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Self-incompatibility in the nitrogen-fixing tree, *Acacia retinodes*: quantitative cytology of pollen tube growth

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Summary. A 10×10 diallel of crosses involving trees in two natural populations of *A. retinodes* (Leguminosae: Mimosoideae) showed a high level of self-incompatibility, with ISI (Index of Self-Incompatibility) of 0.08. In both self- and cross pollinations, pollen tubes grew through the style to the ovules within 11 h. Tube growth rate is approximately similar in both types of mating: 3.3 to 4.5 μm/min for the first 6 h, and 1.2 to 2.0 μm/min for the succeeding 5 h. Three potential explanations for self incompatibility are proposed: (1) an S-gene controlled system in which pollen tubes are arrested in the nucellus of the ovule; (2) sperm: egg interactions at fertilization and (3) action of post-zygotic lethal genes.

Key words: Acacia – Self-incompatibility – Pollen tube growth – Wattle/S-gene – Nitrogen-fixation

Introduction

Acacia is a nitrogen-fixing tree genus of considerable economic importance in many countries (Roy 1956; Sherry 1971; Hall et al. 1972; Garbutt 1980), and there is some evidence for self-incompatibility (SI). Selfing results in reduced seed set in *A. decurrens* and *A. mearnsii* (Philp and Sherry 1946, 1949; Moffett and Nixon 1974). Breeding experiments involving diallels of crosses show that high levels of SI exist in *A. retinodes* (Knox and Kenrick 1983; Bernhardt et al. 1984), and in *A. terminalis* (Kenrick and Knox 1984; Kenrick et al. 1984). Seven other species have shown varying levels of SI (see list in Bernhardt et al. 1984). However, evidence for SI in species of *Acacia* from Asia, Africa and the American continent is fragmentary and empirical (Bernhardt et al. 1984).

We have proposed that there may be a gametophytically inherited system in *A. retinodes* (Knox and Kenrick 1983). In other legumes that have a gametophytic system of SI, cytological studies have indicated that self pollen tubes may be arrested in the style as in *Trifolium pratense* (Sillow 1931; Williams and Williams 1947), on stigma and style as in *T. repens* (Atwood 1941, 1944) and even in the ovule as in *Lotus corniculatus* (Wojciechowska 1963; Spiss and Paolillo 1969).

In this paper, we present detailed results of our breeding experiments in two natural populations of a summer-flowering species. *A. retinodes,* together with quantitative cytological data that may indicate the site of pollen tube arrest, assuming that SI involves a prezygotic mechanism. *A. retinodes* is protogynous, the style extending and becoming receptive before the anther filaments elongate and dehiscence occurs. The stigma is sited terminally on a long narrow style, and is of the wet, non-papillate type (Kenrick and Knox 1981 a). The stigma cup is just large enough to accept a single polyad and 80% of pollinations are of this kind in *A. retinodes* (Knox and Kenrick 1983). The unique 16 grain polyads provide a system for pollen transfer in which all the meiotic products within an anther loculus are retained as a single unit (Knox and Kenrick 1983). Within 30 min of pollination, a post-pollination exudate is secreted and pollen germination occurs soon after (Kenrick and Knox (1981 b).

In initial experiments to monitor pollen tube growth, we found it difficult to obtain satisfactory visualization of pollen tubes using standard methods (Linskens and Esser 1957; Martin 1959). This was probably because the aniline blue fluorochrome did not penetrate through the thick cuticle which surrounds the long narrow style (Kenrick and Knox 1981 a). However, the technique of D'Souza (1972), which provides simultaneous fixation and clearing in lactic acid-

ethanol, gave good results, so that we were able to make qualitative and quantitative analyses of pollen tube growth using a method developed by Williams and Knox (1982) for other systems.

Materials and methods

Ten trees of *Acacia retinodes* var. 'uncifolia' from two natural population at Cape Schanck, Victoria (Bernhardt et al. 1984) were used.

Estimation of pollen quality

Flowering shoots were brought back from the field in polyethylene bags and pollen was examined in the laboratory 6–24 h after collection. Pollen quality was estimated by two methods.

1 The FCR (fluorochromatic reaction) test. An in vitro test performed according to the method of Heslop-Harrison and Heslop-Harrison (1970) and Heslop-Harrison et al. (1984). Polyads were shaken onto a slide and mounted in a drop of pollen growth medium (12% sucrose and nutrients; Brewbaker and Kwack 1963) combined with a saturated solution of fluorescein diacetate. The slides were viewed by fluorescence microscopy. For each treatment 100 polyads were scored from each of six inflorescences. The 16 grain polyads were scored into three classes – 0, 1–8 and 9–16 fluorescent grains – to simplify analysis, since four of the eight central grains are sited behind the other four (see Kenrick and Knox 1979). These classes could be scored quickly and accurately to give an indication of inviability resulting from segregation of pollen lethal genes.

2 Pollen tube counts in styles. Pollen quality was estimated by cytological analysis of pollen tubes in the styles using the aniline blue fluorescence method described below.

Pollination methods

Open flowers and green buds were trimmed from shoots with predominantly yellow buds (Newman 1933). The shoots were then enclosed in cellophane bags to prevent contamination. Three to four days after bagging, the bags were removed, male phase flowers were removed for use in pollen collection and the remaining female phase flowers were pollinated using pollen collected on the inner face of polyvinyl chloride specimen vials (Bernhardt et al. 1984) with either self (pollen from the same tree) or cross pollen (pollen from other trees). The crosses were labelled, rebagged and the time of each cross was recorded.

Fixation and staining methods

Pollinated inflorescences were havested at regular intervals between 6 and 48 h after pollination and fixed immediately. Whole inflorescences were fixed in lactic acid : ethanol, 1 : 2 (D'Souza 1972) and stored at 3 °C for 24 h or longer. Pistils were then dissected into water, rinsed well, and mounted in decolorized aniline blue (Martin 1959) containing 20% glycerol and stored in a refrigerator for at least 2 h before microscopic examination. Squash preparations were examined with a Zeiss Universal fluorescence microscope, using epi-illumination with BG12 exciter filter, FT460 chromatic beam splitter and LP478 barrier filter combination.

Estimation of rate of pollen tube growth

The numbers of pollen tubes in transects of the stigma, style and the ovary were scored according to methods of Williams and Knox (1982).

Results and discussion

Estimation of pollen quality

A. retinodes polyads were quite variable in their FCR scores (Table 1). Inflorescences sampled from two of the trees used for breeding tests showed about 40% of polyads were apparently completely inviable, while the four other trees had significantly reduced pollen quality. Tree 1 had only 12% of polyads of the highest pollen quality class, while trees 2 and 23 had over 70% with more than nine viable grains (as judged by the FCR test, Table 1 A). Within each tree, inflorescences were fairly consistent in their quality scores, with standard error values less than 6.5, except for trees 3 and 6 in which the highest quality polyad class (i.e. 9–16 fertile grains) had SE values more than double most of the others.

Table 1. Estimation of pollen quality in *Acacia retinodes:* **A** by the fluorochromatic reaction (FCR) test; data are mean no. of fluorescent grains/ polyad ± SE. **B** by comparison of FCR data and cytological monitoring of pollen tube growth. In B, only those polyads judged as viable (i.e. containing fluorescent grains by FCR test) have been included in analysis in (i), since in (ii) sterile polyads do not adhere to the stigma, and are lost during cytological processing

A Tree	% polyads with FCR – positive grains		
	0	1–8	9–16
1	43.2±6.5	43.3±4.3	12.0± 3.0
2	6.5±1.2	22.5±3.0	71.2± 3.4
3	17.0±6.4	29.3±6.3	54.3±11.4
6	38.0±5.8	35.5±5.3	28.5± 9.4
23	6.0±1.7	21.2±2.8	74.0± 4.6
34	21.5±5.4	26.8±1.5	52.0± 5.6

B Tree	Polyad quality			
	(i) FCR test		(ii) Cytological analysis of pollinated pistils	
	No. of polyads scored	% of total no. of polyads with 9–16 positive grains	No. of polyads (pistils)	% polyads with 9–16 tubes in style
1	344	21	132 (129)	15
3	502	62	76 (71)	67
6	384	45	99 (94)	46
23	571	78	29 (28)	72
34	473	66	38 (35)	82
Total	2,274	58	374 (357)	45

When polyad quality was estimated by an in vivo test, i.e., monitoring tube growth through styles, the two methods agreed, regarding pollen quality to within 6% in four out of five comparisons (Table 1 B). From these tests, we concluded that the pollen quality of all six trees sampled was adequate for controlled pollinations, and was unlikely to have been a cause of any self-incompatibility detected. The FCR test depends on a laboratory-based facility, fluorescence microscopy, and for these experiments it was necessary to transport inflorescences considerable distances from field to laboratory for testing. There is a need for the development of a reliable field test, as proposed by Stanley and Linskens (1974).

Analysis of the breeding system

Cytological analysis of controlled pollinations between two of the trees in our study showed that less than half of the available pistils in any one globose inflorescence were successfully pollinated (Table 2). In the breeding experiments, only 1 out of 4 cross-pollinated inflorescences developed into an infructescence and only 1 out of 60 when self-pollinated (Table 3). All 10 trees involved in the diallel of crosses set significantly lower numbers of infructescences (Table 3), and thus were self incompatible. The levels of SI varied between trees. This is reflected in the degree of significance of the individual chi-squares (Table 3) and is also demonstrated in the values for the ISI (Index of Self Incompatibility, Zapata and Arroyo 1978). With the exception of tree 23, trees must be considered highly self incompatible, on the basis of the yield of infructescences from self/cross pollination; the composite chi-square based on the differences between observed and expected values for self pollination was significant, $P<0.001$.

The crossing relationships of the individual trees to one another, judged by an analysis of the number of pods per inflorescence showed some variation. The result of the diallel of crosses (Table 4) is typical for a self-incompatible species (Nettancourt 1977) and the pattern is quite consistent with the interpretation of a gametophytically controlled system, governed by a single S-gene with multiple alleles (see also Knox and Kenrick 1983).

Table 2. Success of pollination in controlled crosses of *Acacia retinodes*

Mating		No. of inflorescences (pistils) scored	% stigmas with adhering polyads
A	Self pollination		
	34×34	2 (57)	56
	23×23	4 (110)	24
B	Cross pollination		
	23×34	2 (58)	45
	34×23	2 (61)	39

Table 3. Analysis of the breeding system of *Acacia retinodes*

	A Cross pollination		B Self pollination			7 Chi square df=1	8 ISI[d]
1 Tree	2 No. infl poll[a]	3 No. infr[b]	4 No. infl poll[a]	5 No. infl poll[a]	6 Expected no infr[a]		
1	462	134	65	0	18.9	17.0***	0
2	44	11	43	0	10.8	7.8**	0
3	378	79	94	1	19.7	15.4***	0.05
4	69	27	30	1	11.7	7.1*	0.08
5	161	40	44	2	10.9	5.4***	0.09
6	379	117	117	0	36.1	32.6***	0
20	1,136	315	275	2	76.3	67.0***	0.03
22	152	24	77	0	12.2	10.1**	0
23	730	110	125	7	18.8	5.8*	0.37
34	664	129	96	3	18.7	11.2***	0.16
Total	4,175	986	966	16	228.1		0.07
					Chi square=	205.6*** df=9	

[a] No. of inflorescences pollinated
[b] No. of infructescences, i.e. inflorescences that set 1 or more pods
[c] Expected no. of infructescences (based on cross-pollination data $=(3\times4)/2$
[d] ISI (Index of Self-Incompatibility)$=(5/4)/(3/2)$ i.e. self/cross yield of infructescences (Zapata and Arroyo 1978)

Pathway of pollen tube growth

1 Compatible crosses. The style of *A. retinodes* is approx 1.6 mm in length and 80 µm in width. In the earliest

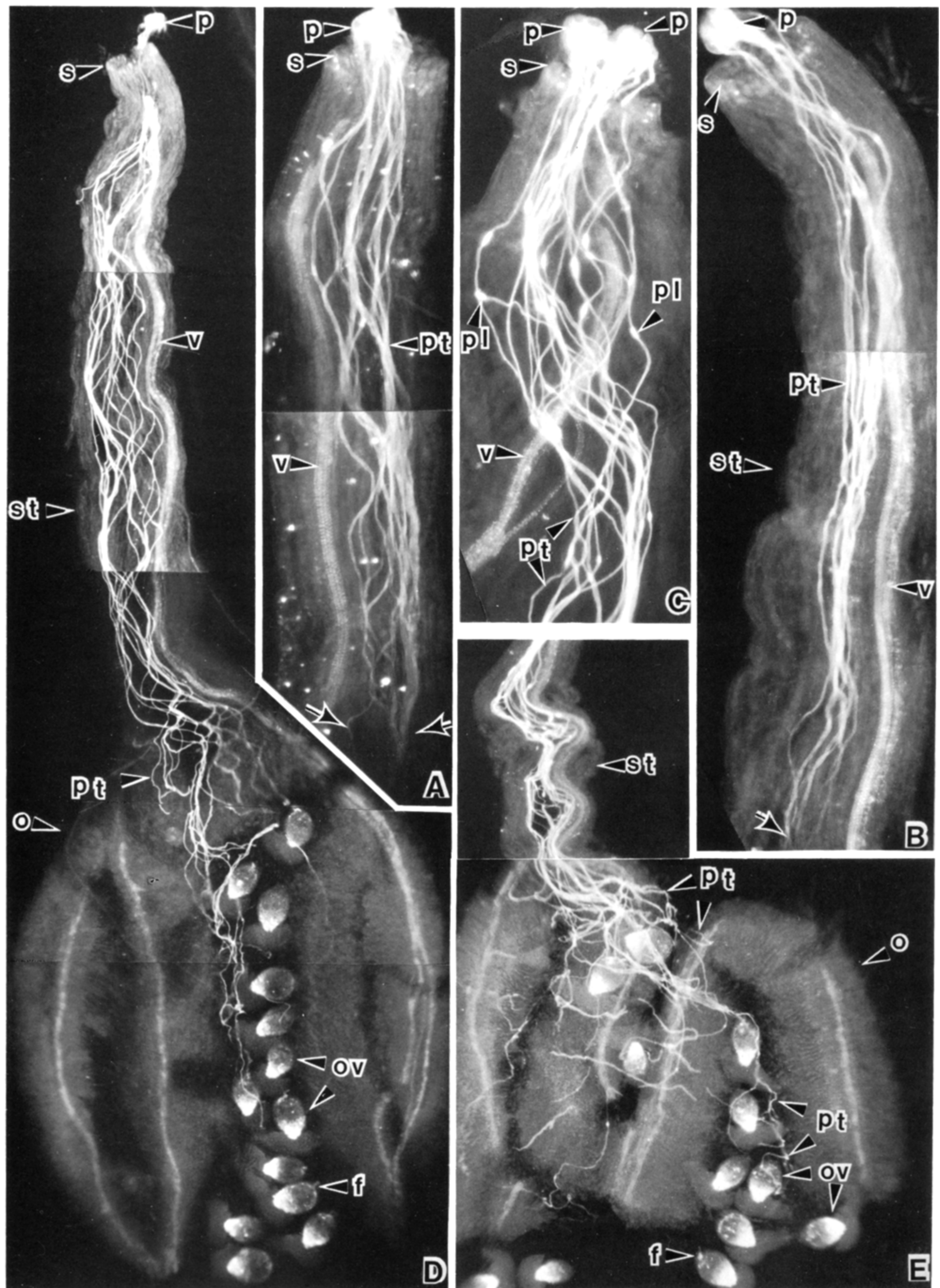
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Table 4. Diallel of controlled pollinations between ten trees of *Acacia retinodes;* data are percentage of pollinated inflorescences that set pods. 0, <5% inflorescences set pods; ±, 5–9% inflorescences set pods; +, >10% inflorescences set pods; ., not tested

Tree no.	1	2	3	4	5	6	20	22	23	34
1	0	+	+	·	·	+	+	·	+	+
2	+	0	+	·	·	·	·	·	+	·
3	±	+	0	·	·	+	+	·	+	+
4	+	·	+	0	·	·	·	·	·	·
5	+	+	·	+	0	·	·	·	·	·
6	+	+	+	·	·	0	+	·	+	+
20	+	±	+	0	+	+	0	+	+	+
22	·	·	·	·	·	·	+	0	+	·
23	+	+	+	±	±	+	+	±	±	+
34	+	·	+	±	·	+	+	·	+	0

fixations after pollination (Fig. 1 A), pollen tubes had grown between the grains of the polyad adjacent to the stigma surface and into the transmitting tissue of the style. Pollen tubes were usually bunched into a rope-like formation as they emerged from the polyad, and generally could not be visualized individually in squash preparations until after they had passed into the transmitting tissue of the style (Fig. 1 A, C, D).

Within the ovary, pollen tubes followed a route along glandular hairs to the ovules. The path of individual pollen tubes were difficult to observe in squash preparations since they tended to break and pull away from the ovules. However, pollen tubes were detected entering ovules in pistils fixed as early as 18 h after pollination (Fig. 1 D).

Each pollen tube formed five or six callose plugs throughout its length in the style. The first plugs in all tubes from a polyad appeared at about the same distance down the style, just below the stigmatic zone. Subsequent plugs formed at increasing intervals apart as the pollen tubes passed down the style (Fig. 1 C). From near the base of the style onwards no further plugs were visible.

Fig. 1 A–E. Cytology of pollen tube growth in pistils of *Acacia retinodes* following self and cross pollination of numbered trees. Epifluorescence illumination of cleared pistils stained with decolorized aniline blue. **A** cross pollination 1×6 at 6 h; pollen tubes have neared the style base, and show only faint fluorescence towards their tips (*arrow*); callose plugs, although visible, show faint fluorescence; **B** self pollination 1×1 at 6 h; showing features similar to **A**. *Arrow* indicates pollen tube tips; **C** cross pollination 1×3 at 11 h; showing two polyads on the stigma. Note intense fluorescence of tubes and callose plugs; **D** cross pollination 1×6 at 48 h; showing pollen tubes in ovary, and entering ovules (*f*); **E** self pollination 1×1 at 48 h; showing pollen tubes in ovary, and entering ovules (*f*). The stigma had been pollinated by two polyads, as in **C**

The intensity of aniline blue fluorescence of pollen tubes and callose plugs varied with the time after pollination at which the material was fixed, and with tube position in the style. In material fixed 6 h after pollination (Fig. 1 A) the plugs and tubes fluoresced faintly and tube tips were not visible against the background tissue. Fluorescence was maximal in fixations between 16 and 42 h. In pistils fixed 48 h after pollination when senescence had begun, tube walls showed faint fluorescence, although the plugs fluoresced strongly and could be up to three or four times the length of plugs in tubes which were fixed only 11 hours after pollination. These observations are essentially similar to those reported by Mulcahy and Mulcahy (1983), who monitored pollen tubes through the styles of *Petunia.*

2 Self pollination. There was no apparent difference, between tube growth in the style after self- (Fig. 1 B, E) or cross pollination (Fig. 1 A–D). In selfs, polyads germinated on the stigma surface (Fig. 1 B), and pollen tubes grew through the style to the ovary (Fig. 1 E) and formed callose plugs in the same manner as after cross pollination. Selfed tubes entered ovules and became appressed to the nucellus (Fig. 1 E).

Quantification of pollen tube growth in styles

Pollen tube growth was monitored in self and cross pollinated pistils of seven trees (Table 5). Self- and cross pollinations gave essentially similar results with the maximum number of tubes growing through styles ranging from 12 to 16 in selfs and from 8 to 16 in crosses; (16 is the maximum number of pollen tubes from a 16 grain polyad).

In crosses between trees 1 and 6, the majority of tubes had grown half-way down the style within 6 h, while a few leading tubes had reached its base (Fig. 2 A). By 11 h, most tubes had reached the base of the

Table 5. Maximum pollen tube number per polyad in styles following self and cross pollinations of *Acacia retinodes.* Numbers in parentheses indicate number of pollinated styles scored

A Self pollination			B Cross pollination		
Pollination	Maximum tube no. in style	Pollen tubes in ovary	Pollination	Maximum tube no. in style	Pollen tubes in ovary
1×1	12 (113)	+			
23×23	16 (26)	+	23×1	12 (6)	+
34×34	16 (45)	+	34×1	14 (36)	+
51×51	16 (12)	+	51×1	14 (30)	+
50×50	15 (8)	+	50×1	15 (5)	+
6×6	14 (16)	+	6×1	8 (15)	+
20×20	16 (12)	+	20×1	16 (21)	+

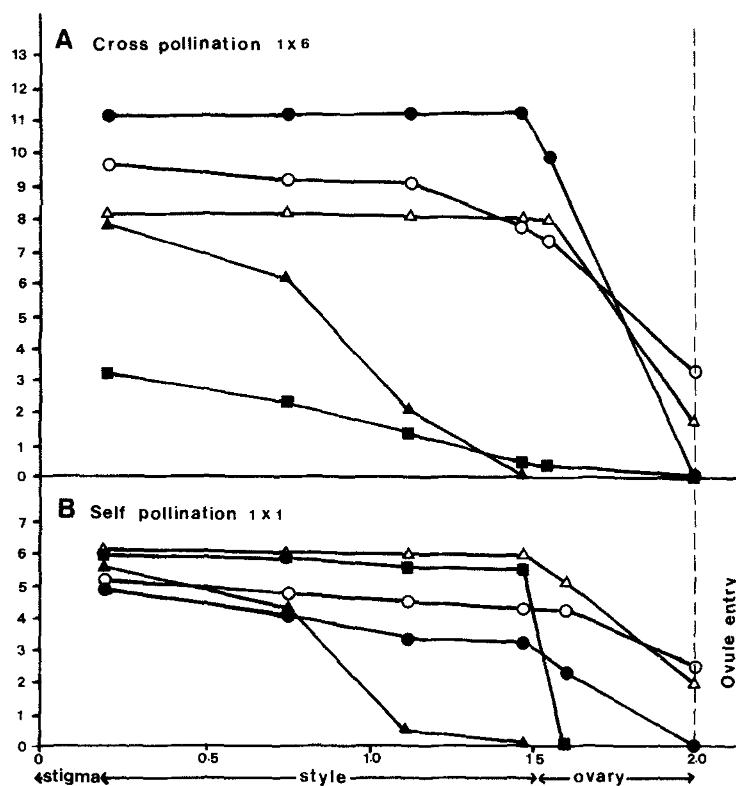

Fig. 2A, B. Quantification of pollen tubes during growth through the pistil using the aniline blue fluorescence method; transects at defined intervals in the pistil scored as described in text **A** cross 1×6; **B** self 1×1. Data are expressed as mean number of tubes per polyad. Each curve represents a different time series after pollination: ▲ 6, □ 11, ● 20, △ 42 and ○ 48 h

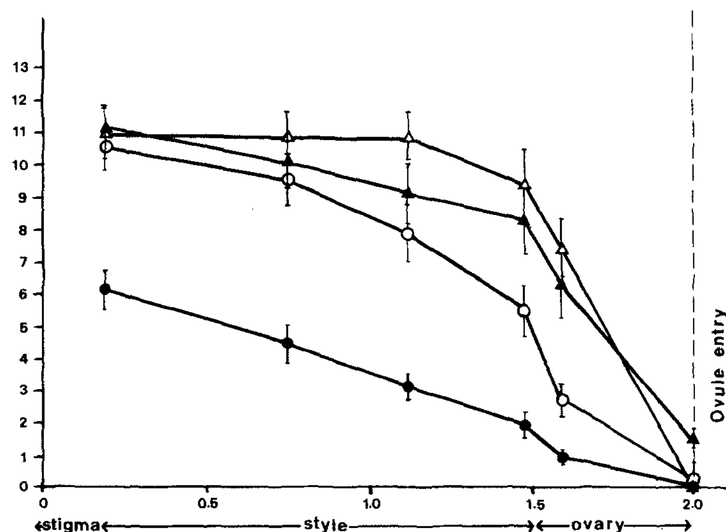

Fig. 3. Cytological monitoring of pollen tube growth in a diallel of crosses between trees 23 and 34, fixed at 16 to 18 h after pollination. Data are expressed as mean number of tubes per polyad. Each curve represents a different cross: Selfs △ 34×34; ○ 23×23; Crosses ▲ 23×34; ● 34×23

style, and leading tubes had entered the ovules. An increased number had reached the ovary by 42 h and almost half the number of tubes recorded in the style had entered the ovules within 48 h of pollination. Self pollinations showed a similar progression of pollen tubes (Fig. 2B). Tube growth was also monitored in two other trees (Fig. 3) where reciprocal crosses were observed. Results were similar, with no significant difference between self- and cross pollination in the pattern of tube growth.

The growth rate of the leading tubes in the styles has been estimated (Table 6A). For tree 1, during the first six hours, the rate was between 3 and 4.5 μm/min, and from 6–11 h, less than 2 μm/min in both self pollinations and cross pollinations with tree 6. In self- and cross pollinations involving trees 23 and 34, over the first 17.5 h the rate of pollen tube growth was between 1.5 and 1.9 μm/min (Table 6B). Some variation in the rate of pollen tube growth was apparent (Fig. 3). This appeared to be associated with the pollen source, since

Table 6. Rate of growth of pollen tubes through the style of *Acacia retinodes.* (**A**) Rate for leading pollen tubes estimated during growth through upper style (0–6 h) and lower style (6–11 h). Numbers in parentheses indicate number of pollinated pistils scored. (**B**) Mean rate of pollen tube growth in the style following self or cross pollination. Numbers in parentheses indicate data for leading pollen tubes

A Pollination	Pollen tube growth rate μm/min	
	(a) 0–6 h	(b) 6–11 h
Self 1×1	3.3 (36)	1.2 (9)
Cross 1×6	4.5 (31)	2.0 (10)

B Cross	No. of pollinated pistils	No. of polyads	Time of fixation	Distance travelled (μm)	% of initial tubes in style[a]	Mean growth rate μm/min
34×34	16	20	16 h 30 min	1,600	69	1.6
34×23	24	40	16 h 30 min	1,600	17	1.6
23×23	20	24	18 h	1,600	25	1.5
				(2,000)	(0.7)	(1.9)
23×34	19	21	18 h	1,600	56	1.5
				(2,000)	(12)	(1.9)

[a] Percentage of tubes counted at the top of the style which had grown the given length by the time of fixation

tree 34 pollen tubes grew faster in styles from both trees 34 and 23 when compared with pollen tubes from tree 23.

A model for self-incompatibility in Acacia

There is little doubt that self-incompatibility is an important outbreeding mechanism in *Acacia* (see Knox and Kenrick 1983; Bernhardt et al. 1984) and in other Mimosoid legumes (see Arroyo 1981). In some Australian species, including *A. retinodes,* trees are highly self-incompatible. In contrast, other species, notably *A. decurrens* and *A. mearnsii,* show moderate levels of selfing, although seed set is depressed when compared to cross-pollination. In these species selfed seed is of reduced viability, and seedlings are of poor vigour, often with chlorophyll defects (Moffett and Nixon 1974). The overall conclusion is that *Acacia* is a highly heterozygous outbreeding tree species.

Because of the high levels of self-incompatibility observed in *A. retinodes,* we have previously proposed a gametophytic model for S-gene control (Knox and Kenrick 1983), in accordance with the system proposed for the Papilionoid legume, *Trifolium* (Williams and Williams 1947; Atwood 1944). In *Trifolium,* pollen tubes may be arrested on the stigma or in the style following self pollinations. In *A. retinodes,* however, both self and cross pollen tubes grow at similar rates towards the ovary and apparently enter the ovules. This situation is similar to that reported for the Papilionoid legume, *Lotus corniculatus* (Spiss and Paolillo 1969; Dobrofsky and Grant 1980a); in this system, the balance of evidence from investigations into the types of proteins present in self or cross-pollinated ovules, suggests that self incompatibility is manifested prior to fertilization (Dobrofsky and Grant 1980b; Wojcieshowska 1963).

In conclusion, there appear to be three possible explanations for self incompatibility in *Acacia retinodes:*

- a gametophytic system, in which pollen tube-ovule interactions result in pollen tube arrest in the micropyle or nucellus;
- gamete-gamete interactions within the embryo sac;
- the action of post-zygotic lethal genes.

To distinguish among these possibilities will require both genetic analysis of the progeny of controlled matings, and the application of high resolution microscopy to the study of pollen tube-ovule interactions, fertilization and early seed development.

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References

Arroyo MTK (1981) Breeding systems and pollination biology in Leguminosae. In: Polhill RM, Raven PH, (eds) Advances in legume systematics, part 2. R Bot Gard, Kew, pp 723–769

Atwood SS (1941) Cytological basis for incompatibility in *Trifolium repens.* Am J Bot 28:551–557

Atwood SS (1944) Oppositional alleles in natural populations of *Trifolium repens.* Genetics 29:428–435

Bernhardt P, Kenrick J, Knox RB (1984) Pollination biology and the breeding system of *Acacia retinodes* (Leguminosae: Mimosoideae). Ann Mo Bot Gard 41 (in press)

Brewbaker JL, Kwack BH (1963) The essential role of Calcium ion in pollen germination and pollen tube growth. Am J Bot 50:859–865

Dobrofsky S, Grant WF (1980a) An investigation into the mechanism for reduced seed yield in *Lotus corniculatus.* Theor Appl Genet 57: 157–160

Dobrofsky S, Grant WF (1980b) Electrophoretic evidence supporting self-incompatibility in *Lotus corniculatus.* Can J Bot 58:712–716

D'Souza L (1972) Staining pollen tubes in the styles of cereals with cotton blue: fixation by ethanol-lactic acid for enhanced differentiation. Stain Technol 47: 107–108

Garbutt DCF (1980) Moisture relationships in wattle wood from chipping to stripping. Wattle Res Inst Rep 1979–80, pp 111–115

Hall N, Boden RW, Christian CMG, Condon RW, Dale FA, Hart AJ, Leigh JH, Marshal JK, McArthur AG, Russel V, Turnbull JW (1972) The use of trees and shrubs in the dry country of Australia. Aust Govt Publ Serv, Canberra

Heslop-Harrison J, Heslop-Harrison Y (1970) Evaluation of pollen viability by enzymically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. Stain Technol 45: 115–120

Heslop-Harrison J, Heslop-Harrison Y, Shivanna KR (1983) The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test proceedure. Theor Appl Genet 67:367–375

Kenrick J, Knox RB (1979) Pollen development and cytochemistry in some Australian species of *Acacia.* Aust J Bot 27:413–427

Kenrick J, Knox RB (1981a) Structure and Histochemistry of the stigma and style of some Australian species of *Acacia.* Aust J Bot 29:733–745

Kenrick J, Knox RB (1981b) Post-pollination exudate from stigmas of *Acacia* (Mimosoideae). Ann Bot 48: 103–106

Kenrick J, Knox RB (1984) Self-incompatibility in two Australian species of *Acacia* (*A. retinodes* and *A. terminalis*). Nitrogen-Fixing Tree Res Rep 2. Hawaii (in press)

Knox RB, Kenrick J, Bernhardt P, Marginson R, Beresford G, Baker I, Baker HG (1984) Extra-floral nectaries as adaptations for bird pollination in *Acacia terminalis.* Am J Bot (in press)

Knox RB, Kenrick J (1983) Polyad function in relation to the breeding system of *Acacia.* In: Mulcahy D, Ottaviano E (eds) Elsevier, North-Holland, pp 411–417

Linskens HF, Esser K (1957) Über eine spezifische Anfärbung der Pollenschläuche in Griffel und die Zahl der Kallosepropfen nach Selbstung und Fremdung. Naturwissenschaften 44: 16

Martin FW (1959) Staining and observing pollen tubes by means of fluorescence. Stain Technol 34: 125–128

Moffett AA, Nixon FM (1974) The effects of self-fertilization on green wattle (*Acacia decurrens* Willd.) and black wattle (*A. mearnsii* De Wild.). Wattle Res Inst Rep 1973–74, pp 66–84

Mulcahy DL, Mulcahy GB (1983) A comparison of pollen tube growth in bi- and trinucleate pollen. In: Mulcahy D, Ottaviano E (eds) Pollen: biology and implications in plant breeding. Elsevier, North-Holland, pp 29–33

Nettancourt D de (1977) Incompatibility in angiosperms. Springer, Berlin Heidelberg New York

Newman IV (1933) Studies in the Australian Acacias II. The life history of *Acacia baileyana* (FM). Part 1. Some ecological and vegetative features, spore production and chromosome number. J Linn Soc London, Bot 49: 145–171

Philp J, Sherry SP (1946) The degree of natural crossing in Green Wattle (*A. decurrens* Willd.) and its bearing on Wattle breeding. J S Afr For Assoc 14: 1–28

Philp J, Sherry SP (1949) The genetics of hybrids between Green Wattle (*Acacia decurrens*) and Black Wattle (*Acacia mollissima*). J S Afr For Assoc 17:6–58

Roy H (1956) La culture du mimosa sur la Cote-d'Azur France. Ministere de l'agriculture et du development rural. Bull Techn Inf Ing Serv Agric 108: 169–178

Sherry SP (1971) The Black Wattle. University of Natal Press, Pietermaritzburg

Silow RA (1931) A preliminary report on pollen-tube growth in Red Clover (*Trifolium pratense* L.). Welsh Plant Breed Stn Bull 12:228–233

Spiss L, Paolillo DJ (1969) Semi-vitro methods in the study of compatibility in birdsfoot trefoil (*Lotus corniculatus* L.). Crop Sci 9: 173–176

Stanley RG, Linskens HF (1974) Pollen. Springer, Berlin Heidelberg New York

Williams EG, Knox RB (1982) Quantitative analysis of pollen tube growth in *Lycopersicon peruvianum.* J Palynol 18: 65–74

Williams RD, Williams W (1947) Genetics of red closer (*Trifolium pratense* L.) Compatibility III. The frequency of incompatibility S alleles in two non-pedigree populations of red clover. J Genet 48:69–79

Wojciechowska B (1963) Embryological studies in the genus *Lotus.* Part 1. Fertilization and seed development following open- and self-pollination of *Lotus corniculatus* L. Genet Pol 4:53–63

Zapata TR, Arroyo MTK (1978) Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica 10:221–230