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The role of the pistil in screening compatible pollen

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Summary. Results of in vitro studies on pollen germination and tube growth in the presence of leachates from bisected pistils in *Crotalaria retusa* provide evidence for the operation of selection pressure during pollen-pistil interaction – a process which stimulates growth of a limited number of pollen tubes giving them an advantage over others in effecting fertilization.

Key words: *Crotalaria retusa* – Pollen-pistil interaction – Pollen competition – Pollen selection – Pollen screening

Introduction

The role of the pistil in inhibiting incompatible pollen is well-known and has been extensively investigated (De Nettancourt 1977; Heslop-Harrison 1978; Shivanna 1982; Linskens 1983).

Many investigations have shown that the pistil also acts as an efficient selection system for compatible pollen: it intensifies competition among pollen tubes and only those which are more vigorous and grow faster than a majority of tubes are favoured for effecting fertilization; the other less vigorous pollen tubes are eliminated (Ottaviano et al. 1980; Mulcahy 1984). This is considered to be one of the important causes of the evolutionary success of angiosperms (Mulcahy 1979).

In this report we present experimental evidence for such a selective function of the pistil on cultured pollen grains of *Crotalaria retusa* L.

Material and methods

We have recently investigated the structural details of the pistil of *Crotalaria retusa* (Malti and Shivanna 1984). The stigma is of the wet type and the style is traversed by a canal through which pollen tubes grow. The stylar canal is bordered by one, or a few layers of glandular cells – the canal cells. In the upper part of the ovary (which is free from ovules) canal cells are elongated into papillae and loosely fill the lumen of the canal. It has been suggested that this 'papillate region' in the upper part of the ovary performs the function of pollen tube screening (Malti and Shivanna 1984). *C. retusa* is self-compatible, but requires insect visits for effective pollination.

Plants grown under field conditions were used for pollen collection. Pollen grains were cultured in small glass vials (6 mg/ml) and kept on a horizontal shaker (100 strokes/min). Before culture, pollen grains were spread uniformly on a glass slide and exposed to high humidity (ca. 95% relative humidity in Petri plates lined with moist filter papers) for 20 min. The culture medium comprised sucrose (100 g/l) and boric acid (100 mg/l) with or without calcium nitrate (300 mg/l). Unpollinated pistils (one day before anthesis) were cut transversely at the base of the style and again approximately 5 mm further down to obtain three parts – stigma and style, the upper non-ovulate part of the ovary and the lower ovulate part of the ovary. Either the stigma and stylar parts, or the upper ovary parts, were bisected longitudinally in the germination medium (25 pistils/ml), left for 30 min for leaching, and removed. The media were then used for culturing pollen. The cultures were maintained at 25±2 °C for 3 h and scored for germination and tube length.

Table 1. Pollen germination and pollen tube growth in the presence of pistil leachates. Average of 200 pollen tubes from 3 replicates

Treatment	Tube length (μm)	
	Culture medium containing calcium	Culture medium lacking calcium
Control	608±154	468±184
Leachate from the stigma and style	730±310*	744±288*
the upper part of the ovary	776±358*	710±320*

* Differences highly significant over the control at $P \leq 0.01$

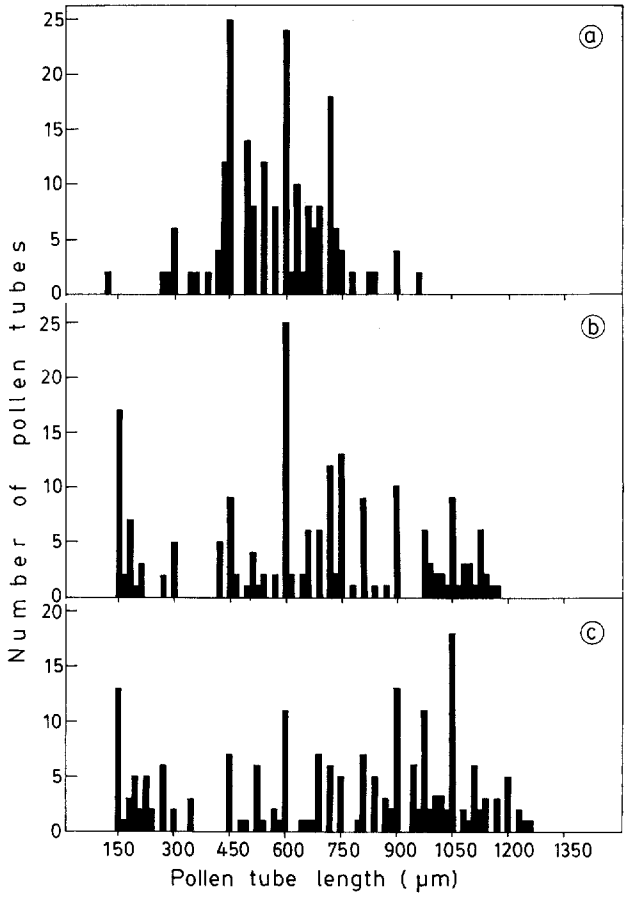

Fig. 1a–c. Frequency distribution of pollen tube lengths in medium containing calcium. **a** control; **b, c** in the presence of leachates from the stigma and stylar parts **(b)** and the upper part of the ovary **(c)**. Data based on groups of 200 pollen tubes

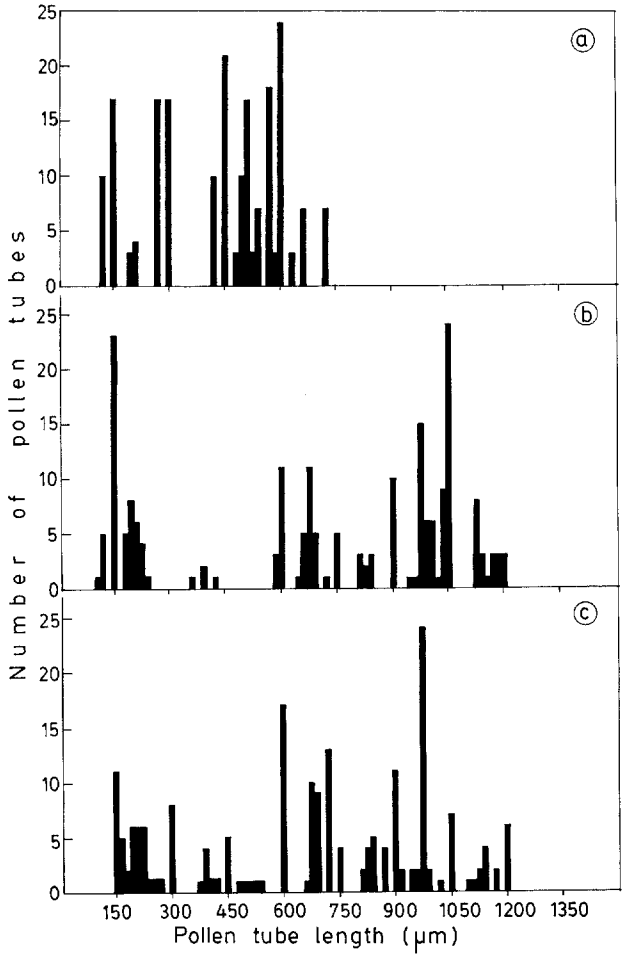

Fig. 2a–c. As in Fig. 1, but in medium lacking calcium

Results and discussion

Table 1 presents the mean values for pollen germination and pollen tube growth in different treatments. The leachates had either no effect, or only marginally inhibited, pollen germination but did markedly increase mean tube lengths. The standard deviations of tube length were much more in the media containing leachates than in that of the control. Fig. 1 presents the frequency distribution of tube lengths in the calcium containing medium. The number of tubes in the lower frequency range was higher in the presence of leachates while that in the median frequency range was lower. More significantly, a proportion of tubes grew much beyond the maximum length attained in the control medium. The increase in mean tube length (Table 1) in the presence of leachates is apparently the result of a significant stimulation of a limited number of tubes rather than a general stimulatory effect.

In the absence of calcium in the culture medium, tube lengths attained in the control medium were much less than those attained in the calcium containing medium (Table 1). In the presence of leachates, a higher proportion of pollen tubes grew beyond those in the control medium (Fig. 2) when compared to those in the calcium medium (Fig. 1). This appears to be the result of calcium leaching from pistillate tissue in addition to the substance(s) responsible for faster growth of a limited number of tubes. The ability of the pistil leachate to discriminate pollen tubes is, therefore, less apparent in the suboptimal germination medium.

Results of the present investigation clearly indicate the operation of selection pressure during pollen-pistil interaction. It stimulates the growth of a limited number of pollen tubes giving them an advantage in effecting fertilization over others. The selection pressure seems to operate throughout the length of the pistil. The in vitro assay we have used provides a rapid method for study-

ing the details of pollen-pistil interaction, pollen competition and selection.

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