:														
	1	**5**	11	22	15	54	3.8	4	7	9	**6**	**1**	27	2.7
Plant wax : absent in V, copious in M, moderately copious in the F_1 = 4:														
	2	**6**	**17**	12	17	54	3.7	14	9	4	-	-	27	1.6
Peduncle angle : erect in V, horizontal in M, nearly horizontal in the F_1 = 4:														
	2	**1**	**15**	24	9	51	3.7	14	4	3	3	**2**	26	2.0
Male rachis angle : erect in V, pendulous in M, sub-pendulous in the F_1 = 4:														
	1	**2**	**7**	11	30	51	4.3	11	3	4	4	**4**	26	2.5
Peduncle pubescence : dense in V, absent in M, rather dense in the F_1 = 2:														
	-	2	3	4	42	51	4.7	10	3	**9**	**2**	**2**	26	2.3
Fruit pubescence : dense in V, absent in M, sparse in the F_1 = 3:														
	-	-	1	3	48	52	4.9	7	7	8	**3**	**1**	26	2.4
Fruit unripe colour : red-magenta in V, green in M, purplish in the F_1 = 3:														
	-	-	14	12	26	52	4.2	**-**	9	15	**1**	**1**	26	2.8
Fruit dehiscence : typical of V, absent in M and in the F_1 = 5:														
	-	-	-	-	24	24	5.0	4	1	9	1	5	20	3.1
Male bud persistence : brief in V, long in M, intermediate in the F_1 = 3:														
	1	**10**	15	12	13	51	3.5	6	6	8	**5**	**1**	26	2.6
SH 79 & 101 *M. flaviflora* Mariani (= M = 5) with *M. ornata* (= O = 1)														
Note: The SH 79 backcrosses were in a better field environment than those of SH 101.														
	x Mariani							**x *M. ornata***						
Stature : O ± 1.5m, M ≥ 2.0m, F_1 intermediate = 3:														
ex SH 79	-	**1**	8	8	18	35	4.2	4	13	9	**9**	**5**	40	3.0
ex SH 101	-	**3**	13	9	9	34	3.7	4	18	14	**4**		40	2.4
Peduncle angle : erect in O, horizontal in M, sub-erect in the F_1 = 2:														
	-	2	27	16	24	69	3.9	70	8	**1**	**1**	-	80	1.2

Appendix 4.1: (continued)

Character	Score frequencies					Total plants	Mean score	Score frequencies					Total plants	Mean score
	1	**2**	**3**	**4**	**5**			**1**	**2**	**3**	**4**	**5**		
Male rachis angle : erect in O, pendulous in M, sub-pendulous in the F_1 = 4:														
ex SH 79	-	**1**	**2**	2	30	35	4.7	12	11	9	6	**2**	40	2.4
ex SH 101	**1**	**3**	**8**	5	17	34	4.0	27	7	5	-	-	39	1.4
Fruit rows : uniseriate at each node in O, biseriate in M and in the F_1 = 5 (intermediates were usually uni- seriate at the base of the inflorescence and biseriate thereafter):														
	-	-	-	-	71	71	5.0	45	18	11	4	1	79	1.7
Male bud persistence : rather brief in O, long in M, intermediate in the F_1 = 3:														
	5	**2**	10	2	7	26	3.1	15	-	16	-	-	31	2.0
Bract outer colour : lilac in O, reddish-purple in M, intense pink in the F_1 = 3:														
	-	**1**	18	31	18	68	4.0	31	29	20	-	-	80	1.9
Anther colour : magenta in O, greyish-yellow in M, light purple in the F_1 = 3:														
	13	**14**	13	6	17	63	3.0	72	6	1	-	-	79	1.1
SH 80 & 81 *M. flaviflora* Mariani (= M = 5) with *M. acuminata* Selangor (= S = 1):														
Note: SH 80 and 81 have *M. flaviflora* as the female and the male parent, respectively.														
	x Mariani							**x Selangor**						
Plant colour : petioles and suckers blotched with red in S, not in M, in the petioles mainly in the F_1 = 3:														
ex SH 80	**7**	**6**	12	6	6	37	2.9!	19	-	8	**1**	**1**	29	1.8
ex SH 81								12	3	1	**1**	-	17	1.5
Plant colour : black pseudostem blotches in S, little evident in M, intermediate in the F_1 = 3:														
ex SH 80	**7**	**7**	11	6	6	37	2.9!	16	-	7	**1**	**5**	29	2.3
ex SH 81								4	2	6	**1**	**4**	17	2.9
Some plants of SH 81 x S were notably redder or darker than Selangor.														
Plant wax : S is only moderately waxy, M is very waxy and so is the F_1 = 5:														
ex SH 80	-	-	**2**	**2**	33	37	4.8	4	1	14	2	8	29	3.3!
ex SH 81								7	4	4	2	-	17	2.1!
Petiole margins : spreading slightly in S, very much in M, much in the F_1 = 4:														
ex SH 80	**4**	**5**	**8**	8	12	37	3.5	23	3	-	2	**1**	29	2.4
ex SH 81								11	2	1	1	**2**	17	1.9
Peduncle pubescence : present in S, absent in F and in the F_1 = 5:														
ex SH 80	-	-	-	1	26	27	5.0	13	2	1	3	10	29	2.9
ex SH 81								6	1	1	1	7	16	3.1
There is a strong suggestion that "glabrous" here behaved as a monogenic dominant.														
Fruit stalks : are short in S, but 2cm long in M and in the F_1 = 5:														
ex SH 80	-	-	**3**	**4**	20	27	4.6	2	4	8	3	12	29	3.7
ex SH 81								-	-	2	4	10	16	4.5
Male rachis angle : horizontal in S, pendulous in M, sub-pendulous in the F_1 = 4:														
ex SH 80	-	-	1?	-	22	23	4.9+	7	2	3	3	**14**	29	3.5
ex SH 81								2	3	3	-	**6**	14	3.4

Appendix 4.1: (continued)

Character	Score frequencies					Total plants	Mean score	Score frequencies					Total plants	Mean score
	1	**2**	**3**	**4**	**5**			**1**	**2**	**3**	**4**	**5**		
Male bud apex : convolute in S and in the F_1 = 1, much imbricate in M:														
ex SH 80	8	6	2	2	5	23	2.6	21	**3**	**4**	**1**	-	29	1.5
ex SH 81								13	**1**	**1**	**1**	**1**	17	1.6
Bract apex form : acute in S, sub-obtuse in M and in the F_1 = 5:														
ex SH 80	-	-	**5**	**4**	14	23	4.4	2	2	10	4	11	29	3.7
ex SH 81								3	4	5	2	3	17	2.9
Bract base colour (non-anthocyanin) : creamy in S, orange-yellow in M and in the F_1 = 5:														
ex SH 80	-	**1**	**3**	**7**	10	21	4.2	18	2	7	-	2	29	1.8
ex SH 81								6	2	3	2	3	16	2.6
Male flower colour : yellowish in S, orange in M and in the F_1 = 5:														
ex SH 80	-	**1**	**1**	**3**	18	23	4.7	11	2	3	6	7	29	2.8
ex SH 81								7	3	2	3	2	17	2.4
Stigma colour : orange in S and in the F_1 = 1, yellow in M:														
ex SH 80	9	1	2	1	10	23	3.1	17	**2**	**1**	**-**	**9**	29	2.4
ex SH 81								9	-	**4**	-	**4**	17	2.4

APPENDIX - Chapter 5
M. schizocarpa and *M. acuminata*

Appendix 5.1. Some quantitative morphological characters of *M. schizocarpa*, *M. acuminata* ssp. *banksii*(IR 448), *microcarpa* (IR 303), *malaccensis* (IR 53); variation within F_1 hybrid families of the latter subspecies obtained with pollen of *M. schizocarpa*.

Character	*schiz.*	IR 448	F_1 range	IR 303	F_1 range	IR 53	F_1 range
First crop - plants:			0		9		5
Plant height (m)	± 4		no record	2-2.5	2.7-3.3	<2	2.0-2.8
Girth/height	?		no record	0.17	0.18-0.24	0.17	0.15-0.18
Leaf n-2:							
Petiole length (cm)	?		no record	?	34-45	?	?
Lamina length (cm)	250		no record	150	218-253	125	147-198
Lamina L/B	3.8		no record	3.0	2.7-3.4	2.4	2.4-2.9
Hands/bunch	many		no record	many	10-12	few	5- 7
Fruits/hand	20+		no record	16+	17-20	15	13-18
♂ bract - L/B	low?		no record	?	1.7-2.1	2.1	1.9-2.4
Ratoons - plants	:		3		11	9	
Plant height (m)	5-6?	tall	3.9-4.8	3?	3.5-4.5	2.5+	3.0-4.5
Girth/height	?	?	0.15-0.18	?	0.14-0.17	?	0.12-0.14
Leaf n-2:							
Petiole length (cm)	?	?	35-40	?	45-60	?	54-70
Lamina length (cm)	?	?	275-290	?	240-290	?	230-250
Lamina L/B	(3.8)	?	3.3-3.7	(3.0)	3.5-4.0!	(2.4)	3.2-3.6!
Hands/bunch	many	± 10	9-12	many	7-12	few	6-10
Fruits/hand	20+	few	14-20	16+	14-20	15	16-20
♂ bract - L/B	low?	?	1.8-2.0	?	1.6-2.1	(2.1)	1.5-2.2
♂ flowers/cluster	(20)	?	18-21	(18)	18-26	(12-14)	16-24

* Values for the parents are approximate and **not** derived from observation in the same environment.

Appendix 5.2. Some quantitative morphological characters of *M. schizocarpa*, *M. acuminata* ssp. *burmannica* (IR 124) and *siamea* (IR 403 and 476)*; variation within F_1 hybrid families of the latter subspecies obtained with pollen of *M. schizocarpa*.

Character	*schiz.*	IR 124	F_1 range	IR 403	F_1 range	IR 476	F_1 range
First crop - plants			10+		9		10
Plant height (m)	4?	< 2	1.9-2.7	2.5	2.3-2.7	1.3	2.0-2.4
Girth/height	?	0.23	0.20-0.24	0.13	0.16-0.19	0.20	0.19-0.21
Leaf n-2:							
Petiole length (cm)	?	?	31-45	?	?	37	26-40
Lamina length (cm)	250	170	170-210	140	157-202	125	183-220
Lamina L/B	3.8	2.6	2.3-3.0	2.5	2.3-2.9	2.4	2.6-3.1
Bunch angle ** record	60	60	15-60	0	?	0	no
Hands/bunch	many	5-7	?	6	5-8	4	6-8
Fruits/hand	20+	18	16-20	12	11-17	11-14	15-19
♂ bract - L/B	low?	1.7	1.8-2.3	1.3	1.6-2.0	?	1.7-1.9
Ratoons - plants			10		9		(1-10)
Plant height	5-6?	2.5	3.5-4.5	3+	3.0-5.0	?	3.5-4.0
Girth/height	?	?	0.12-0.14	?	0.12-0.15	?	(0.13-0.14)
Leaf n-2:							
Petiole length	?	?	50-70	?	50-70	?	(52-55)
Lamina length	?	?	220-270	?	190-310!	?	(250-290)
Lamina L/B	(3.8)	(2.6)	3.3-3.7	(1.3)	3.1-3.3	(2.4)	(3.5)
Bunch angle ** record	60	60	0-75!	(0)	?	?	no
Hands/bunch	many	5-7	6-10	6	3-9!	?	(7)
Fruits/hand	20+	18	15-24!	12	10-18!	?	(15-18)
♂ bract - L/B	low?	(1.7)	1.7-2.1	1.3	1.5-2.1	?	1.7-2.1
♂ flowers/cluster	(20)	16	no record	16	16-23	12?	(15-18)

* Values for the parents are approximate and **not** derived from observation in the same environment.
** Angles estimated as degrees below the horizontal.

APPENDIX - Chapter 7
Australimusa and Callimusa

Appendix 7.1. Pollen fertilities and grain diameters (x 4µ) in accessions and hybrids of Australimusa.

Plan	% sound	Frequencies of pollen diameters (unit = 4?) 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39	Means
Accessions:			
M. textilis			
St. Vincent	100	1 - 6 10 20 14 22 22 4 1	23.19 ± 0.17
Kundasan	95	1 5 8 20 38 18 7 2 1	24.87 ± 0.14
cv Libuton	95	12 25 26 20 13 4	24.09 ± 0.13
cv Tangongon	90	4 8 15 15 11 13 13 11 6 4	25.31 ± **0.24**
M. lolodensis	95	3 11 29 34 13 10	27.73 ± 0.12
M. peekelii	100	4 5 7 11 31 24 15 3	28.07 ± 0.16
M. maclayi	100	1 2 6 12 21 30 24 4	32.56 ± 0.14
IR 585-1	100	2 3 15 30 26 16 4 2 2	29.61 ± 0.15
IR 585-3	100	2 5 7 27 31 13 8 4 3	30.89 ± 0.16
IR 585-5	90	1 1 3 4 12 22 24 20 12 1	30.66 ± 0.17
IR 585-6	80*	2 3 16 21 25 19 7 5 2	31.86 ± 0.16
IR 585-7	95	2 1 2 1 4 14 20 22 17 13 3 1	31.62 ± 0.20
M. fehi 'Ink'	35*	2 2 3 5 12 14 24 17 15 3 3	30.81 ± 0.21
Hybrids:			
StV x lol	95	1 - 2 2 7 16 23 18 19 7 4 1	25.48 ± 0.19
lol x StV	85	2 3 5 8 19 20 19 11 7 2 3 1	26.19 ± 0.22
StV x ang	70	1 1 2 2 10 22 25 17 13 4 3	26.00 ± 0.18
Tan x mac	90	1 - 2 3 10 19 14 18 19 11 1 2	26.41 ± 0.20
lol x ang	90	1 4 5 13 18 18 20 14 3 3 1	25.94 ± 0.20
lol x pkl	—	not studied	
pkl x lol	**45**	5 3 8 18 27 20 12 4 2 1	28.06 ± 0.18
mac x lol	85	1 1 4 6 27 28 15 11 7	26.98 ± 0.16
pkl x ang	75*	1 6 8 9 7 16 22 23 6 2	27.16 ± 0.21
pkl x mac	85	1 1 8 27 28 17 9 5 1 3	30.19 ± 0.16

* These were less reliable estimates, because of difficulty in identifying bad grains.

**Australimusa hybrids are listed in the same order of Table 7.3.

*** 20 larger and presumably diploid grains were excluded, since the distribution was apparently bimodal; in the cases of SH 135 and SH 145, bimodality was not conspicuous and some diploids may have been included.

APPENDIX - Chapter 8
Abnormal spore development

Appendix 8.1. Pollen diameters for round grains of plants of backcross family 4552 (units approximating to 4μ).

Plant nº	Grains	Diameter units of round grains:																														
		<24	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	>50		
Normal plants:																																
-13	100			1	2	16	26	33	16	4	2								(mean 27.62 ± 0.13)													
-16	100			3	13	39	29	14	2										(mean 26.44 ± 0.10)													
-18	100			1	14	43	32	8	2										(mean 26.38 ± 0.09)													
-22	100				4	22	34	32	5	2			**1**						(mean 27.25 ± 0.12)													
Abnormal plants:																																
- 9	503	3	3	2	8	**43**	**75**	**65**	**46**	21	17	10	15	**32**	**30**	**30**	12	13	8	4	7	8	5	**12**	**13**	8	4	3	3	3		
-12	400	2		2	1	6	11	**20**	**33**	**19**	**22**	13	8	**28**	**36**	**27**	**18**	13	10	5	4	12	10	**18**	**20**	**19**	6	9	9	**19**		

Appendix 8.2. Diameters of "Siamese twins" and other strange pollen forms in plants of family 4552B (units approximating to 4μ).

Plant	"Siamese" ± equal		"Siamese" unequal		Others	
	parts	notes	parts	notes	parts	form
4552B- 9	29 + 28		26 + 21	anastomosis	31 + 25	± ovoid
(28)	29 + 29		30 + 26	"	42 + 34	"
	30 + 27	anastomosis	31 + 25	"	42 + 36	"
	32 + 32	plus a "bud"	31 + 25	"	43 + 34	"
	38 + 37	anastomosis	31 + 27	"		
			31 + 27	"		
			32 + 23			
			32 + 26	anastomosis		
			34 + 20	"		
			35 + 26	"		
			38 + 30		"	
			39 + **21**			
			40 + **21**	anastomosis		
			40 + 33			
			41 + 25	anastomosis		
			42 + 25	"		
			42 + 25	"		
			42 + 28			
			48 + 21	anastomosis		
4552B-12	31 + 30		33 + **10**	one a "bud"	29 + 26	ellipsoid
(16)	32 + 30		33 + 22			(Fig. 8.2C)
	33 + 32		33 + 28	empty	35 + ?	± ovoid
			36 + **16**			(Fig. 8.2B)
			36 + 24	(Fig. 8.2A)	**45 + 18**	± ovoid
			36 + 25			
			36 + 32			
			38 + **17**			
			38 + 27	+ "buds" 14+13		
			42 + **20**			
4552B-16	26 + 24		28 + 24			
(18)	27 + 25	wall-less	29 + 20	anastomosis		
	28 + 26	anastomosis	29 + 24	"		
			29 + 24	wall-less		
			30 + 22			
			30 + 24			
			30 + 26			
			31 + **11**			
			31 + **13**			
			31 + 19	anastomosis		
			31 + 21	"		
			32 + **12**			
			32 + 23	double anastomosis		
			33 + 19			
			34 + 24	anastomosis		

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