NOTES ON SELF-INCOMPATIBILITY IN THE GENUS *IPOMOEA* L.

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The existence of self-incompatibility and the associated phenomenon of cross-incompatibility in the sweet potato (*Ipomoea batatas* (L.) Lam.) has long been recognised (Mendiola, 1921 ; Stout, 1926 ; Tioutine, 1935 ; Togari *et al*, 1942 ; Poole, 1952). In a breeding programme, one of the important effects of incompatibility is to reduce the potential genetic base available for the generation of seedling populations on which selection for improved types can be practised. Self-incompatibility also precludes or narrowly restricts the use of conventional techniques of parental evaluation such as progeny testing and it retards fixation of desirable heritable characters because it so severely limits inbreeding.

An investigation of the various aspects of the floral and reproductive biology of the species in question is the traditional approach to the study of incompatibility in plants. The important morphological, physiological and genetic features normally implicated in the manifestation of self-incompatibility, have been reviewed by Pandey (1960). Martin (1965) has adequately reviewed this subject in the sweet potato within such a framework. With the exception of the sweet potato, the authors know of no reported investigations of the phenomenon of self-incompatibility within the Convolvulaceae.

By elucidating and describing more precisely the nature of the mechanism of genetic control of incompatibility, attempts to catalogue the characteristics associated with the expression of incompatibility in a crop species such as the sweet potato could be expected to permit a more meaningful approach to the establishment of breeding programmes. The present study was directed towards obtaining a better understanding of incompatibility in the sweet potato by examining (i) the relationship between heterostyly and fertility, (ii) cross-compatibility relations among cultivars and by (iii) a preliminary survey of the characteristics of the incompatibility reaction in two wild species of *Ipomoea.* The outcome of interspecific crosses among the three species was also examined.

MATERIALS AND METHODS

Twenty West Indian sweet potato cultivars, selected as parents in the U.W.I. breeding programme, and two wild species, *Ipomoea trichocarpa* Ell. and *Ipomoea gracilis* R., comprised the main plant materials used in the present study. Seeds of the wild species were obtained from Dr. G. Chapman of the Department of Botany, U.W.I., Jamaica, from a collection he made in Mexico. Dr. Chapman's identification of these species has been tentatively accepted because confirmation of their identity has not yet been secured. In particular, the legitimacy of the name *I. trichocarpa,* as Shinners (1962) has pointed out, is still in doubt. The somatic chromosome number of I. *trichocarpa* was determined to be 2n = 30. Its type locality is listed as Carolina and it is reported to be distributed throughout tropical America (House, 1908). Chapman and Wedderburn (1966) reported that I. *gracilis* was also a diploid (2n = 30) but the authors were not able to confirm this. The distribution of I. *gracilis* is said to be circumtropical although most reports of the species have come from the tropical American and Caribbean areas (House, 1908).

Three plants of each of the wild species (designated as IT or IG 1, 2, or 3) were raised from seed and reciprocal intra- and inter-specific pollinations made among them. The sweet potato cultivar C26 was also crossed with these plants, as a pollen parent. For a preliminary survey of the segregation of incompatibility phenotypes, ten plants representing progenies from each of the six families derived from all possible combinations of the three parent plants within each species (reciprocals being counted separately) were raised. Analysis of the cross IT (1) x IT (2) and its reciprocal was the most complete at the time of writing and details of only these results will be presented.

Cross- and self-pollinations within the set of 20 sweet potato cultivars were started in 1964 and have been continued since then.

In the determination of incompatible matings, two methods were employed. First, incompatibility reaction was determined as the number of seeds set per flower crossed or selfed. In crosses among sweet potato cultivars, variation in intensity and duration of flowering very often prevented the use of the same number of flowers within each parental combination. Accordingly, estimates of self-fertility were based on totals of 20 to 97 flowers and estimates of cross-fertility on 20 to 160 flowers manipulated. In the wild species, seed-set was based on 100 to 500 flowers in the case of crosses among the original parents and on 10 to 20 flowers in the case of their progenies.

The second method of defining incompatibility reaction was based on *in vivo* tests of pollen germination on stigmas and pollen tube penetration into the style at intervals ranging from 3 to 24 hours after pollination. At least five pistils from each cross were used for this assessment. Pistils with ovaries removed were examined by a rapid squash technique. In addition, whole pistils from selected crosses were reserved for detailed histological investigation. The study of all microtome sections has not been completed and results of *in vivo* tests presented in this paper are largely confined to data on incompatibility reaction as determined from pollen germination on stigmas.

A sample of ten flowers per cultivar was used to determine stamen lengths in the sweet potato. Measurements of length were made from the point of insertion of these organs in the floral receptacle.

RESULTS

Heterostylic variation and fertility in the sweet potato

Among crosses between sweet potato parent cultivars, seed set data from 26 compatible combinations were available for the present analysis. Pistil length of female parents ranged from 16.6 mm., to 22.9 mm., mean stamen length of male parents ranged from 10.9 mm. to 17.7 mm.; (Table 1).

Table 1. Features of heterostylic variation in a sample of 20 sweet potato cultivars.

Character	Mean (mm.)	C.V. (%)	Range (mm.)
Pistil length	20.0	9.4	16.6 — 22.9
Stamen length	15.5	12.5	10.9 — 18.9
Elevation of stigma over stamen	4.5	52.8	0.9 — 7.7

An analysis of the correlation between female fertility and the mean difference between pistil length of the female and mean stamen length of the male was performed. The correlation coefficient (r — 0.26) between them did not attain significance at the five per cent point.

Intra-incompatible, inter-compatible groups in the sweet potato

On the basis of seed set and *in vivo* determinations of pollen tube germination on the stigma, cultivars were found to fall into four intra-incompatible, inter-compatible groups (Table 2). The pattern of distribution of fertiliy of matings was the typical hyperbolic-type curve with higher frequencies skewed toward the zero and low fertility classes and progressively diminishing frequencies in the higher fertility classes with truncation in the vicinity of the class interval centred at 2.0 seeds per flower. Forty-three point eight per cent of all matings set no seed ; of the remaining matings which set seed, fertility ranged from 0.1 to 2.0 seeds per flower. All cultivars were self-incompatible and in matings within intra-incompatible groups, there were only two instances of crosses which set viable seed. A common feature of crosses between groups was the existence of substantial differences in reciprocal fertility. In particular, cultivars of group I crossed as female to cultivars of group II were, on the average, more fertile than their reciprocals. At least three clear instances of unilateral incompatibility were established. In such combinations, matings compatible in one direction were totally incompatible in the other.

Compatibility relations in intra- and inter-specific crosses and their progenies.

The cytology of pollen reaction on stigmas and pollen tube growth in styles was substantially similar in the three *Ipomoea* species investigated. Some results of detailed observations carried out in the sweet potato are presented in Table 3.

In incompatible pollinations, the mean number of pollen grains retained on the stigma was 50.7. The number of abnormal pollen grains observed in these matings was very low and pollen germination was negligible. In these matings, no penetration of pollen tubes into the style was observed. In compatible matings, on the other hand, the mean number of pollen grains retained on the stigma was approximately 107. The percentage of abnormal pollen grains was again negligibly small. The mean number of germinated pollen grains per stigma was 16.2 with a range from 2 to 40 and the mean percentage of germinated pollen was 14.8 with a range from 2.5 to 42.2.

Table 2 Sweet potatoes: Intra-incompatible, cross-compatible groups

♀ \ ♂		G I							G II						G III		G IV
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
G I	1	-	-	-	-	-	-	-	+	±	+	+	±	+	+	+	
	2	-	-	-	-	-	-	-	±	+	+	+	±	±	±	±	
	3	-	-	-	-	-	-	-	+	+	+	±	+	+	+	+	+
	4																
	5	-	-	-	-	-	-	-	±	+	±	+	±	+	+	+	±
	6	-	-	-	-	-	-	-			+	+	+			+	
	7	-	-	-	-	-	-	-	+	+	+	+	+	-	±	+	+
G II	8	±	±	-	-	±	±	±	-	-	-	-	-	±	+	+	±
	9	+	+	±	±	+	+	+	-	-	-	-	-	-			
	10				+		±				-			-	+	+	
	11	±	±	±	-	±	±	+	-	-	-	-	-	-	±	+	
	12					+				-			-		+		
	13	+	±	±	±	+	±	±	-	-	-	-	-	-	±		±
G III	14			+		+		±						+	-		
	15			±	±	±	±	±			±	±	-	±	-	-	
G IV	16	+	±	+	+	+	+	+	±	+	+	+	+	+	+	+	-

– Fertility 0.00 – 0.10 seeds per flower
± " 0.10 – 0.20 " " "
\+ " > 0.20 " " "

Table 3. Comparative pollen germination and penetration into style : Sweet potatoes

Type of Cross	$\bar{x}$	C.V. (%)	ABN (%)	POLLEN GRAINS ON STIGMA GERMINATING			
				$\bar{x}$/Stigma	Range	$\bar{x}$ %	Range
Compatible	107.3	29.0	1.1	16.2	2 — 40	14.8	2.5 — 42.2
Incompatible	50.7	44.6	4.0	—	—	0.1	—

		POLLEN TUBES IN STYLE			
		$\bar{x}$	Range	Modal Class (% Freq.)	Range of Modal Class
Compatible	Top	6.9	2 — 21	32.7	4 — 6
	Mid	3.9	1 — 10	25.7	2 — 3
	Lower	1.3	0 — 5	55.4	0 — 2

In the lower part of Table 3 details of pollen tube penetration into upper, mid and lower style are presented. A decreasing trend in the mean number of pollen tubes observed at each stylar level was noted. The range in the number of pollen tubes observed at top- and mid-style is fairly wide and may more appropriately be taken as being representative of the variation in the lower limits of pollen tube density observed in these crosses. In most cases more pollen tubes than were observed were probably present, but the squash technique employed coupled with the intermittent attenuation and sinuous growth of pollen tubes in the style prevented more accurate determinations. In compatible matings, four to six pollen tubes were most frequently observed in the upper portion of the style; corresponding ranges for mid and lower style were 2-3 and 0-2 pollen tubes, respectively.

Fertility relations in intra- and inter-specific crosses involving *I. trichocarpa, I. gracilis* and *I. batatas* are presented in Table 4. The three parental clones of *I. trichocarpa* and *I. gracilis* were self-incompatible. Cross combinations within each species were mostly fertile but in some parental combinations, differences in reciprocal fertility of about 50 per cent were noted. In the cross IG (3) x IG (1) there was an indication of unilateral incompatibility : IG (3) x IG (1) set 0.76 seeds per flower while the reciprocal cross had a seed-set of 0.1 seeds per flower.

Table .4—Fertility relations (seeds/flower) in intra- and inter-specific crosses : I. trichocarpa, I. gracilis and I. batatas

♀ ♂→	IT (1)	IT (2)	IT (3)	IG (1)	IG (2)	IG (3)	IB (1)
IT (1)	0.00	1.43	1.05	0.00	0.00	0.00	0.00
IT (2)	0.53	0.00	0.43	0.00	0.00	0.00	0.00
IT (3)	0.94	1.06	0.00	0.00	0.00	0.00	0.00
IG (1)	0.13	0.13	0.00	0.00	1.14	0.01	0.07
IG (2)	0.02	0.05	0.08	0.52	0.00	0.60	0.00
IG (3)	0.11	0.04	0.07	0.76	1.05	0.00	0.23

In inter-specific crosses between *I. trichocarpa* as female and *I. gracilis* as male, no seed-set occurred. In the reciprocal combinations, however, seed-set ranged from 2 to 13 per cent. Two inter-specific hybrid plants from the cross IG x IT were tested as male parents against a number of sweet potato cultivars: Pollen germination and penetration into the stigma was noted in four such combinations. Cultivar C26 (*I. batatas*) as a pollen parent stimulated normal capsule development in IG (1) and IG (2) of 7 and 17 per cent, respectively. Seeds from these crosses were shrivelled and failed to germinate. No such stimulation occurred when *I. trichocarpa* was used as female parent.

Histograms of fertility of balanced matings among progenies of families within *I. trichocarpa* and *I. gracilis* are presented in Figure 1. In both cases the skewed distribution of fertility reminiscent of the distribution of fertility in the sweet potato was clearly evident. In *I. trichocarpa,* truncation of the curve was at a seed-set value of 4.0 seeds per flower; in *I. gracilis* the range of fertility was narrower and truncation was at a fertility of 1.5 seeds per flower.

In the determination of incompatible matings in cross combinations among progenies and parents in families of *I. trichocarpa,* a number of discrepancies were found between the seed set and *in vivo* methods of establishing incompatibility reaction. Table 5 presents results of an analysis of the comparative efficacy of these two criteria in differentiating alternative mating types.

Table 5.—Comparison of two methods of determining incompatibility reaction in matings among progenies of I. trichocarpa.

	Unadjusted	Adjusted
No. assessed alike	244	232
No. assessed differently	40	52

Computed $\chi^2 = 1.57 <$ Critical $\chi^2 = 3.84$, $P = 0.05$

When matings assessed as incompatible included only those in which absolutely no seed-set occurred, the percentage of mis-classification was 18.3 When this restriction was removed and matings with less than ten per cent seed set were included in the incompatible class, there was a reduction in mis-classification to 14.1 per cent. This reduction, however, did not represent a significant gain from this adjustment.

The ratio of incompatible to compatible matings was also affected by the criteria of assessment.

In Table 6 results of the analysis of this ratio are presented. When incompatible matings were unadjusted, there was a significant departure ($P = 0.5$) from the 1 : 1 ratio with both methods of assessment of incompatibility reaction. When incompatible matings were adjusted to include crosses which did not exceed 10 per cent seed set, the departure of the ratio of incompatible matings to compatible matings from the hypothesised 1 : 1 ratio was not significant at the five per cent level.

Fig. 1 Histograms of Fertility (seeds/flower)

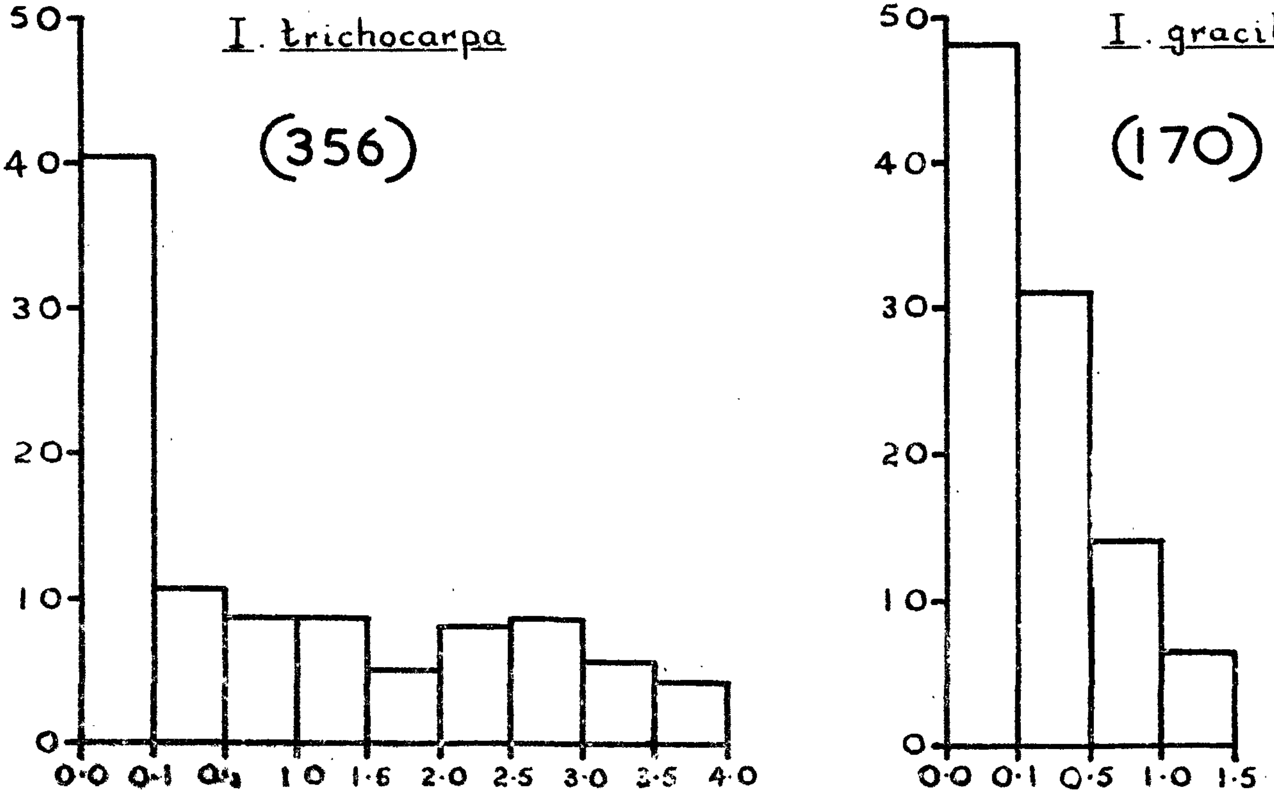

Table 6.—Comparison of ratio of incompatible to compatible matings in families of I. trichocarpa in relation to method of determination of mating type.

Unadjusted

Method of determination	Type of Mating —	 +	 TOTALS
In vivo tests	111	173	284
Seed Set	134	150	284
TOTALS	245	323	

Computed $\chi^2 = 14.0$ Tabular $\chi^2 = 3.84$ at $P = 0.05$

Adjusted

Method of determination	—	+	TOTALS
In vivo tests	142	142	284
Seed Set	133	151	284
TOTALS	275	293	

Computed $\chi^2 = 1.02$ Tabular $\chi^2 = 3.84$ at $P = 0.05$

The relatively high incidence of misclassification of incompatibilty reaction among these matings did not substantially affect the identification of incompatibility phenotypes among progeny of the crosses IT (1) x IT (2) and its reciprocal. This was due to the fact that it was fairly easy to determine, by inspection, the consistency of crossing behaviour within a set of plants provisionally assessed alike with regard to incompatibility reaction by either criterion.

In Table 7 a tentative summary of the distribution of incompatibility phenotypes in the family IT (1) and IT (2), mainly on the basis of seed set, but supplemented by *in vivo* tests of compatibility reaction of pollen on stigmas is presented. The relatively small number of flowers used for estimates of seed set and the fact that *in vivo* tests were restricted to examination of pollen reaction on the style only, may have resulted in an inadequate assessment of the possible significance of some observed cases of zero or low seed-set and differences in reciprocal fertility which were judged compatible by the *in vivo* method. The results presented in Table 7, therefore, must be regarded as a first approximation to a relationship which might be much more complex.

Table 7.—Incompatibility relations among incompatibility phenotypes in family IT (1) x IT (2) and IT (2) x IT (1)

♀ \ ♂→	IT (1)	IT (2)	(1 x 2) A	(1 x 2) B	(2 x 1) A	(2 x 1) B
IT (1)	—	+	—	+	—	+
IT (2)	+	—	+	—	+	—
(1 x 2) A	—	+	—	+	—	+
(1 x 2) B	+	—	+	—	+	—
(2 x 1) A	—	+	—	+	—	+
(2 x 1) B	+	—	+	—	+	—

DISCUSSION

Heterostylic variation and fertility in the sweet potato

Heterostylic variation has a wide distribution in the plant kingdom, occurring in some 15 orders (Ford, 1964). In species of the genera *Primula* and *Lythrum*, heterostyly is intimately integrated with the expression of incompatibility through closely linked blocks of genes which control anther height, style length, rate of pollen tube growth together with pollen size and length of stigmatic papillae (Ernst, 1933 in Ford, 1964).

The existence of heterostylic variation in the sweet potato has been noted by several workers (Poole, 1952 ; Van Schreven, 1954 ; Yen, 1961). In his Pacific, Asiatic and tropical American collection of sweet potatoes, Yen (1961) encountered a range from extreme pin heterostyly to homostyly. Van Schreven (1954) considered that heterostyly was not associated with the expression of incompatibility in the sweet potato, apparently on the basis of observation. Martin (1965) pointed to the absence of supporting experimental evidence for this relationship and suggested that the extrorse dehiscence of anthers combined with their various levels may serve only to ensure good distribution of pollen on visiting insects.

The analysis of matings involving females of differing pistil and anther heights provide little evidence in support of the view that fertility in the sweet potato is related to heterostylic variation. In spite of this indication, however, the authors consider that the question of the probable association between heterostyly and—at least—fertility in *I. batatas* remains unresolved. This view is taken because of the wide variation in heterostyly encountered in this species.

In Yen's (1961) South American collection, pistil length varied from 17mm. to 26 mm. This range of variation was similar to that recovered in the present study (Table 1). In a randomly inter-breeding experimental population of seedling sweet potatoes, Jones (1966) found style lengths ranging from 8 to 29 mm. Considering that, under natural conditions, wilting and deterioration of the floral parts of the sweet potato flower are generally complete within 48 hours, the rate of pollen tube growth down the stigma and style would seem to be a critical factor in the potential for setting seed. It is not inconceivable, therefore, that the existence of differences of up to 21 mm. in pistil length among a set of cross-compatible cultivars, under natural conditions, could be of importance in determining whether the male gametes are delivered to the placental tissue before the onset of deterioration of the stigma and die back of the style.

In a large inter-breeding population in which representation of cultivars in cross-compatible groups is not limiting, the influence of differences in style length may be negligible. However, in small breeding blocks where there may be restrictions on compatible pollen sources, differences in pistil length could conceivably exert some influence on fertility. Differences in pistil lengths either independently, or in concert with the incompatibility mechanism, may very well be implicated in some cases of low fertility, differences in reciprocal compatibility and even unilateral incompatibility, in the sweet potato.

The fact that anthers are borne at different levels may be of significance in this context as well. In *Primula*, the level at which anthers are borne determines what the incompatibility reaction of the pollen will be. In *Lythrum*, where heterostyly has been further elaborated to feature three pistil and three anther levels, a similar relationship exists. Ford (1964) has pointed out that the genetic system controlling the heterostyle-polymorphism in such species, permits the reconstruction of the mating system from one that is primarily outbreeding to one that is mainly inbreeding, under certain conditions of stress. In *Primula*, this transition is accompanied by a transference from heterostyly to pin homostyly (Ford, 1964).

Yen's (1961) extensive study of variation in the sweet potato indicated that the range of variation of Asiatic and Pacific populations sampled for a number of characters including anther height and pistil length, was exceeded by the range in material from his South American collection. In particular, the range in pistil length in the Asiatic and Pacific collections was narrower than the range recovered in the South American collection. This feature may be nothing more than a reflection of the types of cultivars which were originally transferred to these areas or which may have been generated from a relatively small, initial source subsequent to its introduction. However, the frequently reported higher incidence of self-fertility particularly in Old World sweet potato cultivars, might possibly be indicative of a trend towards a change in the mating system analogous to that reported by Ford (1964).

Heterostyly, by itself, is a fairly effective means of ensuring out-breeding and in *I. batatas,* may no longer be under the control of the genetic system responsible for self-incompatibility. In fact, the sweet potato may well be a species in transition from an incompatibility system originally integrated with heterostyly to one in which heterostyly is now only a relict feaure of floral morphology of dubious adaptive worth. On the other hand, this species may be one in which the evolution of an efficient incompatibility mechanism has been held in suspense through the mediation of domestication and vegetative propagation by man.

A critical experimental examination of the association between heterostyly and fertility is very difficult because of the peculiarities of this plant species. This fact, no doubt, may explain the absence of reliable information on its possible significance in the breeding system. The authors consider that critical studies on the significance of heterostylic variation in the sweet potato may yield important evidence, not only about its probable relationship with self-incompatibility, but also towards the elucidation of a number of unresolved questions concerning the centre of origin and pattern of distribution of this species.

Intra-incompatible, inter-compatible groups in the sweet potato

Apart from its obvious utility in a breeding programme, the establishment of intra-incompatible — inter-compatible groups could contribute important evidence on the probable mode of genetic control of the incompatibility mechanism.

The character of the distribution curve of fertility, the existence of reciprocal differences in fertility and the presence of unilateral incompatibilities observed in the present study, were substantially similar to those reported by other investigators working with similar material (Hernandez and Miller, 1962 ; Martin and Cabanillas, 1965). The number of cross-compatible groups found in other studies in the sweet potato, have been relatively few (Terao, 1934 ; Togari and Kawahara, 1942 ; Fujise, 1964, Hernandez and Miller, 1962 ; Martin and Cabanillas, 1965).

These characteristics of breeding behaviour in the sweet potato have already led Martin (1965) to conclude that self-incompatibility in this species is of the sporophytic type and probably under the control of a small number of genes. The presence of unilateral incompatibilities also suggests the existence of dominance relations among alleles involved in the control of self-incompatibility.

Compatibility relationships in intra- and inter-specific crosses and their progenies

The cytology of pollen reaction and pollen tube growth.

The cytology of pollen reaction on stigmas and pollen tube growth in the conductive tissue of the style was closely similar in the three *Ipomoea* species investigated.

The first feature of significance in pollen grain reaction on stigma was the fact that in matings which were clearly incompatible, approximately half the number of pollen grains was retained on the stigma as were retained in compatible matings (Table 3). Secondly, in incompatible pollinations, pollen grains failed to germinate. Similar findings by Van Schreven (1953) and Martin and Cabanillas (1965) identify the stigmatic papillae in these species as a major site of inhibition of pollen tube growth. Simultaneous cytokinesis during microsporogenesis have been reported in some *Ipomoea* species. There have also been reports that mature pollen grains in some species of this genus are trinucleate. The total evidence, provided by these results endorses Martin's (1965) suggestion that self-incompatibility in the sweet potato is of the sporophytic type.

In figure 2, photomicrographs illustrate the sequence of pollen germination on the stigma and pollen tube penetration into the style in a compatible mating in *I. trichocarpa.* This pattern was fairly typical of fertile matings in the three species. The greater retention of pollen grains on stigmas in compatible crosses, was no doubt due to the mechanical retention afforded by germinated pollen tubes growing into the stylar tissue. Pollen tubes with swollen tips were frequently discerned in stigmas (Fig. 2A and D). There appeared to be some variation among matings assessed as compatible with regard to the number of advancing pollen tubes which had inflated tips. It was difficult to quantify such variability in cytological preparations of stigmas fixed six to seven hours after pollination, however, because of the massive congression of pollen tubes converging towards the stigma-style insertion.

The occurrence of pollen tubes with dilated tips is a feature of the incompatibility reaction in some self-incompatible species. Their regular occurrence in *I. batatas*. *I. trichocarpa* and *I. gracilis* points to the possible inadequacy of the *in vivo* method of assigning incompatibility reaction on the basis of pollen germination on the stigma alone. More importantly, however, it raises the issue as to whether these swellings represent a permanent termination of pollen tube growth and a consequent non-delivery of gametes to the ovules.

The stigmatic tissue is rapidly traversed by advancing pollen tubes (Van Schreven, 1953; Martin and Cabanillas, 1965). These tubes converge on the stigma-style insertion in a solid cone in which it was possible to count up to 40 tubes in favourable preparations in some combinations (Table 3). Although actual counts of the number of pollen grains placed on the stigmas in cross pollinations were not made prior to fixation, stigmas were pollinated to saturation in order to ensure that pollen availability was not limiting. The wide range in the percentage of germinated pollen grains, therefore, could be expected to relate to the character of the distribution curve of fertility reported in these species. In order to quantify this relationship more accurately, however, more critical studies need to be undertaken.

In a high proportion of compatible combinations, it seemed evident that the large number of pollen tubes converging into the stigmatic cone, could not be physically accommodated into the conductive tissue of the upper position of the style. The mean number of pollen tubes observed at this stylar level was 6.9 but the actual number was subject to wide variation (Table 3). It has been suggested elsewhere (Martin and Cabanillas, 1965) that mechanical impedance of pollen tube growth in the stylar tissue may constitute a barrier to pollen penetration into the style. In crosses in which high percentages of pollen tubes germinated on the stigma, the relatively large numbers observed entering the conductive tissue, in this investigation, was more than adequate to effect full fertilization of the four ovules characteristically present in these species. Pollen tubes with inflated tips similar to those encountered in the stigma, were found at all stylar levels but more frequently in the upper portion (Fig. 2E). In the upper style where pollen tube density was greatest, tubes with inflated tips apparently forced adjacent pollen tubes to arc around them (Fig. 2E). In both crushed and microtome preparations this feature of pollen tube behaviour tended to accentuate the obstructing pollen tube tip (s) and, where reconverging pollen tubes disappeared from the plane of focus, sometimes gave the impression that a single, large swelling existed in the path of the pollen tube, or that the large swelling terminated the pollen tube(s).

Because of the irregular pathway followed by pollen tubes in their progress through the style, the authors were not able to establish whether the swellings observed on the stigma were identical to those encountered in the style. In a number of instances pollen tubes in the stigma and style appeared to dilate and then continue growth, but the limitations of the squash technique prevented more critical cataloguing of the regularity of this occurrence.

Inflated pollen tube tips in stigmas and styles, is an invariable feature of the cytology of pollen tube growth in *I. batatas, I. trichocarpa and I. gracilis.* It is remarkable, therefore, that this phenomenon does not appear to have been commented on before. Martin and Cabanillas (1965) made detailed investigations of pollen tube growth in relation to stigmatic and stylar anatomy in the sweet potato but failed to make explicit mention of this characteristic.

The question of the possible biological significance of swellings in pollen tubes of these species has been alluded to above. The decreasing trend in the mean number of pollen tubes from upper to lower style was associated with a similar trend in the number of swellings observed in pollen tubes. This constitutes strong, circumstantial evidence that these inflations, at whatever level they occurred, might well represent sites of gametic extinction. Martin and Cabanillas (1965) have proposed that elimination of gametes occurs in compatible matings in the sweet potato but they have suggested that this is due to the failure of tube growth of pollen grains containing weak or unbalanced gametes. These investigators did not appear to relate gametic elimination to type of pollen tube cytology in the pistil. They may have been led to attribute cases of low seed set in the sweet potato to gametic extinction in consideration of evidence for the existence of partial homologies between chromosomes of the presumably different genomic constituents of the sweet potato (Ting and Kehr), 1953: Jones, 1965). However, both Ting and Kehr (1953) and Jones (1965) have discounted the influence of meiotic irregularity—and its associated pollen abnormality— as important components in the seed setting process in *I. batatas.* It will be recalled that the percentage of aborted pollen grains noted in the present study was negligibly small (Table 3). If swellings in the course of pollen tube growth identify sites of gametic loss due to unbalanced duplicate-deficient gamete formation in the hexaploid sweet potato, their regular occurrence in the diploid species *I. trichocarpa* and *I. gracilis* remains unexplained.

These considerations led the present authors to propose that matings which exhibit a wide range from low to high seed set in the species *I. batatas, I. trichocarpa* and *I. gracilis,* are associated with the occurrence of inflations in the progress of pollen tube growth: These inflations represent sites of gametic extinction associated with a genetic incompatibility mechanism which allows the expression of an intergrading series of compatibilities. Evidence for the existence of dominance relations among self-incompatibility alleles have already been presented by the present authors in the foregoing. We have also suggested that the evolution of an efficient incompatibility mechanism in the sweet potato may not be very far advanced. Th existence of incomplete dominance relations among incompatibility alleles resulting in failure to attain full fertility in a certain proportion of matings is, therefore, within the range of expectation for these phylogenetically retarded species.

The character of the distribution curve of fertility in *I. gracilis* and *I. trichocarpa* reported herein (Fig. 1) and their similarity to that reported in the sweet potato by other investigators would seem to support this hypothesis. Incomplete dominance of incompatibility alleles related to modification by the genetic background could result in a subjugation of their full penetrance and expressivity. This effect could explain the occurrence in these species of an intergrading series of fertilities from low to high levels. In the polyploid *I. batatas,* the super-imposition of competitive interaction in heterogenic gametes could lead to even finer graduations in compatibilites and fertilities.

The contribution of sterilities associated with unbalanced gametes cannot be completely discredited as a factor related to low seed set in the sweet potato. However, the cytological features of pollen tube growth in pistils may possibly point to a unique mode of origination of sterility systems in plants which could be highly correlated with the operation of the incompatibility system. Since pollen tube growth would seem to be a critically time dependent process in these species, mechanical obstruction to penetration of fully compatible tubes by neighbouring pollen tubes with inflated tips, could conceivably retard their rapid delivery of gametes.

Intra- and inter-specific compatibilities in relation to ancestry in the sweet potato.

Intra-specific compatibilities found within *I.trichocarpa* and *I. gracilis,* in addition to other evidence concerning breeding behaviour in the sweet potato, document the authors' proposal that the sporophytic mode of self-incompatibility exists in the three species. Of particular significance in this context, was the recovery of phenotypes among progenies which recapitulated parental compatibility relationships in *I. trichocarpa* (Table 7).

The results of inter-specific hybridizations in the three *Ipomoea* species impinge on the issue of the mode of origin of the sweet potato. In the present study, apparently normal fruit development resulted from crosses between *I. gracilis* and *I. batatas* but seeds were non-viable. Chapman and Wedderburn (1966) achieved similarly successful fruit stimulation, but only in combinations in which *I. trichocarpa* was used as the female parent. These workers found that embryo development following such crosses was slow and ceased before cotyledon formation. Such results are typical of inter-specific crosses between species differing in chromosome number. The successful stimulation of embryo and fruit development in these hybridizations, suggest the existence of partial homologies between the genomes of each diploid species and sectors of the chromosome complement of *I. batatas.*

The experimental demonstration of the recovery of viable inter-specific hybrids between the diploid *I. gracilis* and *I. trichocarpa,* furnish more decisive information on the existence of homologies between the genomes of *I. gracilis* and *I. trichocarpa.* Chromosome relationships in the synthesised hybrid plants have not yet been examined, but the relatively high frequency of pollen abnormality observed indicates that substantial irregularities may exist at meiosis. The two hybrid plants proved to be completely sterile when selfed and when cross-pollinated. Germination of hybrid pollen on stigmas of some sweet potato cultivars indicated that some pollen grains were functional and suggest, in addition to the foregoing, the existence of a tripartite combination of genomic homologies among *I. trichocarpa, I. gracilis* and *I. batatas.*

The recovery of viable, inter-specific hybrids between the diploid species of *Ipomoea* and a consideration of cross-compatibility relationships among the three species, led the authors to propose a new hypothesis to explain the method of origination of the sweet potato: This species most likely arose by chromosome doubling of a sterile hybrid between two related diploid species one of which supplied an un-reduced gamete. At least one of these presumed progenitors must have been self-incompatible. But even if both species were self-incompatible, the proposition may still be valid, if it is conceded that the male species involved, was the parent which supplied the un-reduced gamete.

The implications of the findings presented in this study are wide ranging and perhaps, in some places, somewhat speculative. In this discussion, we have given a high degree of freedom to our thinking in the hope that our thoughts would stimulate further research into the many exciting avenues which await exploration within the genus *Ipomoea* L.

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