GENETICAL AND CYTOLOGICAL STUDIES OF *MUSA*

X. STOMATAL SIZE AND PLANT VIGOUR IN RELATION TO POLYPLOIDY

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(With Five Text-figures)

1. Introduction

An extensive literature dealing with the phenotypic characters of natural and experimentally induced polyploids has been reviewed by Goodspeed & Bradley (1942), Dermen (1940), and Muntzing (1936). While plants of different ploidy usually differ in respect of stature, vigour, cell size, physiology, metrical relations, etc., characters that in general may be called quantitative (Muntzing, 1936), there appears to be variation in the degree and even in the direction of such differences.

Recently, it has been found that certain interspecific hybrids of *Musa* produce large numbers of polyploid offspring (Dodds & Pittendrigh, 1946; Dodds & Simmonds, 1946), and this material affords an unusually favourable opportunity for the study of the phenotypic effects of polyploidy.

First, stomatal characters are shown to afford, with certain specified limitations, a reliable indication of ploidy and, with this established, a method for the determination of ploidy is defined and illustrated. Other results concern the relation between ploidy and vigour, dry weight and rate of cell division of seedlings; and between ploidy and stomatal characters and vigour in mature plants. An incidental result relates to variability in the production of polyploid progeny by one of the hybrid female parents concerned.

2. Plant Material

Details of the seedling families used are set out in Table 1 (cf. Table 1 of Dodds & Simmonds, 1946). All were derived from backcross pollinations of one or other of three diploid hybrid siblings, interspecific F_1's between the diploid seeded species, *Musa Balbisiana* Colla, clone Ceylon (I.R. 100) (female) and *Musa laterita* Cheesman (I.R. 225) (male); these three hybrid siblings are referred to respectively as S.H. 51(1), (2) and (3). It will be noticed that numerous seedlings were raised only from S.H. 51(1) and, accordingly, the bulk of the present results are based on the behaviour of the progeny of this one plant.

It may be assumed that the male gametes functional in the production of the seedlings were all haploid, the polyploid gametes being contributed by the hybrid female parents. The two aneuploids found in 851 A and B reflect the pairing irregularities of S.H. 51(1); they must have arisen from the functioning of aneuploid female gametes derived from pollen mother cells in which premature division of unpaired univalents at the first division was followed by a complete failure of the second division in the manner described by Dodds & Simmonds (1946). Indeed, their occurrence is itself evidence that failure of the second division was responsible and not restitution of the first division followed by a successful second division.

There were striking phenotypic differences between the two backcrosses, 851 A and B; plants of the former were smaller, more compact and more glaucous than those of the latter. I.R. 100 is a large plant with somewhat glaucous foliage, while I.R. 225 is much smaller and not at all glaucous. Evidently, each backcross resembled its male parent in respect of waxiness, corresponding with a preponderance of the chromosomes of that parent in each offspring. The apparent inverse relationship which seems to hold between seedling and male parental size, on the other hand, is probably of no significance for, as mentioned below, the two families 851 A and B were not raised under identical environments.

Table 1. *Backcross progenies of certain diploid interspecific hybrids of* Musa

Family	Parentage	Total seedlings	Nos. of seedlings with $2n=22$	$2n=33$	$2n=55$
803 A	S.H. 51 (3) × I.R. 100	3	1	1	1
819 A	S.H. 51 (2) × I.R. 100	4	1	1	2
819 B	S.H. 51 (2) × I.R. 225	5	0	1	4
851 A	S.H. 51 (1) × I.R. 100	Numerous	0	20*	12
851 B	S.H. 51 (1) × I.R. 225	Numerous	0	19†	24
953	S.H. 51 (1) × I.R. 225	Numerous	0	39‡	72‡

* Includes one aneuploid with $2n=34$.
† Includes one aneuploid with $2n=32$.
‡ Determined by the stomatal method; see § 3 (*a*), (*d*).

3. Results

(*a*) *The stomatal characters of triploid and pentaploid seedlings*

Seeds of 851 A and B were sown separately in boxes of compost and a random selection of seedlings were transplanted into a sand-bed for intensive study. Three-cm. samples of the third or fourth leaf of each seedling were stored in alcohol; the tissues rapidly became decolorized and, when mounted in glycerine, the stomata could readily be counted and measured under the microscope by means of strong transmitted light. The upper surface of the leaf alone was used, owing to its fewer stomata. Stomatal size was determined by measuring the length of ten guard cells with an eyepiece micrometer scale and density by counting the numbers of stomata in ten high-power fields. Chromosome counts of each seedling were made on root-tips fixed in Craf and stained in gentian violet-iodine.

As 851 A and B were raised in separate seed boxes, precise quantitative comparisons between these two families are not possible, though comparisons between seedling members of the same family are clearly justified.

Results are presented in Figs. 1 and 2, wherein means per plant of stomatal size and density are plotted in the form of scatter diagrams. It will be seen that pentaploids had markedly longer stomata but a lower stomatal density than triploids. The analysis of variance of the data is given in Table 2. In every case the sum of squares due to ploidy was highly significant, as might be expected from an examination of Figs. 1 and 2. In addition, there was, with one exception, significant variation between the triploids and pentaploids of each group. The exception (stomatal density in the pentaploids of 851 A) was based on only 11 degrees of freedom and corresponded with a suspiciously low probability. Thus a larger sample might have shown the heterogeneity that was exhibited by all the other categories. Its cause cannot certainly be stated, but, since the seedlings of each family were kept in the same box and the same sand-bed throughout their life, it

would seem likely, *a priori*, that genetic variation was contributory. Greater genetic variability might be expected to occur within triploids than within pentaploids, since the former arose from diploid eggs (the products of first divisions) while the latter arose from tetraploid eggs (the products of complete meiotic failure). This was not evidently the case, however.

Table 2. *Mean stomatal lengths* (μ), *stomatal densities* (*numbers per sq.mm.*) *and tests of significance in two polyploid seedling families of* Musa

Family	Ploidy		Stomatal Density	Stomatal Length
851 A	Triploid	Mean	25·45	28·23
		v.r. 'between'	6·01 (19; 288)*	4·32 (19; 288)
		P	<0·001	<0·001
	Pentaploid	Mean	15·53	37·54
		v.r. 'between'	1·45 (11; 288)	6·24 (11; 288)
		P	0·2–0·05	<0·001
		t 'ploidy'	15·5 (288)	24·7 (288)
		P	<0·001	<0·001
851 B	Triploid	Mean	24·33	30·03
		v.r. 'between	7·48 (18; 387)	4·05 (18; 387)
		P	<0·001	<0·001
	Pentaploid	Mean	12·98	37·94
		v.r. 'between'	3·02 (23; 387)	5·63 (23; 387)
		P	<0·001	<0·001
		t 'ploidy'	22·6 (387)	32·5 (387)
		P	<0·001	<0·001
Comparisons of means between families	Triploid		Density $t=2·08$, $P=0·05$	
			Length $t=6·14$, $P=<0·001$	
	Pentaploid		Density $t=4·16$, $P=<0·001$	
			Length $t=1·19$, $P=0·2–0·3$	

* In brackets: degrees of freedom.

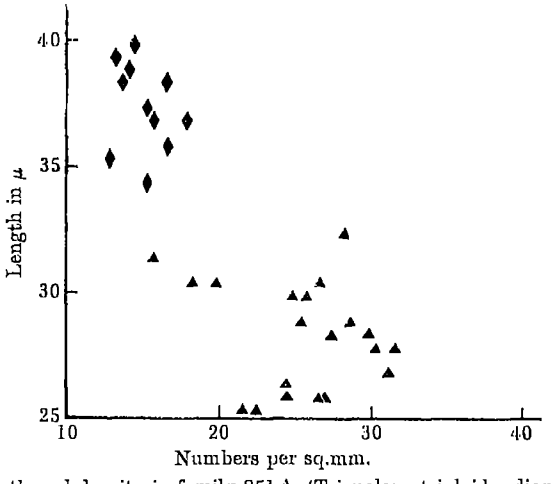

Fig. 1. Stomatal length and density in family 851 A. (Triangles—triploids; diamonds—pentaploids.)

As mentioned previously, a close comparison between the two families was not possible. Means of stomatal density and length are given in Table 2. Comparable groups have comparable means, but there are some rather marked differences.

The two small seedling families, 803 and 819, were sampled at a slightly later stage of development than 851. The results are summarized in Fig. 3, where it appears that the

two diploids had smaller stomata than triploids, though the difference was less than that found between triploids and pentaploids, as would, of course, be expected.

It is now fully established that the production of a range of diploid and polyploid offspring is a characteristic feature of some hybrids of *Musa*, and it therefore becomes a matter of considerable importance to find some means of classifying such offspring in respect of ploidy without having recourse to the direct but prohibitively laborious technique of making numerous chromosome counts.

The results set out above show that the stomatal method affords such a technique for the classification of seedling progenies containing only triploids and pentaploids. The stomatal differences between families 851 A and B, whether they arise from genetic or environmental causes, show that the standard of comparison must be internal. Thus limits cannot be given for stomatal distribution and size into which any polyploid category in all families will fall, but it is clearly quite justifiable to assume that a sharply bimodal

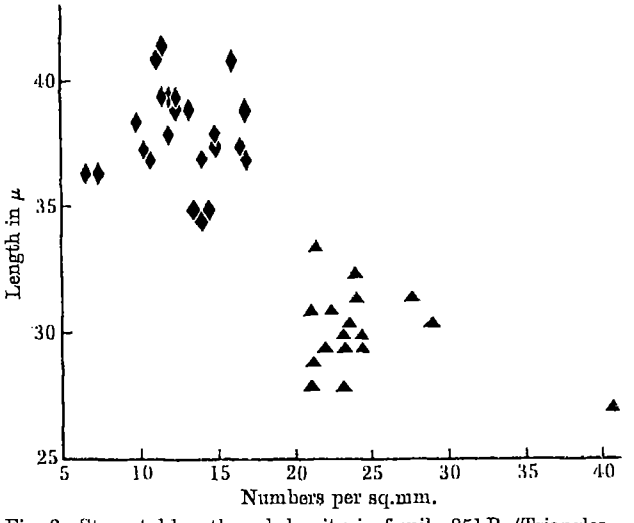

Fig. 2. Stomatal length and density in family 851 B. (Triangles—triploids; diamonds—pentaploids.)

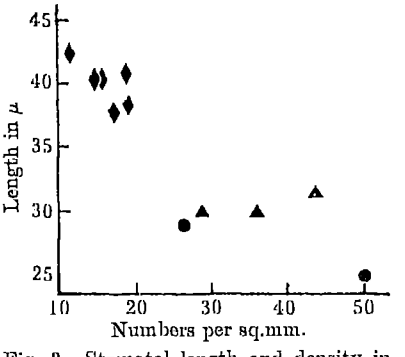

Fig. 3. Stomatal length and density in families 803 and 819. (Circles—diploids; triangles—triploids; diamonds—pentaploids.)

distribution in the size-number graph within a family indicates the presence of plants of different ploidy. This established, the chromosome numbers of a few sample plants may be determined, together with those of any other plants that appear at all anomalous in their stomatal characters or in general appearance. By this method, an accurate and easy classification may be achieved. This procedure was adopted in the seed-weight experiment described below.

In a study of the cytology of the origin of polyploid spores in certain *Musa* hybrids, Dodds & Simmonds (1946) have shown that, with haploid pollen, the offspring may be diploid, triploid or pentaploid. There is, in such hybrids, no known mechanism for the production of tetraploids, so that difficulties resulting from their presence need not be anticipated. Diploids, however, are not likely to be so sharply distinguished from triploids as are triploids from pentaploids (Fig. 3), and the chief function of the stomatal method would be the definition of plants for which cytological data were necessary.

A minor disadvantage of the method is that aneuploids would probably be missed, but this is of no great importance, since the main object of such work would be the classifica-

tion of hybrid female parents in respect of their capacity to produce polyploid progeny and variation in this same capacity.

(*b*) *Vigour*

Vigour was estimated in 851 A and B about the time that leaf-samples and root-tips were taken. Estimation was made by eye and was based on size, colour and general appearance, each plant being classified as 'weak', 'intermediate' or 'vigorous'. The process was repeated several times and was checked by two independent observers. Consistent results were obtained despite the subjective nature of the method and they were therefore accepted as showing genuine differences between plants. Of the forty-four plants of 851 A and the thirty-two of 851 B, one only failed to yield root-tips satisfactory for cytological purposes; examination of its stomata showed clearly that it was pentaploid. It has therefore been included in the following data from which the two aneuploids have been excluded.

Table 3. *Vigour of triploids and pentaploids in the seedling stage*

		Nos. of offspring			
Family	Vigour	Triploid	Pentaploid	χ_c	P
851 A	'Weak' and 'intermediate'	5	3	0·194	0·8–0·9
	'Vigorous'	14	19		
851 B	'Weak' and 'intermediate'	7	19	2·505	0·01
	'Vigorous'	12	5		

Table 4. *Mean dry weights of triploid and pentaploid seedlings in* 851 *A and B*

Family	Ploidy	No. of plants	Dry weight (mg.)	S.E.	t	P
851 A	Triploid	19	54·8	5·45	0·75	0·4–0·5
	Pentaploid	12	48·3	6·83		
851 B	Triploid	18	74·6	8·55	2·58	0·01–0·02
	Pentaploid	23	45·2	7·57		

A summary of the results, in the form of two contingency tables, is given in Table 3. The vigour classes 'weak' and 'intermediate' have been combined since some very small values occurred in certain cells of the tables. In the case of 851 B, χ_c is 2·505, which is significant at the 1% level; triploids, therefore, were more vigorous than pentaploids in this cross. In contrast, there was no evidence of any association between vigour and ploidy in 851 A for $\chi_c = 0{\cdot}194$.

(*c*) *Dry weights and the inference of rates of cell division*

The aerial parts of each seedling in 851 A and B were placed individually in bags, sun-dried for a few days, oven-dried at 100° C. for 24 hr. and weighed. Two plants were lost and the two aneuploids were omitted, so that data were available for thirty-one and forty-one plants respectively in the two families, 851 A and B. In both cases triploids were heavier than pentaploids, though only in 851 B was the difference significant (Table 4). The results agree with those obtained from the estimation of vigour and were, perhaps, not unexpected, as size was one of the criteria of vigour.

Assuming that the ratio of linear measurements of pentaploid to triploid cells is the same in all parts of the plant as it is for guard cells, and that dry weight is proportional to the bulk of the seedling, a rough estimate of the relative numbers of cells and rates of

cell division may be made. Combining data for 851 A and B, the ratio of pentaploid to triploid cell volume is $(38/29)^3 = 2{\cdot}25$ (Table 2) and the corresponding ratio of dry weights is $(94/129) = 0{\cdot}73$ (Table 4). The ratio of cell numbers per seedling is, then, 0·73/2·25, or about 0·32. An alternative approach to the problem is to consider seedlings having dry weight W, composed of N cells, each of weight C. Then, $W_p/W_t = (N_p/N_t)\,(C_p/C_t)$, where p = pentaploid, t = triploid. Assuming that the dry weight of a cell is equal to the weight of the cell wall and that this is of equal thickness and density in triploids and pentaploids, $C_p/C_t = kL_p^2/kL_t^2$ and $W_p/W_t = (N_p/N_t)\,(L_p/L_t)^2$, where k is a constant and L is a linear measurement of cell size. Hence $N_p/N_t = (W_p/W_t)\,(L_t/L_p)^2 = (94/129)\,(29/38)^2 = 0{\cdot}42$. Neither method is very exact, but it seems safe to conclude that triploid seedlings had between two and three or more times as many cells as pentaploid seedlings of the same age and hence that cell division proceeded correspondingly more rapidly in them than in pentaploids.

(*d*) *Seed weight and germination rate*

One hundred and fifty seeds of 953 were weighed individually to 0·1 mg. and planted shallowly in a box of compost which was kept in the greenhouse for the entire course of the experiment. Planting was 'on the square', each seed being 1 in. from its neighbours, its position being marked by means of a string grid. The date of each germination was recorded, and the ploidy of the seedlings was determined by the stomatal method, supplemented by root-tip counts where necessary. The experiment was terminated after 4 months, when no more germinations were considered likely.

In all, eighty-seven seeds germinated out of the 150 that were planted. Two seedlings were lost before their ploidy could be determined. Root-tip chromosome counts were needed only in the case of six plants having anomalous stomatal attributes as judged by inspection of the size-number scatter diagram.

The numbers of triploids and pentaploids germinating in successive time intervals are given in Table 5, for which $\chi^2 = 3{\cdot}1126$, $P = 0{\cdot}4$; there is no evidence of difference in germination rate between triploids and pentaploids. The analysis of variance of seed weights classified according to germination category and ploidy is given in Table 6. This analysis has been adjusted for disproportionate subclass numbers according to the method described by Snedecor (1946, ch. 11). There is no evidence that seeds bearing triploid and pentaploid embryos differ in weight or that seed weight and rate of germination are related. The general mean weight of germinated seeds was $96{\cdot}3306 \pm 1{\cdot}0531$ mg., while that of ungerminated seeds was $93{\cdot}7742 \pm 1{\cdot}9562$ mg. This difference corresponds with $t = 1{\cdot}15$, for which $P = 0{\cdot}2$–$0{\cdot}3$. There is no evidence of any relation between seed weight and germinability.

It is to be noted that this experiment touches only upon the difference between triploids and pentaploids. The material is not available in which to compare diploids and polyploids.

(*e*) *Variation in the production of polyploid female spores by S.H.* 51(1)

The seedling families 851 A and B derived from a single bunch of S.H. 51(1), whole hands being pollinated alternately by I.R. 100 and I.R. 225. Family 953, used in the experiment on seed weight, derived from a different bunch pollinated at a different time by I.R. 225 alone. It is of interest, therefore, to inquire whether, (*a*) there is any paternal

effect in determining the ploidy of the surviving seedlings, and (*b*) whether there is any variation between bunches in respect of the proportions of triploid and pentaploid offspring. The relevant data are summarized in Table 7, wherein are included data from some seedlings of 953 additional to those used in the experiment on seed weight.

Comparing first 851 A and B, $\chi^2_c = 1{\cdot}7862$, $P = 0{\cdot}1$–$0{\cdot}2$; there is no evidence of any paternal effect within a single bunch. Combining the data for these families, therefore, the comparison between 851 and 953 gives $\chi^2_c = 4{\cdot}5584$, for which $P = 0{\cdot}02$–$0{\cdot}05$; there is good evidence that the proportion of triploid and pentaploid offspring produced by S.H. 51(1) varies from bunch to bunch. The latter result accords with the inference of Dodds & Simmonds (1946) that the amount and degree of meiotic breakdown depends on a variable physiological unbalance which is now shown to be susceptible to environmental as well as genetic control (cf. Dodds & Simmonds, 1947, 1948).

Table 5. *Germination rates of triploids and pentaploids*

	Seeds germinating in the periods (December 1945)				
	20–23	24–27	28–31	After 31	Totals
Pentaploid	9	26	13	8	56
Triploid	4	12	4	9	29
Totals	13	38	17	17	85

Table 6. *Analysis of variance of seed weight; adjusted for disproportionate subclass numbers*

Item	D.F.	S.S.	M.S.	V.R.	*P*
Ploidy	1	14512·52	14512·52	1·52	> 0·2
Period	3	4645·03	1548·34	0·16	> 0·2
Interaction	3	33558·76	11186·25	1·17	> 0·2
Error	77	734542·75	9539·52	—	—

Table 7. *Polyploid offspring produced by S.H.* 51 (1)

	Nos. of seedlings	
Family	Pentaploid	Triploid
S51 A	12	20
S51 B	24	19
953	72	39

(*f*) *Stomatal characters of mature plants*

The stomatal attributes of a range of mature diploids and polyploids in the collection of *Musa* material maintained at this Institution were studied. Strips of the lamina were torn out of the mature leaf about one-third of the length from the apex, stored and decolorized in alcohol. Freehand surface sections were cut in the centre of the upper surface, mounted in glycerine and 20–40 readings of stomatal length and density were taken.

The results are presented in Table 8 and Figs. 4 and 5. In general, they agree with the observations of other investigators; the higher the ploidy, the fewer and larger the stomata. The relationship is approximately linear over the range of ploidy studied (Fig. 5), that is, there is no sign of any inhibiting effect upon stomatal size by high ploidy such as has been reported in *Raphano-brassica* by Karpechenko (1928).

Size is clearly the most reliable index of ploidy, but even on this basis no class was quite distinct from any adjacent one. Density was even more variable though, again, the general relation with ploidy is quite clear.

I.R. 242F, I.R. 188, I.R. 185, and I.R. 240, and S.H. 1 among the seeded diploids; I.R. 12, I.R. 21 and I.R. 90 among the edible triploids; and I.C. 55, I.C. 56, I.C. 57 and I.C. 61 among the experimental tetraploids all have noticeably low stomatal densities. Now, one of the seeded diploids (I.R. 242F) is referred to *Musa Balbisiana*; the phenotypes of all three edible triploids suggest an origin from this species (cf. Dodds & Simmonds, 1947), and the four experimental tetraploids all derive from one or other of these same triploids. Furthermore, the density given in Table 8 for *M. Balbisiana* clone Ceylon (I.R. 100) comprises two separate estimations made at different times; in one the density was 94·6/mm.^{2}, in the other only 4·4/mm.^{2} Similarly, two separate leaf-samples of I.R. 32 (type 20) gave respectively 70·5 and 3·3/mm.^{2} with a mean of 48·1/mm.^{2} (Table 8). This

Table 8. *Stomatal length and density in representatives of* Musa

(1) Seeded diploids				Stomatal	
Accession no. etc.	Species	Clone	Chromosome no.*	Length (μ)	Density (nos./mm.^{2})
I.R. 100	*Balbisiana* Colla	Ceylon	22 (2)	22·4	64·5
I.R. 242F	*Balbisiana* Colla	Assam	22 (7)	19·2	5·6
I.R. 53	*acuminata* Colla	A	22 (2)	21·8	65·1
I.R. 124	*acuminata* Colla	Calcutta 4	22 (2)	22·2	123·2
I.R. 144	*acuminata* Colla	Annam	22 (7)	23·9	65·5
I.R. 187B	*acuminata* Colla	Long Tavoy	22 (7)	20·9	86·6
I.R. 139	*Banksii* F.v.M.	Bloomfield	22 (7)	26·1	40·3
I.R. 1	*ornata* Roxb.	A	22 (2)	24·4	53·9
I.R. 78A	*Basjoo* Sieb.	—	22 (2)	24·6	15·9
I.R. 188	*Nagensium* Prain	Kermode 197A	22 (7)	17·4	5·0
I.R. 225	*laterita* Cheesm.	—	22 (6)	21·8	86·2
I.R. 209	Undetermined	Mariani	22 (7)	20·0	59·4
I.R. 185	*itinerans* Cheesm.	Tagwin 4	22 (7)	20·4	2·2
S.H. 1	(I.R. 1 × I.R. 6)		22 (5)	22·2	8·3
S.H. 6	(I.R. 100 × I.R. 53)		22 (6)	21·8	47·8
S.H. 51(1)	(I.R. 100 × I.R. 225)		22 (6)	22·9	48·9
I.R. 71	*textilis* Nee	St Vincent	20 (2)	21·0	29·0
I.R. 240	*textilis* Nee	Tangargon	20 (7)	20·0	6·1
I.R. 108	*violascens* Ridl.	—	20 (2)	24·2	38·5
I.R. 142	*coccinea* Andr.	—	20 (2)	23·7	39·5
I.R. 118	*borneensis* Becc.	Sarawak	20 (7)	28·0	47·7
I.R. 201	Undetermined	Buka B	20 (7)	25·2	78·3
(2) Edible diploids					
Accession no. etc.		Clone			
I.R. 11 (type 22)		Bande	22 (4)	24·5	111·8
I.R. 32 (type 20)		Guindy	22 (4)	22·1	48·1
I.R. 56 (type 21)		Palembang	22 (4)	25·4	43·4
I.R. 143 (type 32)		Pisang Lilan	22 (4)	22·0	69·1
I.R. 252 (type 33)		Tongat	22 (7)	23·5	77·7
(3) Edible triploids					
Accession no. etc.		Clone			
I.R. 12 (type 11)		Bluggoe	33 (2)	26·6	1·1
I.R. 17 (type 8)		Orotava	33 (2)	29·8	44·8
I.R. 21 (type 14)		Celat	33 (2)	27·8	4·4
I.R. 24 (type 4)		Congo	33 (2)	30·9	37·8
I.R. 31 (type 1)		Gros Michel	33 (2)	30·5	47·2
I.R. 38 (type 2)		Dwarf Chinese	33 (2)	31·5	44·4
I.R. 43 (type 13)		King	33 (2)	29·2	30·0
I.R. 49 (type 16)		Marathuva	33 (2)	31·4	47·8
I.R. 52 (type 6)		Mysore	33 (2)	29·8	65·7
I.R. 54 (type 15)		Pome	33 (2)	27·3	34·4
I.R. 60 (type 7)		Red	33 (2)	30·2	37·8
I.R. 61 (type 10)		Rio	33 (2)	32·2	35·5
I.R. 63 (type 18)		Rajah	33 (2)	27·0	28·9
I.R. 90 (type 12)		Awak Legor	33 (2)	29·2	0·8

Table 8 (*cont.*)

(4) Experimental polyploids					
	Parentage			Stomatal	
Label	Female	Male	Chromosome no.*	Length (μ)	Density (nos./mm.^{2})
163 (1)	I.R. 143	I.R. 124	33 (4)	29·4	51·1
S.H. 56	I.R. 1	I.R. 143	33 (7)	26·1	18·9
I.C. 1	I.R. 31	I.R. 6	44 (1)	31·5	35·5
I.C. 2	I.R. 31	I.R. 6	44 (1)	32·2	29·4
I.C. 22	I.R. 52	I.R. 6	44 (3)	29·2	30·6
I.C. 55	I.R. 12	I.R. 83	44 (3)	29·7	4·0
I.C. 56	I.R. 90	I.R. 78A	44 (3)	30·3	1·7
I.C. 57	I.R. 90	I.R. 110	44 (3)	30·6	10·5
I.C. 60	I.R. 61	I.R. 78A	44 (3)	32·6	33·9
I.C. 61	I.R. 90	I.R. 53	44 (3)	29·7	13·0
I.C. 62	I.R. 54	I.R. 124	44 (3)	30·5	53·4
103 (1)	S.H. 6	I.R. 53	55 (4)	35·1	26·9
S.H. 2	S.H. 1	S.H. 1	55 (5)	32·6	9·5
S.H. 3	S.H. 1	I.R. 1	55 (5)	35·0	9·6
S.H. 4	S.H. 1	I.R. 6	55 (5)	34·6	5·3
	Means: Diploid (27 plants)			22·9	46·9
	Triploid (16 plants)			29·3	34·6
	Tetraploid (9 plants)			30·7	18·3
	Pentaploid (4 plants)			34·7	13·5

* Numbers in brackets refer to references to authorities for chromosome numbers, as follows:

(1) Cheesman (1932).
(2) Cheesman & Larter (1935).
(3) Cheesman & Dodds (1942).
(4) Dodds (1943).
(5) Dodds & Pittendrigh (1946).
(6) Dodds & Simmonds (1946).
(7) Unpublished.

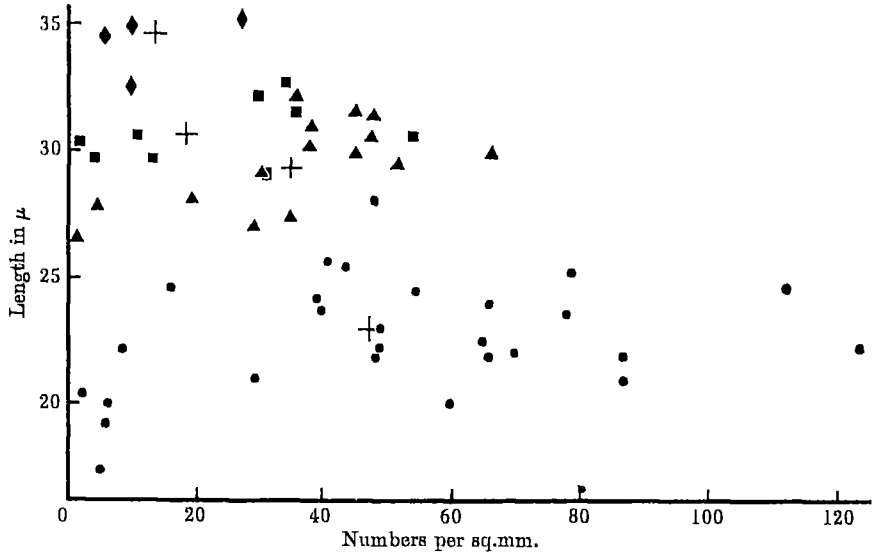

Fig. 4. Stomatal lengths and densities in mature diploids and polyploids of *Musa*. (Circles—diploids; triangles—triploids; squares—tetraploids; diamonds—pentaploids. Crosses—means of the four diploid and polyploid classes.)

clone has been shown to have originated from an interspecific cross between *M. Balbisiana* and *M. acuminata* (Dodds & Simmonds, 1947). Thus it seems that there is a tendency, whose expression is dependent on environmental conditions, for *M. Balbisiana* to bear leaves having very low stomatal densities, and that this character is heritable, being transmitted to its diploid, triploid and tetraploid descendants.

That this behaviour is not confined to *M. Balbisiana*, however, is shown by its occurrence in I.R. 185, I.R. 188 and I.R. 240, three quite distinct species, the last, indeed, belonging to the 20-chromosome group of *Musa*. There is no evidence of its occurrence in *M. acuminata*, a point which might be of value in tracing the origins of established edible types.

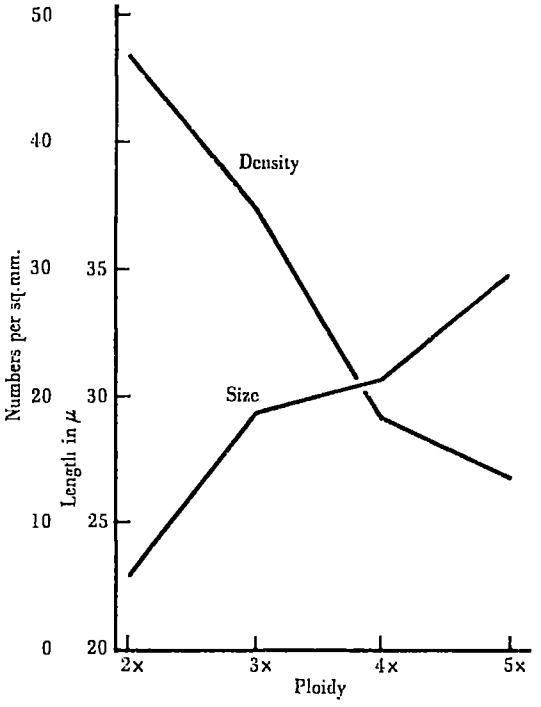

Fig. 5. The relation between stomatal characters and ploidy in *Musa*.

(*g*) *Vigour of mature plants*

The following comments are based on general field observation and experience with *Musa* rather than on any exact experimental measurements. However, despite the subjective errors to which such results might thus be susceptible they are thought to be of sufficient interest to justify inclusion in this paper.

In the *acuminata* complex, at least, triploids are rather more vigorous than diploids, forming taller and thicker pseudostems, and larger leaves. *Balbisiana* diploids, however, differ but little from the triploids, and no consistent superiority of one or the other category has been detected. Thus the seeded diploid clones (Ceylon) I.R. 100 and (Assam) I.R. 242 F are distinctly more vigorous than certain edible triploids believed to have derived from *M. Balbisiana*, e.g. Bluggoe (I.R. 12, type 11); on the other hand, the edible triploid clone Awak Legor (I.R. 90, type 12) is probably the most massive and vigorous clone in the entire collection.

It is difficult to judge whether triploids differ in vigour from the tetraploids derived from them. In the *acuminata* complex tetraploids are perhaps superior, but the difference is, at most, slight. However, they often show signs of the weak petiole and midrib which are characteristic of the higher polyploids, and which result in an abnormal drooping of

the leaves, a tendency to breakage near the base of the lamina and a loose pseudostem (cf. Simmonds, 1948). Most of these points are well seen in Plates XIX–XXI of Cheesman (1932). On the other hand, triploids occasionally show some of these leaf characters, though they are much less frequent and well-marked than in tetraploids.

Of the higher polyploids, pentaploids alone have been grown to maturity. They produce a few small stems and always show the leaf and pseudostem characters just mentioned. They appear to lie at the threshold of viability in *Musa* for still higher polyploids grow excessively slowly, rarely survive the seedling stage and, if they do, never flower. They may often be classed as 'thick-leaved dwarfs' (Cheesman, 1932; Cheesman & Dodds, 1942). One such plant (approximately heptaploid) attained a height of 3 ft. 6 in. after growing for 4 years and thereafter died (Cheesman, 1932, Plate XXII).

Thus maximal vigour occurs in *Musa* somewhere between diploid and tetraploid, depending on specific origins, and with this is to be associated the prevalence of triploids amongst established edible clones (Dodds & Simmonds, 1947).

4. The Effects of Polyploidy in *Musa*

Seeds bearing triploid and pentaploid embryos do not differ in weight or rate of germination; the young seedlings to which they give rise may or may not differ in vigour and dry weight, but there is evidence that cell division proceeds more slowly in the pentaploids than in the triploids. At a later stage of growth there are pronounced differences in vigour between plants of different ploidy, triploidy lying near the optimum, pentaploidy close to the threshold of viability. Thus, besides the immediate generalized effect of polyploidy on cell size, there is an effect on vigour manifested developmentally and becoming cumulatively greater through the life of the individual plant.

This developmental effect on the plant as a whole is paralleled by a similar effect on individual organs. Simmonds (1948) has shown that, in the development of a leaf, polyploidy had the greatest effect on the thickness of those parts formed latest in the ontogeny of the leaf, and this applied not only to the increase in thickness due to an increase in ploidy from diploid to triploid but also to a reduction in thickness between tetraploidy and pentaploidy. In addition, the existence of a mechanical restriction upon the development of the lamina by polyploidy was inferred; this was not apparent in seedlings but was well marked in older plants and was found to become greater, the higher the ploidy.

It may be concluded that ploidy has certain immediate generalized effects (such as that on cell size), and a number on secondary, less easily defined effects concerned with such characters as vigour. The latter are expressed developmentally and the effect of polyploidy on them can only properly be defined in relation to some particular stage of development, whether of the entire plant or one of its organs.

5. Summary

1. Seedling families from certain diploid interspecific hybrids of *Musa*, pollinated with haploid pollen, and consisting mainly or entirely of triploids and pentaploids, were used to study the phenotypic effects of polyploidy.

2. The stomata of triploids were smaller and more numerous than those of pentaploids. Some triploids were more vigorous and had greater dry weight than pentaploids, but there was no evidence of any difference in seed weight or germination rate.

3. Significant variation in the proportions of triploid and pentaploid offspring from different bunches of one diploid interspecific hybrid was demonstrated.

4. A comparison of size and distribution of stomata of a polyploid series of mature plants showed that the higher the ploidy, the larger and less numerous were the stomata, the relationship being approximately linear. Some of the considerable variation which occurred at each level from diploidy to pentaploidy probably resulted from genetic causes; thereby suggesting the use of stomatal characters for taxonomic purposes.

5. There is a tendency towards maximal vigour at triploidy in mature plants of *Musa*, depending on specific origins. Pentaploids are weakly, higher polyploids mostly inviable. All mature polyploids show a greater or lesser degree of weakness of the pseudostems, petiole and leaves, and this becomes progressively greater the higher the ploidy.

6. It is concluded that, apart from certain generalized effects, such as that on cell size, polyploid characters are subject to developmental control, several examples of which are adduced. The later the stage in the development of the plant or the ontogeny of an organ, the greater is the effect of polyploidy.

7. Stomatal characters offer an effective means of assessing ploidy in seedling families though chromosome counts cannot be entirely eliminated.

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