Sex Plant Reprod (1990) 3:7–17

Sexual Plant Reproduction

Relationships of pollen size, pistil length and pollen tube growth rates in *Rhododendron* and their influence on hybridization

E.G. Williams[1] and J.L. Rouse[2]

[1] Department of Botany, University of Georgia, Athens, GA 30602, USA
[2] School of Physics, The University of Melbourne, Parkville, Victoria 3052, Australia

Summary. Pollen size and pistil length data have been collected for 93 species of *Rhododendron* (Ericaceae) belonging to a number of different subgeneric taxa. For a sample of eight species in section *Vireya*, pollen tube growth in the style after self- or interspecific pollination has been quantified. Pollen volume and the time taken for pollen tubes to reach the ovary were both related to pistil length. Pollen-tube growth rates were generally greater for species with longer pistils and larger pollen. Increasing temperature increased the rate of pollen-tube growth. There was no detectable effect of pollen tube density on tube growth rate in the style. After interspecific pollinations tube growth rates in foreign styles could be faster or slower than in self styles. A semisterile individual with two viable pollen grains per tetrad and a plant grafted as scion to a longer-styled stock both showed more rapid pollen-tube growth than expected on the basis of pistil size. Data collected for 26 species in section *Vireya* showed that where extreme disparity of pollen/pistil size causes failure of interspecific crosses, one or more bridging species with intermediate pollen/pistil size can generally be selected.

Key words: Ericaceae – Hybridization – Pistil length – Pollen size – Pollen-tube growth rate – *Rhododendron*.

Introduction

The genus *Rhododendron* (Ericaceae) contains some 900 species among which interspecific pollinations produce a variety of outcomes ranging from full compatibility with vigorous fertile progeny to total incompatibility with pollen tubes arrested before fertilization (Williams et al. 1982; Williams et al. 1985; Kaul et al. 1986). An understanding of the nature of these barriers is necessary before manipulations can be designed to achieve hybridization.

Within section *Vireya*, a group of some 270 species endemic to south-east Asia, the ability to cross is related primarily to comparative flower size of the parent species such that successful hybridization is unlikely if the style-length ratio is either much greater or much less than one (Williams and Rouse 1988). Within this group few other major incongruity barriers (Hogenboom 1975) appear to be operating. Our observations of interspecific pollinations within section *Vireya* suggested a possible relationship of pistil or style length, not only to the ultimate success or failure of crosses, but also to the size of pollen and the rate of pollen-tube growth. Here we present data collected to test for such relationships, and discuss their significance for interspecific hybridization.

Materials and methods

The following species from subgenus *Rhododendron*, section *Vireya*, were selected on the basis of a range of style lengths (Fig. 1) to obtain detailed measurements of pollen-tube growth in styles: *R. anagalliflorum* Wernh., *R. rubineiflorum* Craven, *R. inconspicuum* J.J. Smith, *R. macgregoriae* F. Muell, *R. stenophyllum* Hook., *R. gracilentum* F. Muell., *R. javanicum* (Bl.) Benn, *R. laetum* J.J. Smith, *R. multinervium* Sleumer, and *R. konori* Becc.

The following additional *Vireya* species were used to obtain seed set data in interspecific pollinations: *R. aequabile* J.J. Smith, *R. commonae* Foerster, *R. lochae* F. Muell., *R. zoelleri* Warb., and *R. leucogigas* Sleum., 'Hunstein's Secret'.

The following additional *Rhododendron* species were used to provide pistil length and pollen size data. Subgenus *Rhododendron*, section *Vireya: R. alticolum* Sleumer, *R. bagobonum*, Copel., *R. beyerinckianum* Koord., *R. caliginis* Kores, *R. christianae* Sleumer, *R. dielsianum* Schltr., *R. ericoides* Low ex

Offprint requests to: E.G. Williams

Hook., *R. hellwigii* Warb., *R. herzogii* Warb., *R. intranervatum* Sleumer, *R. jasminiflorum* Hook., *R. kawakamii* Hay. var. 'flaviflorum' Liu et Chuang, *R. leptanthum* F. Muell., *R. loranthiflorum* Sleumer, *R. malayanum* Jack, *R. nervulosum* Sleumer, *R. pauciflorum* K. and G., *R. perakense* K. and G., *R. phaeopeplum* Sleumer, *R. polyanthemum* Sleumer, *R. quadrasianum* Vidal var. 'rosmarinifolium' (Vidal) Copel., *R. rarum* Schltr., *R. retusum* (Bl.) Benn., *R. rugosum* Low ex Hook., *R. santapaui* Sastry et al., *R. saxifragoides* J.J. Smith, *R. schoddei* Sleumer, *R. vitis idaea* Sleumer, *R. yongii* Argent.; section *Rhododendron: R. augustinii* Hemsley, *R. ciliicalyx* Franchet, *R. cubitii* Hutch., *R. dalhousiae* Hooker, *R. davidsonianum* Rehder and Wilson, *R. formosum* Wall., *R. johnstoneanum* Hutch., *R. keiskei* Miquel, *R. lindleyi* T. Moore, *R. lutescens* Franchet, *R. lyi* Léveille, *R. maddenii* Hooker, *R. moupinense* Franchet, *R. mucronulatum* Turcz, *R. nuttallii* T.W. Booth, *R. scintillans* Balfour and Smith, *R. scottianum* Hutch., *R. sinonuttallii* Balfour and Forrest, *R. supranubium* Hutch., *R. veitchianum* Hooker; section *Pogonanthum: R. trichostomum* Franchet. Subgenus *Hymenanthes,* section *Ponticum: R. arboreum* Smith subsp. *arboreum, R. arboreum* Smith subsp. *delavayi* (Franchet) Chamberlain, *R. hyperythrum* Hayata, *R. macabeanum* Watt ex Balfour, *R. magnificum* Ward, *R. metternichii* Siebold and Zuccarini var. 'metternichii' *R. metternichii* Siebold and Zuccarini var. 'hondoense' Nakai, *R. ponticum* L., *R. yakushimanum* Nakai. Subgenus *Azaleastrum,* section *Azaleastrum: R. ovatum* Maxim; section *Choniastrum: R. amamiense* Ohwi, *R. championae* Hemsley, *R. ellipticum* Maxim., *R. latoucheae* Franchet. Subgenus *Pentanthera,* section *Pentanthera: R. calendulaceum* (Michx.) Torrey, *R. japonicum* (Gray) Suringar, *R. nudiflorum* (L.) Torrey (Syn. *R. periclymenoides* Michx.), *R. occidentale* (Torr. and A. Gray) A. Gray. Subgenus *Tsutsusi,* section *Tsutsusi: R. indicum* Sweet, *R. oldhamii* Maxim., *R. rubropilosum* Hayata, *R. serpyllifolium* Miq., *R. simsii* Planch; section *Brachycalyx: R. schlippenbachii* Maxim; section *Tsusiopis: R. tashiroi* Maxim.

All materials were grown in the private collection of J.L. Rouse in Melbourne, except for one plant each of *R. rubineiflorum, R. saxifragoides* and *R. laetum* (semisterile) which were kindly loaned by G. Snell, Shrublands Nursery, 970 Mountain Highway, Boronia, Victoria 3155, present address, 7 Lawrence Place, Maleny, Queensland, 4552. He also supplied a sample of *R. polyanthemum* pollen.

Pistil length

Pistil length (Fig. 1) was measured directly from the stigma surface to the base of the ovary. For intraspecific pollinations the important criterion is the total length of the pistil since pollen must be capable of reaching ovules at the base of the ovary. For interspecific pollinations the combined stigma plus style length, which is referred to hereafter as style length, is a more useful criterion since hybrids may be obtained even if pollen tubes penetrate only to the level of the uppermost ovules.

Pollen size

In *Rhododendron*, pollen is shed in persistent tetrahedral tetrads linked by vicin threads. The diameter of a tetrad is approximately equivalent to twice the diameter of a single pollen grain. Pollen was hydrated on a microscope slide in a 12% (w/v) sucrose solution and covered with a coverslip. The diameters of ten tetrads were measured with an eyepiece micrometer to obtain a mean estimate of pollen size for each species.

Since growth potential of pollen must be related to cytoplasmic volume rather than cross-sectional area or diameter, we sought a parameter for pollen size related to cytoplasmic volume and corrected for wall thickness. Although detailed measurements were not performed for all species, wall thickness appeared to vary relatively little over the range of tetrad diameters measured. Most estimates of tetrad diameter involved measurement across two adjacent pollen grains, including four thicknesses of wall. Best fit of a cubic function of tetrad diameter plotted against pistil length was obtained by subtracting a constant 25 ± 5 µm from the measured tetrad diameter. This implies a pollen-wall thickness of approximately 6.5 ± 1.5 µm, which we accepted as being sufficiently close to an upper limit for real wall thickness values. For *R. laetum*, the wall thickness measured from electron micrographs was approximately 4 µm.

Pollination procedure

Pollinations were made by hand on emasculated flowers, using either fresh pollen from a dehiscing anther or pollen stored over $CaCl_2$ at $-20°$ C (Rouse 1984). Pollinated pistils were either harvested at various intervals in the first 3 weeks for examination of pollen-tube growth, or were allowed to remain on the plant for recording of abscission or seed set. Temperatures during the pollen-tube growth period were recorded using maximum and minimum thermometers sited above ground level near the plants. The effect of pollen-tube density in the style was examined for *R. javanicum* using two series of self-pollinations, one with excess pollen applied to the entire stigma surface, and the other with only a few grains applied to one stigma lobe.

Pollen-tube growth

Pollen-tube growth was assessed at daily intervals after pollinations by measuring the distance down the pistil penetrated by the pollen tubes (Williams et al. 1982). Pistils were fixed 2 h in 1:3 acetic acid:ethanol, stored in 70% ethanol at room temperature, autoclaved in 10% (w/v) sodium sulphite (Na_2SO_3 anhydrous) for 30–40 min at 104 kPa (15 psi), and stained overnight in decolorized aniline blue stain (Merck Anilinblau WS, 0.1% in 0.1 *M* K_3PO_4 decolorized overnight in the dark). Each pistil (or segment of large pistils) was laid out straight on a microscope slide and squashed gently in stain beneath a coverslip. Where possible, ovary-wall tissues were removed to expose placentae within the ovary. Coverslips were sealed with white petroleum jelly to prevent evaporation. Pistil squashes were examined with an Olympus CHC/BHZ-RFL incident light fluorescence microscope using blue light excitation (exciter filter BP-490 dichroic mirror DM-500 +0-515).

Pollen-tube growth rates were estimated from plots of pollen-tube length against elapsed time from pollination. Since pistils contracted slightly during autoclaving, rates were corrected to fresh pistil length. Temperature was found to markedly affect tube growth rate. Thus growth rate comparisons should be made at the same temperature. This was not technically possible for large shrubs growing outdoors and flowering at different times. We therefore had to assume that temperature affects tube growth rates similarly for all species, and applied a standard correction to 17° C (the temperature requiring the least correction over all pollinations).

Results

Pollen size and pistil length

The relationship between pollen size and pistil length is shown for *Vireya* species in Fig. 2a and for all *Rhododendron* species examined in Fig. 2b. Measured tetrad diameters ranged from 40 to 100 μm. Log/log plots of pollen tetrad diameter corrected approximately for wall thickness (*d*-25) against pistil length (L_p) produced the cubic function

$$\frac{(d\text{-}25)^3}{L_p} = 1.42 \times 10^3 \, k$$

where d = measured tetrad diameter in μm, L_p = pistil length in mm, and with 1.0 as the optimum value for the constant k. All measured values for the genus fell within the region $1/3 \leq k \leq 3$. This indicates that pollen cytoplasmic volume is related to pistil length – i.e., to the distance pollen tubes must grow to achieve full seed set.

Pollen-tube growth

Observations of pollen-tube growth in the pistil are summarized in Table 1 and illustrated for representative self-pollinations and crosses in Figs. 3–5. The relationship between pollen volume and pollen-tube growth rate is shown in Fig. 6a, that between style length and pollen-tube growth rate is shown in Fig. 6b, and that between style length and time taken for pollen tubes to reach the base of the style is shown in Fig. 6c. Several major points emerge from the data:

Pollen-tube growth rates in self pistils were generally more rapid for species with longer pistils (Table 1, Figs. 3, 5a) and were also positively correlated with pollen size for six species (*R. anagalliflorum, R. rubineiflorum, R. inconspicuum, R. stenophyllum* (normal tubes), *R. javanicum, R. konori*) ($r = 0.94$, Fig. 6a).

Pollen-tube growth rate was greater at higher temperatures in the range 13° to 21° C (Table 1, *R. javanicum* selfed (Fig. 3c); *R. multinervium* × *R. konori* (Fig. 5b)). The pollen-tube growth rate was apparently unaffected by the density of pollen tubes in the stylar canal, as can be seen by comparing heavy and light pollinations of *R. javanicum* (Table 1, Fig. 3c).

When pollen from a small-flowered species was applied to a much larger pistil, the pollen grew to 1.5–2 times the length of its own pistils but did not reach the foreign ovary if it lay beyond this distance (Table 1, Fig. 4).

When pollen from a large-flowered species was applied to a much smaller pistil, pollen tubes either reached the base of the ovary without entering the ovules (Table 1, *R. konori* pollen on *R. anagalliflorum*) or showed infrequent and often abnormal entries involving overgrowth of the pollen tube beyond the egg apparatus within the embryo sac as described by Williams et al. (1986) (Table 1, *R. javanicum* pollen on *R. anagalliflorum* and *R. inconspicuum*, and *R. konori* pollen on *R. inconspicuum* and *R. macgregoriae*).

When growing in a foreign pistil, pollen tubes could grow either more slowly or more rapidly than after self-pollination. For example, on pistils of *R. anagalliflorum* and *R. inconspicuum*, pollen tubes of both *R. javanicum* and *R. konori* grew more slowly than in self pistils, and on *R. multinervium* pistils, pollen tubes of *R. konori* grew more rapidly than on self pistils (Table 1, Figs. 5, 6b).

Pollen-tube growth rates of two plants, *R. gracilentum* and *R. laetum* were clearly exceptions to the pattern of data produced by other individuals in the study (Figs. 6a, b). These tube growth rates were markedly more rapid than expected on the basis of pistil and pollen sizes.

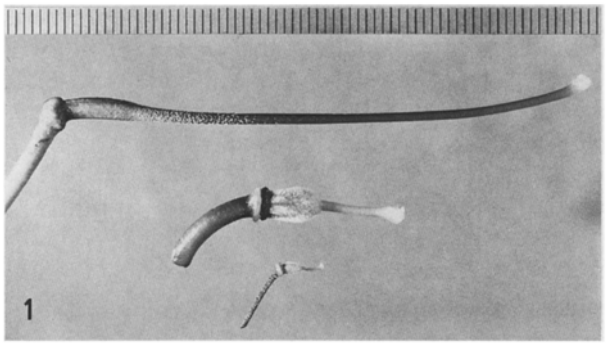
Two classes of pollen tubes were observed in all self-pollinated pistils of the single plant of *R. stenophyllum* (Table 1). These were slow-growing, wide, spiralling tubes with heavily callosed walls, and normal, narrow tubes. Although the frequencies of these two classes were not quantified, the abnormal tubes appeared to comprise the majority. The abnormal tubes did not penetrate further than 4–7 mm down the pistil, whereas the normal tubes were seen to enter ovules apparently normally. When pollen of this *R. stenophyllum* plant was applied to foreign pistils of *R. macgregoriae* and *R. multinervium*, only a single class of apparently normal tubes was observed.

Seed set after self- and interspecific pollinations

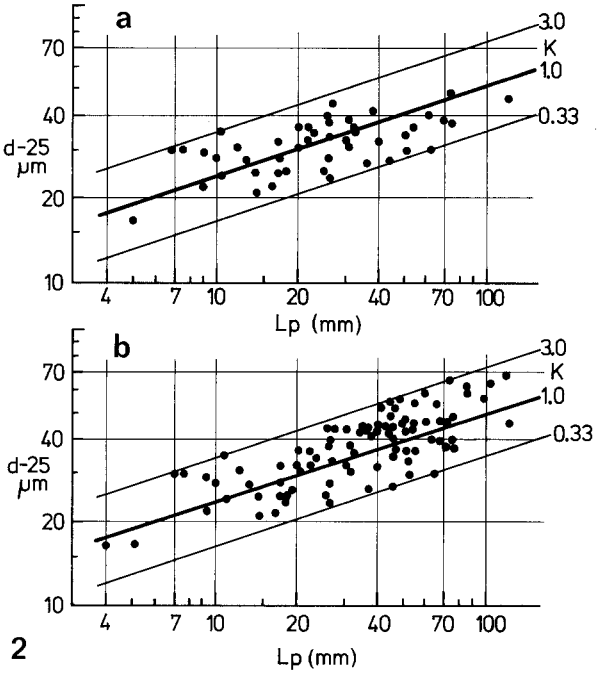
Seed set after self- and interspecific pollinations is shown in Table 2 and displayed in the form of a chart of successful interspecific crosses in Fig. 7. Although these data are limited in terms of both numbers of plants tested and numbers of flowers pollinated, several points are worthy of note:

No seed was produced by crosses with a male/female style length ratio (SLR) outside the range $0.2 \leq \text{SLR} \leq 6$.

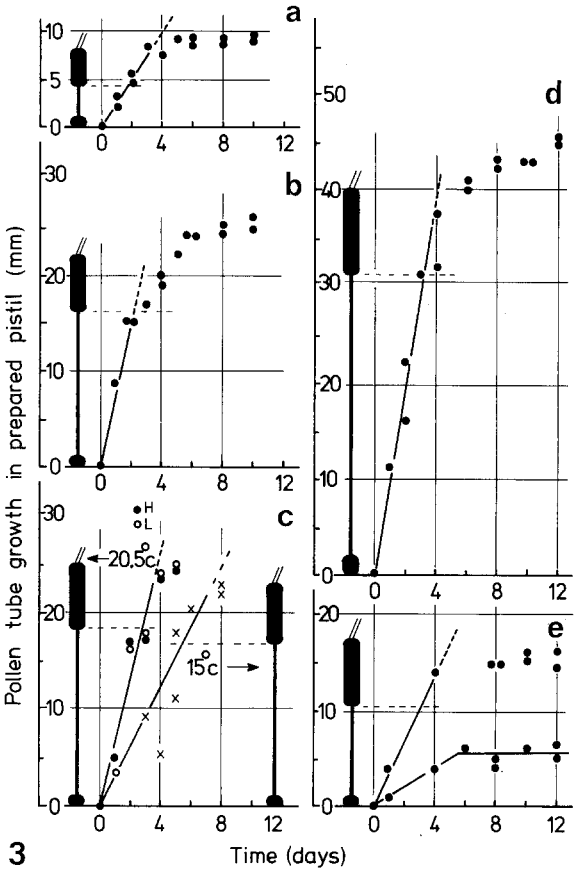
R. stenophyllum was the only plant producing no selfed seed during the present study, although it had produced selfed seed previously. When this individual was used as pollen parent in crosses to



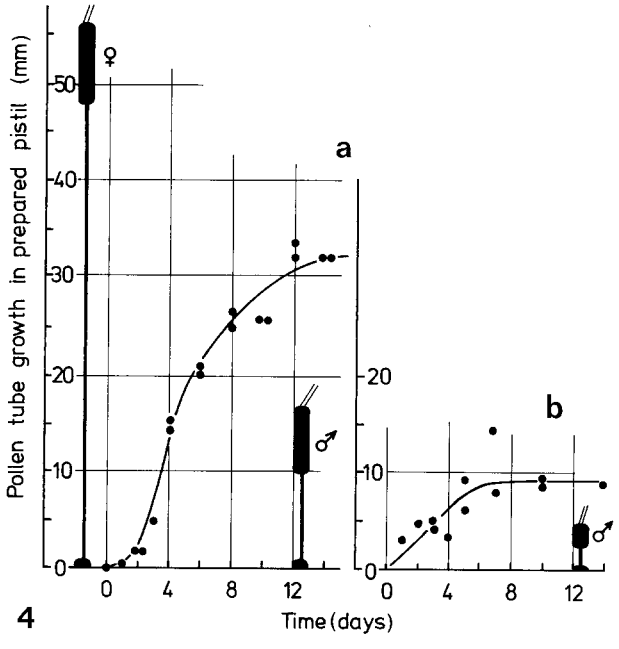
1


a
b
d-25 µm
Lp (mm)
3.0
K
1.0
0.33

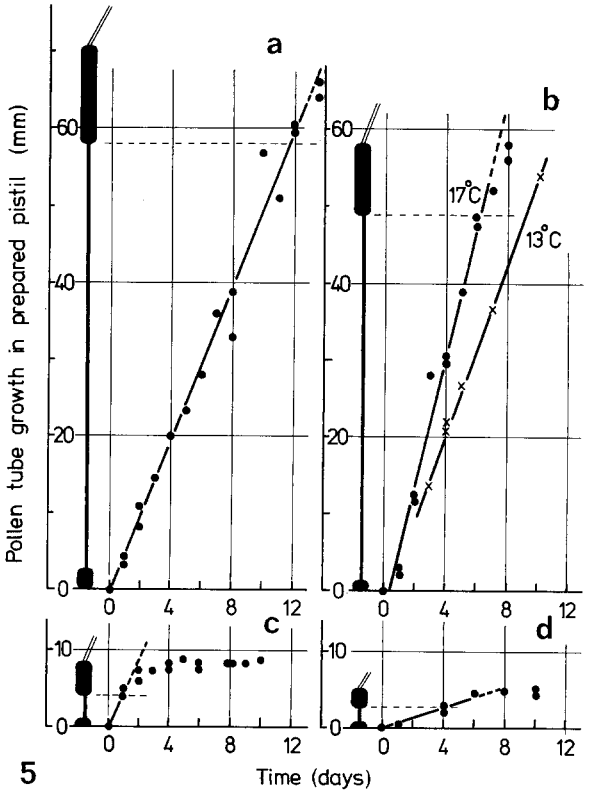
2


a
b
c
d
e
Pollen tube growth in prepared pistil (mm)
Time (days)
20.5c
15c
H
L

3


a
b
Pollen tube growth in prepared pistil (mm)
Time (days)
♀
♂

4


a
b
c
d
Pollen tube growth in prepared pistil (mm)
Time (days)
17°C
13°C

5

R. anagalliflorum and *R. macgregoriae* seed was produced.

R. inconspicuum proved to be particularly ineffective as a pollen parent and also failed to produce seed as a pistillate parent in two out of four interspecific crosses with SLR inside the range given above. Selfed seed set was also relatively poor although placentae in pistil squashes were covered with pollen tubes to the ovary base and many ovules were entered. Crosses showing infrequent but normal ovule entries in pistil squashes were associated with poor retention of control pistils on the plants and frequent abscission (compare Tables 1 and 2); e.g., *R. javanicum* × *R. inconspicuum* and *R. javanicum* × *R. konori*. This presumably indicates some variation in the frequency of fertilization among different pistils and a threshold proportion of developing ovules required for prevention of abscission of the capsule.

Discussion

We have shown that for a sample of 93 species of *Rhododendron* belonging to a number of different subgeneric taxa, pollen volume is directly related to pistil length. When small pollen from a species with short pistils is placed on a very much longer foreign pistil, the pollen tubes may grow to 1.5–2 times the length of their own pistil but will not reach the foreign ovary if it lies beyond this distance. Labarca and Loewus (1972, 1973) have shown for *Lilium* that radioactively labeled carbohydrates are taken up from the stylar mucilage into the cytoplasm and walls of growing pollen tubes. Our results suggest, however, that the pollen tubes cannot grow indefinitely through the pistil, even if heterotrophic uptake occurs. Size of pollen presumably reflects to some extent a form of prepackaging to grow only the distance required for intraspecific fertilization. In *Nicotiana*, pollen tubes have been found to synthesize no rRNA (Suss and Tupy 1976) and only very small amounts of mRNA during the early growth phase (Tupy et al. 1986). These workers concluded that pollen tube growth did not require de novo synthesis of RNA. Pollen-tube growth may therefore be limited at the transcriptional level, and possibly also the translational level, with growth continuing only so long as synthetic capability remains. Our data for *Rhododendron* species suggest a considerable safety margin since pollen tubes were able to grow 1.5–2 times the length of their own pistils.

For a small sample of one to two plants each of six species in section *Vireya*, pollen size was positively correlated with pollen-tube growth rate ($r = 0.94$). For these species there was a linear relationship between style length and time taken by pollen tubes to traverse the style. Thus, for each species, pollen size and pollen-tube growth rate were related to pistil length. These observations are similar to those of Perez and Moore (1985), who demonstrated correlations of pollen-tube growth rate with pistil length and pollen volume for 16 species of *Prunus*. Two interesting exceptions were the individuals used to represent *R. gracilentum* and *R. laetum*. Both showed more rapid pollen-tube growth than expected on the basis of pollen and pistil sizes. The *R. gracilentum* plant is a scion grafted to a hybrid *Vireya* stock. Both species involved in the hybrid (*R. christianae* × *R. jasminiflorum*) have larger flowers than *R. gracilentum*, and the hybrid itself has a pistil length of 49 mm compared with 23 mm for *R. gracilentum*. The tube growth rate of scion pollen may reflect an influence of the stock. Effects of a grafted stock on pollen-tube growth and fertilization on the scion have been reported previously for *Lycopersicon* (Nirk 1959) and *Oenothera* (Kivilaan and Chang 1963). The individual plant of *R. laetum* was found to be semisterile with two fertile and two sterile pollen grains per tetrad. It is possible that the two fertile grains may have received more than the usual complement of resources during pollen maturation.

Although pollen-tube growth rate in self pistils is related to pollen size, a clear effect of the pistil

Fig. 1. *Vireya* rhododendron pistils, from *top: R. multinervium, R. stenophyllum, R. anagalliflorum.* (Scale in millimeters)

Fig. 2a, b. The relationship between pollen size and pistil length for *Rhododendron* species shown on a log/log plot as the function $(d\text{-}25)^3/L_p = 1.42 \times 10^3$ K where L_p is pistil length in mm and d is pollen tetrad diameter in micrometers. The function is plotted for $K = 0.33$, 1.0 and 3.0. *Data points* lie in the range $0.33 \leq K \leq 3.0$. **a** Species in section *Vireya*, **b** species in all taxonomic groups

Figs. 3–5. Pollen-tube penetration into pistils compared with pistil proportions, shown with stigma pointing down on *left* of plots. Temperatures during pollen tube growth are given in Table 1. **Fig. 3a–e.** Extent of pollen tube growth in the pistil plotted against time after self-pollination for **a** *R. inconspicuum*, **b** *R. gracilentum*, **c** *R. javanicum* at a mean temperature of 20.5° C or 15° C (*H* heavy pollinations, and *L* light pollinations, at 20.5° C), **d** *R. laetum*, **e** *R. stenophyllum*. **Fig. 4a, b.** Extent of tube growth of pollen from short-styled species on larger foreign pistils **a** *R. stenophyllum* pollen on *R. multinervium* (SLR = 0.21), **b** *R. anagalliflorum* pollen on *R. multinervium* (SLR = 0.073). **Fig. 5a–d.** Growth of *R. konori* pollen tubes in self and foreign pistils **a** self-pollination, **b** on *R. multinervium* at mean temperatures of 17° C and 13° C, **c** on *R. inconspicuum*, **d** on *R. anagalliflorum*

Table 1. Summary of pollen tube growth observed after self- and interspecific pollinations of *Vireya* rhododendrons. Table is divided horizontally by pollen type. Pollen tube tips and ovule entries classified: N, Normal; S, tips swollen; D, distorted; R, rare; O, pollen tube overgrowths in ovules

Pistillate parent (♀)		Pollen parent (♂)				Pollen tubes						Mean temperatures (°C)		
Species	Pistil dimensions (mm): stigma; stigma and style; pistil	Species	Style length (mm)	Pollen fresh (F) or stored (S)	Style length ratio ♂/♀	Reach style base (day)	First enter ovules (day)	Pollen tubes (normal/abnormal)	Ovule entries (normal/abnormal)	Growth rate in style mm d^{-1}	Growth rate corrected to mean temperature of 17° C	Max.	Min.	Mean
R. anagalliflorum	0.5 3.8 6.5	Selfed	3.8	14F 2S	1.0	2	3	N	N	1.6	1.6	22.5	11.5	17.0
R. multinervium	2.0 52.0 61.5	*R. anagalliflorum*	3.8	F	0.073	–	–	SD	–	1.5	2.1	22.0	10.0	16.0
R. rubineiflorum	0.5 3.8 7.3	Selfed	3.8	F	1.0	1.5	2	N	N	2.7	2.1	22.0	13.5	18.0
R. inconspicuum	1.0 5.0 9.5	Selfed	5.0	F	1.0	1.5	3	N	N	2.9	3.2	21.0	12.0	16.5
R. javanicum	1.0 17.0 23.0	*R. inconspicuum*	5.0	F	0.29	7.5	10	N	NR	2.3	1.7	24.5	11	18.0
R. multinervium (2)	2.0 52.5 61.5	*R. inconspicuum*	5.0	F	0.095	–	–	NS	–	0.8 ±0.3[a]	(3.2)[a]	19.5	6.5	13.0
R. stenophyllum	2.0 11.7 18.7	Selfed	11.7	F	1.0	3 –[b]	4 –[b]	N SD	NR –	3.6 1.0[b]	3.6 1.0	22.5	12.0	17.0
R. macgregoriae	1.0 12.2 16.2	*R. stenophyllum*	11.7	F	0.96	3	4	N	NO	3.8	3.5	24.0	11.0	17.5
R. multinervium (1)	1.5 55.5 64.5	*R. stenophyllum*	11.7	S	0.21	–	–	N	–	3.7	3.7	22.0	12.5	17.0
R. gracilentum	1.0 17.0 24.0	Selfed	17	F	1.0	2.3	3	N	N	7.5	7.5	20.0	14.0	17.0
R. anagalliflorum	0.5 2.7 5.2	*R. javanicum*	20	F	7.4	1.4	12	ND	NOR	2.0	2.0	21.5	13.0	17.0
R. inconspicuum	1.0 4.5 8.5	*R. javanicum*	20	F	4.4	1.4	4	ND	NOR	4.1	4.1	21.0	13.0	17.0
R. javanicum	1.0 18.5 24.5	Selfed	18.5	F	1.0	5.5	7–8	N	N	3.7	4.9	21.5	8.5	15.0

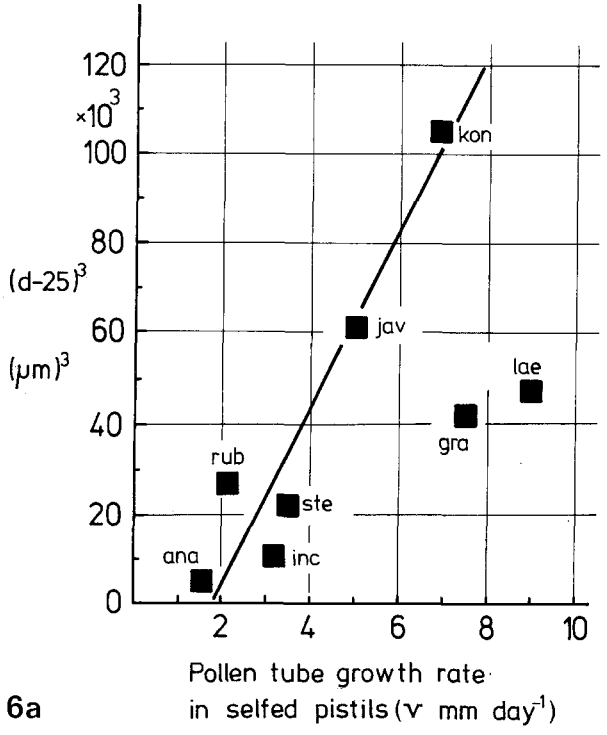
Table 1 (continued)

Pistillate parent (♀)		Pollen parent (♂)				Pollen tubes						Mean temperatures (°C)		
Species	Pistil dimensions (mm): stigma; stigma and style; pistil	Species	Style length (mm)	Pollen fresh (F) or stored (S)	Style length ratio ♂/♀	Reach style base (day)	First enter ovules (day)	Pollen tubes (normal/abnormal)	Ovule entries (normal/abnormal)	Growth rate in style mm d^{-1}	Growth rate corrected to mean temperature of 17° C	Max.	Min.	Mean.
R. javanicum (cont.)	1.0 20.5 27.5	Selfed (heavy)[c]	20.5	6F 5S	1.0	2.7	4	N	N	7.1	5.0	28.0	13.0	20.5
	1.0 20.5 27.5	Selfed (light)[c]	20.5	6F 5S	1.0	2.7	4	N	N	7.1	5.0	28.0	13.0	20.5
R. laetum	2.0 33.5 42.5	Selfed (semi-sterile)[d]	33.5	F	1.0	3.2	4	N	N	10.8	9.0	24.5	15.0	20.0
R. anagalliflorum	0.5 2.5 5.0	*R. konori*	60	7F 8S	24	4.1	Nil[e]	D	–	0.6	0.6	22.0	12.0	17.0
R. inconspicuum	1.0 4.5 8.0	*R. konori*	60	3F 13S	13	0.8	2	ND	NOR	5.1	5.1	21.0	13.0	17.0
R. macgregoriae	1.0 13.0 17.5	*R. konori*	60	7F 3S	4.6	3.2	8	ND	NOR	4.1	4.7	21.0	11.5	16.0
R. javanicum	1.5 20.0 26.5	*R. konori*	60	9F 4S	3.0	4	10	ND	NR	5.0	6.2	21.0	9.0	15.0
R. multinervium (1)	2.0 54.5 63.5	*R. konori*	60	F	1.1	6–6.5	11	N	NR	9.5	9.5	22.0	12.5	17.0
R. multinervium (2)	2.0 54.0 64.0	*R. konori*	60	S	1.1	9–9.5	?[f]	N	?[f]	6.1	8.5	18.5	7.5	13.0
R. konori	3.0 60.0 74.0	Selfed	60	11F 7S	1.0	11.5–12	13–14	N	N	5.3	6.8	20.5	8.5	14.5

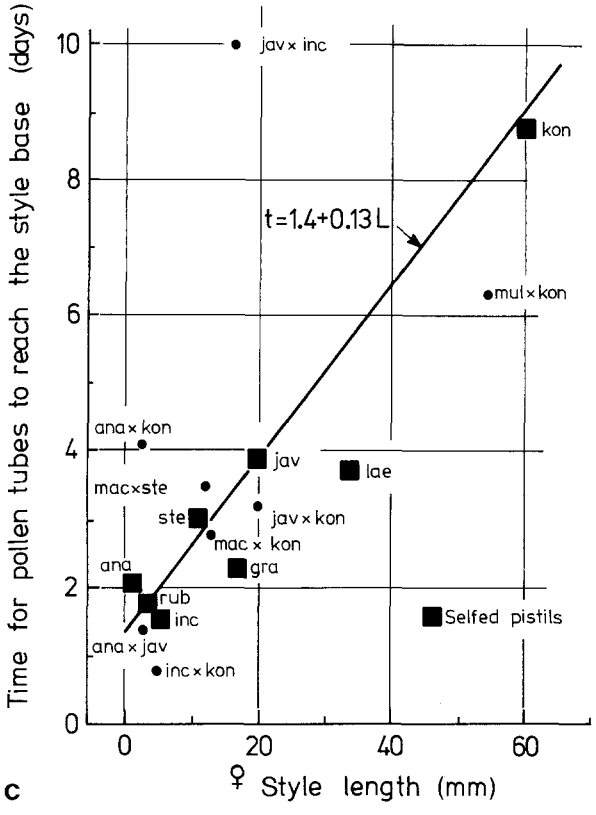
[a] Insufficient data for accurate estimates
[b] Self-pollinations of *R. stenophyllum* showed two distinct classes of pollen tubes
[c] Heavy and light pollinations
[d] Semi-sterile plant with two full and two empty grains per tetrad
[e] Pollen tubes reached base of ovary, but no ovule entries were observed
[f] Observations only to 10 days after pollination

on pollen-tube growth can be seen when interspecific crosses are compared with selfs using the same pollen. Pollen tubes may grow more slowly (e.g., *R. anagalliflorum* × *R. konori*) or more rapidly (e.g., *R. multinervium* × *R. konori*) in a foreign pistil.

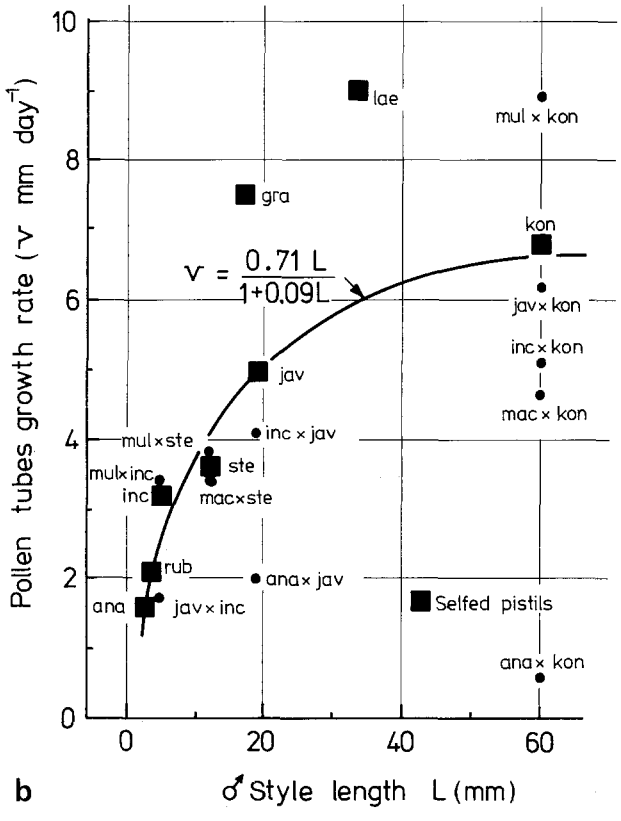
Observed pollen-tube growth rates were relatively slow but of the same order of magnitude


120
×10³
100
80
(d-25)³
(µm)³
60
40
20
0
2
4
6
8
10
kon
jav
lae
gra
rub
ste
ana
inc
Pollen tube growth rate
in selfed pistils (v mm day⁻¹)

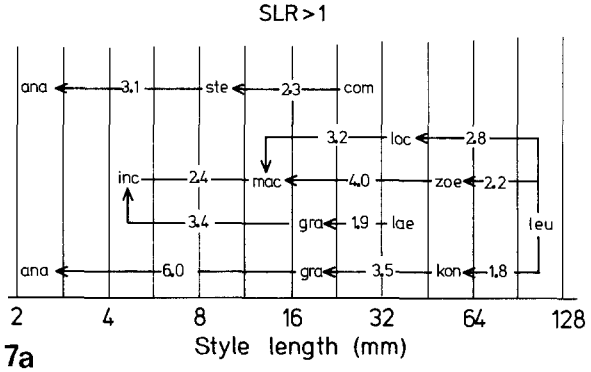
6a


Time for pollen tubes to reach the style base (days)
10
8
6
4
2
0
jav × inc
kon
t=1.4+0.13L
mul × kon
ana × kon
jav
lae
mac×ste
ste
jav × kon
mac × kon
ana
gra
rub
inc
Selfed pistils
ana × jav
inc × kon
0
20
40
60
♀ Style length (mm)

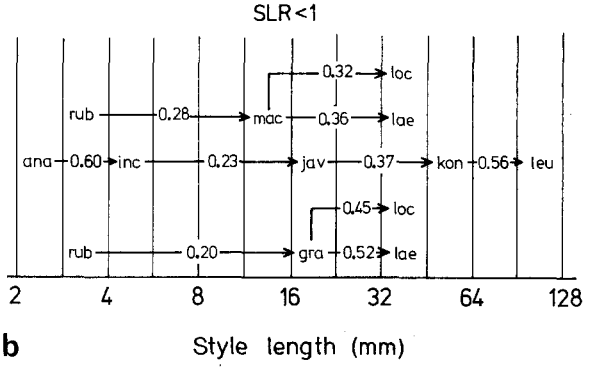
c


Pollen tubes growth rate (v mm day⁻¹)
10
8
6
4
2
0
lae
mul × kon
gra
kon
v = 0.71 L / 1+0.09L
jav × kon
inc × kon
jav
mac × kon
inc × jav
mul×ste
ste
mul×inc
inc
mac×ste
rub
ana × jav
ana
jav × inc
Selfed pistils
ana × kon
0
20
40
60
♂ Style length L (mm)

b


SLR>1
ana
3.1
ste
2.3
com
3.2
loc
2.8
inc
2.4
mac
4.0
zoe
2.2
3.4
gra
1.9
lae
leu
ana
6.0
gra
3.5
kon
1.8
2
4
8
16
32
64
128
Style length (mm)

7a


SLR<1
0.32
loc
rub
0.28
mac
0.36
lae
ana
0.60
inc
0.23
jav
0.37
kon
0.56
leu
0.45
loc
rub
0.20
gra
0.52
lae
2
4
8
16
32
64
128
Style length (mm)

b

as tube growth rates reported for other bicellular pollen types. In the present study, growth rates of normal tubes, corrected to 17° C, ranged from 1.6 to 9.5 mm d^{-1} (1.1–6.6 μm min^{-1}), compared with 3.8 to 8.7 mm d^{-1} (2.6–6.0 μm min^{-1}) for *Prunus* species (Perez and Moore 1985), 8.6 mm d^{-1} (6 μm min^{-1}) for *Tradescantia* and 17.3 mm d^{-1} (12 μm min^{-1}) for *Lilium* (Knox 1984). Tricellular pollen types, e.g., *Secale* and *Zea* show more rapid tube growth rates of up to 346 mm d^{-1} (240 μm min^{-1}; Knox 1984).

For two types of pollination performed at two different temperatures in the range 13°–21° C, pollen-tube growth rate was found to be more rapid at the higher temperature. Insufficient data were available to confirm that all species are affected alike, as assumed in calculating standard rates at 17° C. Small variations in temperature response might account for a proportion of observed variation in the relationships of tube growth rate to pistil length and pollen size. We were unable to detect any effect of pollen tube population density on growth in the style as found by Cruzan (1986) for *Nicotiana*.

The wide disparity in time of first entry of pollen tubes into ovules in the *Vireya* species studied (day 2–day 13 after pollination) implies that the relative timing of male and female gametophyte maturity must also differ among species with different pistil lengths. In *R. nuttallii*, which has a pistil length of 115 mm, female gametophytes have barely commenced development at the time of pollination, about 10–12 days before pollen tubes reach the ovules (Palser et al. 1989). In species with small pistils, e.g., *R. anagalliflorum*, in which ovule entries occur 2 days after self-pollination, female gametophytes may be more nearly mature at the time of pollination. We have not studied in vivo the timing of the male gametophyte mitosis that produces two sperms from the generative cell during tube growth through the pistil. However, divisions have been observed in *R. laetum* pollen tubes cultured in vitro after 24–48 h at 25° C (J. Kenrick, personal communication) and after 72 h) at ambient room temperature (Kaul et al. 1987). In the present work ovule entries were first observed for this species 4 days after pollination. We believe that mis-match of male and female gametophyte maturity periods may contribute to the failure of fertilization in crosses between species with widely different pistil lengths (Williams and Rouse 1988). Mis-match of this type may be responsible for the failure of pollen tubes of the long-styled *R. konori* to enter ovules of the shorter-styled *R. anagalliflorum* (SLR = 24) and for a tendency of the same pollen to produce tube overgrowths inside embryo sacs of *R. inconspicuum* (SLR = 13.3) and *R. macgregoriae* (SLR = 4.6) without forming viable seed. Pollen tubes of *R. konori* are presumably adapted for ovule entry after 13–14 days of growth and are likely to be immature, possibly even with an undivided generative cell after only 2–8 days of growth.

Two classes of pollen tubes, normal and abnormal, were observed in all self-pollinations of *R. stenophyllum*, but not when *R. stenophyllum* pollen from the same plant was used to pollinate *R. macgregoriae* and *R. multinervium*. The abnormal tubes failed to reach the ovary of self pistils. In interspecific pollinations, pollen germination was sufficiently prolific to make unlikely the arrest of an equivalent abnormal class of pollen before germination. It appears that styles of the *R. stenophyllum* plant used here discriminate among self pollen tubes. The genetic basis for this apparent self discrimination is unknown, but a somewhat similar phenomenon has been reported for *Crotalaria* by Malti and Shivanna (1985). Pistils were found to exert selection pressure on pollen tubes during their growth in the style by selective stimulation of a limited number of tubes. In self-pollinations of *R. stenophyllum* normal pollen tubes appeared to be a minority class so that only a small proportion of ovules were entered even after heavy pollination. We presume that failure to achieve a threshold number of developing ovules was the reason for abscission of capsules and failure of seed set after self-pollination. Other individuals of *R. stenophyllum* have been found to set abundant seed after self-pollination (J.L. Rouse, unpublished observations). Thus the unusual form of self sterility observed in the present study is unlikely to be general for the species.

Figs. 6, 7. Abbreviations: *ana R. anagalliflorum, com R. commonae, gra R. gracilentum, inc R. inconspicuum, jav R. javanicum, kon R. konori, lae R. laetum, leu R. leucogigas, loc R. lochae, mac R. macgregoriae, mul R. multinervium, rub R. rubineiflorum, ste R. stenophyllum, zoe R. zoelleri.* **Fig. 6a–c.** Relationships of pollen-tube growth rate to **a** pollen size, **b** style length of the plant producing the pollen, **c** time taken for pollen to reach the base of self or foreign styles, plotted against length of the style in which the pollen is growing. The *vertical scale* is a function of tube growth rate. Lines of best mathematical fit are shown for data from self-pollinations. **Fig. 7a, b.** Successful interspecific crosses presented as a function of style length in millimeters for **a** SLR > 1, **b** SLR < 1. *Arrows* indicate direction of pollen transfer. Species of intermediate style length which cross with both members of a pair that lie outside the range $0.2 \leq SLR \leq 6$ can be used as bridging species for gene exchange. For example, *R. gracilentum* and *R. javanicum* are suitable bridging species for *R. inconspicuum* and *R. konori*

Table 2. Seed set after self- and interspecific pollinations of *Vireya* rhododendrons. The table is divided horizontally by pollen type

Pollination		Style length ratio	Number of pistils		Seed germination	Seedlings vigorous
♀	♂		Total	Producing seed		
R. anagalliflorum	Selfed	1.0	3	3	+	+
R. inconspicuum	*R. anagalliflorum*	0.76	10	10	+	+
R. gracilentum	*R. anagalliflorum*	0.22	15	14	−	
R. rubineiflorum	Selfed	1.0	8	1	+	−
R. anagalliflorum	*R. rubineiflorum*	1.0	13	12	+	+
R. gracilentum	*R. rubineiflorum*	0.22	4	3	+	−
R. inconspicuum	Selfed	1.0	3	1	+	−
R. anagalliflorum	*R. inconspicuum*	1.3	7	0		
R. rubineiflorum	*R. inconspicuum*	1.3	4	0		
R. gracilentum	*R. inconspicuum*	0.29	9	0		
R. javanicum	*R. inconspicuum*	0.29	6	1	+	+
R. laetum	*R. inconspicuum*	0.15	6	0		
R. multinervium	*R. inconspicuum*	0.095	3	0		
R. stenophyllum	Selfed	1.0	3	0		
R. anagalliflorum	*R. stenophyllum*	3.1	7	1	+	+
R. inconspicuum	*R. stenophyllum*	2.3	6	3	−	
R. macgregoriae	*R. stenophyllum*	0.96	1	1	+	−
R. multinervium	*R. stenophyllum*	0.21	2	0		
R. konori	*R. stenophyllum*	0.20	5	0		
R. gracilentum	Selfed	1.0	7	7	+	+
R. anagalliflorum	*R. gracilentum*	5.6	8	1	+	+
R. rubineiflorum	*R. gracilentum*	4.5	7	1	+	+
R. inconspicuum	*R. gracilentum*	3.4	8	8	+	+
R. javanicum	*R. gracilentum*	0.85	4	4	+	+
R. konori	*R. gracilentum*	0.28	4	0		
R. javanicum	Selfed	1.0	4	1	+	+
	Selfed (light)[a]	1.0	2	1	+	+
R. anagalliflorum	*R. javanicum*	7.4	4	0		
R. inconspicuum	*R. javanicum*	4.4	8	0		
R. laetum	Selfed (semi-sterile)[b]	1.0	5	5	+	+
R. leucogigas	*R. laetum*	0.25	2	0		
R. konori	Selfed	1.0	3	3	+	+
R. anagalliflorum	*R. konori*	24	7	0		
R. inconspicuum	*R. konori*	13	7	0		
R. macgregoriae	*R. konori*	4.6	6	0		
R. gracilentum	*R. konori*	3.5	13	10	+	+
R. javanicum	*R. konori*	3.0	6	2	+	+
R. multinervium	*R. konori*	1.1	2	0[c]		
R. leucogigas	*R. konori*	0.56	2	2	+	+
R. leucogigas	Selfed	1.0	2	2	+	+
R. rubineiflorum	*R. leucogigas*	28	4	0		
R. gracilentum	*R. leucogigas*	6.2	7	0		
R. javanicum	*R. leucogigas*	5.3	4	0		
R. laetum	*R. leucogigas*	3.1	8	7	−	

[a] Light pollination on one stigma lobe only
[b] Two fertile and two sterile grains per tetrad
[c] One pistil lost, possibly abscised

R. inconspicuum proved to be a poor parent in both self-pollinations and interspecific crosses. Without data from further individuals, however, it is not possible to determine whether failure to produce germinable seed in several crosses with style length ratios in the range ($0.2 \leq SLR \leq 6$) was due to the particular plant as an individual, or to the operation of an additional incongruity barrier between *R. inconspicuum* and the other species tested.

Variable capsule retention and seed set were observed for self-pollinations of several species in the present study. With the exception of *R. stenophyllum*, however, all individuals used were known from previous work to be capable of setting healthy seed after self-pollination. Since we were working in most instances with plants growing in an outdoor species collection, weather, season, temperature, daylength, etc. may have contributed to variability. Many *Vireya* species endemic to the tropical regions of south-east Asia flower intermittently throughout the year. When grown in a temperate climate such as that of Melbourne, seasonal differences in temperature and daylength may affect the size and reproductive performance of flowers produced at different times.

Although these studies have involved limited plant samples grown without full environmental control, seed set data have been found to correlate well with observations of pollen tube growth in pistils. The work has also confirmed our earlier observation that success of interspecific crosses within *Rhododendron* section *Vireya* is substantially dependent on comparative style lengths. In this study, successful crosses were only achieved with a male/female style length ratio in the range $0.2 \leq SLR \leq 6$. Genomes of species with SLR outside this range can be combined by the use of an appropriate bridging species of intermediate style length.

Acknowledgements. We are grateful to Professor R.B. Knox for helpful discussions, to Mr. G. Snell, for provision of plant materials, and to Miss Jillian Champ for assistance in preparation of the manuscript. Much of the work was carried out in the Plant Cell Biology Research Centre, University of Melbourne, under a grant from the Australian Federal Government (C.T.E.C). Supporting grants were also received from the American Rhododendron Society and the USA – Australia Program of the National Science Foundation, USA. We gratefully acknowledge additional donations from the National Council of the Australian Rhododendron Society, and the Victorian Branch of the Australian Rhododendron Society.

References

Cruzan MB (1986) Pollen tube distributions in *Nicotiana glauca:* evidence for density dependent growth. Am J Bot 73:902–907

Hogenboom NG (1975) Incompatibility and incongruity: two different mechanisms for the non-functioning of intimate partner relationships. Proc R Soc London Ser B 188:361–375

Kaul V, Rouse JL, Williams EG (1986) Early events in the embryo sac following intra- and inter-specific pollinations in *Rhododendron kawakamii* and *R. retusum.* Can J Bot 64:282–291

Kaul V, Theunis CH, Palser BF, Knox RB, Williams EG (1987) Association of the generative cell and vegetative nucleus in pollen tubes of *Rhododendron.* Ann Bot (London) 59:227–235

Kivilaan A, Chang CW (1963) Effect of grafting on the incompatibility response of *Oenothera organensis.* Nature 197:1130–1131

Knox RB (1984) Pollen-pistil interactions. In: Linskens HF, Heslop-Harrison J (eds) Cellular interactions. (Encyclopedia of plant physiology, NS, vol 17) Springer, Berlin Heidelberg New York, pp 508–608

Labarca C, Loewus F (1972) The nutritional role of pistil exudate in pollen tube wall formation in *Lilium longiflorum.* I. Utilization of injected stigmatic exudate. Plant Physiol 50:7–14

Labarca C, Loewus F (1973) The nutritional role of pistil exudate in pollen tube wall formation in *Lilium longiflorum.* II. Production and utilization of exudate from stigma and stylar canal. Plant Physiol 52:87–92

Malti D, Shivanna KR (1985) The role of the pistil in screening compatible pollen. Theor Appl Genet 70:684–686

Nirk H (1959) Interspecific hybrids of *Lycopersicon.* Nature 184:1819–1820

Palser BF, Rouse JL, Williams EG (1989) Coordinated timetables for megagametophyte development and pollen tube growth in *Rhododendron nuttallii* from anthesis to early post-fertilization. Am J Bot 76:1167–1202

Perez S, Moore JN (1985) Prezygotic endogenous barriers to interspecific hybridization in *Prunus.* J Am Soc Hortic Sci 110:267–273

Rouse JL (1984) Pollen storage and *Rhododendron* breeding. In: Williams EG, Knox RB (eds) "Pollination '84". School of Botany, University of Melbourne, pp 185–186

Süss J, Tupý J (1976) On the nature of RNA synthesized in pollen tubes of *Nicotiana alata.* Biol Plant 18:140–146

Tupý J, Süss J, Rihova L (1986) RNA synthesis and ribosome status in pollen tube growth of *Nicotiana tabacum* L.: effects of external pH. J Plant Physiol 123:467–476

Williams EG, Rouse JL (1988) Disparate style lengths contribute to isolation of species in *Rhododendron.* Aust J Bot 36:183–191

Williams EG, Knox RB, Rouse JL (1982) Pollination sub-systems distinguished by pollen tube arrest after incompatible interspecific crosses in *Rhododendron* (Ericaceae). J Cell Sci 53:255–277

Williams EG, Rouse JL, Knox RB (1985) Barriers to sexual compatibility in *Rhododendron.* Notes R Bot Gard Edinburgh 43:81–98

Williams EG, Kaul V, Rouse JL, Palser BF (1986) Overgrowth of pollen tubes in embryo sacs of *Rhododendron* following interspecific pollinations. Aust J Bot 34:413–423