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# POLLEN-PISTIL INTERACTIONS IN TRISTYLOUS PONTEDERIA SAGITTATA (PONTEDERIACEAE). II. PATTERNS OF POLLEN TUBE GROWTH[1]

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Pollen tube behavior was compared after legitimate and illegitimate pollinations of tristylous *Pontederia sagittata*. All pollinations resulted in substantially fewer pollen tubes in the style than pollen grains on the stigma surface. Number of pollen tubes showed a characteristic attrition, particularly by the midpoint of the style in all pollination types. Two classes of illegitimate pollination could be identified. In pollinations with pollen smaller than the legitimate size class for a given morph, pollen tubes ceased growth in characteristic regions of the style. In pollinations with pollen larger than the legitimate size class, pollen tubes entered the ovary. In the latter class of pollination, pollen tube behavior in the M morph was similar to that of legitimate pollinations. In the S morph illegitimate pollen tube behavior was distinctive. Pollen tubes entered the ovary but rarely contacted the obturator most often growing down the funiculus of the ovule and terminating growth at its base. The associations between pollen size, pollen tube length and width, and callose plug length regardless of pollination type suggest that incompatibility in *P. sagittata* is governed by the interaction of heteromorphic characters rather than by an active rejection mechanism mediated by molecular specificities. The distinctive features of incompatibility in *P. sagittata* are discussed in relation to structural features of the pollen tube pathway, incongruity mechanisms, and gametophytic incompatibility in monocotyledonous families.

Information on pollen tube growth is limited in heterostylous plants in comparison with that available for taxa with homomorphic incompatibility. The available data indicate fundamental differences in the functioning of self-incompatibility between heteromorphic and homomorphic self-incompatibility systems (Gibbs, 1986; Barrett, 1988b; Lloyd and Webb, In press a). In homomorphic sporophytic systems, rejection characteristically occurs on the stigma surface through either the inhibition of pollen adhesion and hydration or pollen tube growth before stigma penetration (Knox, 1984; Gaude and Dumas, 1987; Elleman et al., 1989). In contrast, in distylous species the site of rejection differs between the floral morphs. Most typically, in the long-styled morph, cessation of growth of illegitimate pollen tubes occurs in the stigma or style, whereas in the short-styled morph inhibition results from failures in pollen adhesion, germination, and pollen tube entry into the stigma (Gibbs, 1986; Richards, 1986). Differences in the nature of self-incompatibility reactions between heteromorphic and homomorphic systems support the view that they have independent evolutionary origins (Charlesworth, 1982).

There has been little detailed work on the sites of pollen tube inhibition in the tristylous families Lythraceae, Oxalidaceae, and Pontederiaceae. Observations of pollen tube growth in tristylous species are of particular interest because of their unique intrafloral variation in pollen characteristics. Pollen from the two anther levels within a flower differ in size, number, and incompatibility behavior (Darwin, 1877; Dulberger, 1970; Weller, 1976; Barrett, 1977). In *Lythrum salicaria* (Kostoff, 1927; Esser, 1953) and *L. junceum* (Dulberger, 1970) inhibition of pollen tubes in the stigma or upper part of the style in illegitimate pollinations has been reported. Pollen tubes also appear to be inhibited in the stigma or upper part of the style in *Oxalis* spp. (Weller, 1975). In contrast, in *Pontederia sagittata* and *P. cordata*, cessation of pollen tube growth occurs in both the style and ovary (Glover and Barrett, 1983; An-

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derson and Barrett, 1986). The occurrence of "late-acting incompatibility" (Seavey and Bawa, 1986) in the Pontederiaceae is unique to species with heteromorphic incompatibility. Whether ovarian inhibition in *Pontederia* spp. involves pre- or postzygotic phenomena is unknown.

Scribailo and Barrett (1991) noted the close similarity in the structural features of the pollen tube pathway in *Pontederia sagittata* to that of several monocotyledonous taxa with gametophytic self-incompatibility (e.g., in the Liliaceae, Iridaceae, and Commelinaceae). Work on self-incompatible genera in the Liliaceae in particular has indicated that the sites of pollen tube inhibition are often similar to that reported in *Pontederia,* occurring in both the style and ovary (see Seavey and Bawa, 1986). This observation and the possible phylogenetic affinities of Liliaceae and Pontederiaceae (see Eckenwalder and Barrett, 1986) suggested that similarities in the structural properties of the pollen tube pathway may be important in determining the nature of heteromorphic incompatibility behavior in the Pontederiaceae.

In this paper we describe the general properties of self-incompatibility in tristylous *Pontederia sagittata.* The specific questions addressed in this study were: Is failure to set seed in illegitimate pollinations where pollen tubes regularly enter the ovary attributable to pre- or postzygotic mechanisms? What are the patterns of pollen tube growth in the various legitimate and illegitimate pollen-pistil combinations? Are distinct pollen-pistil behaviors identifiable, and what cytological features distinguish illegitimate from legitimate pollinations? Is there evidence supporting the view of a functional role for floral heteromorphism in mediating incompatibility behavior in *P. sagittata*?

## MATERIALS AND METHODS

***Plant material***—Information concerning the source of plant material used in this study and its glasshouse culture is given in a companion paper (Scribailo and Barrett, 1991). For information on the tristylous syndrome of *P. sagittata,* details of the reproductive ecology of populations, and data on seed set following controlled legitimate and illegitimate pollinations, see Glover and Barrett (1983).

***Embryo abortion***—To investigate whether postzygotic abortion, rather than prezygotic inhibition of incompatible pollen tubes, could explain the reduced seed set in L × m, M × l, S × l, and S × m self-pollinations (style morph, uppercase × pollen size, lowercase), comparative studies of embryo and endosperm development were undertaken. Only these pollinations were examined, since observations by Glover and Barrett (1983) and our own preliminary observations indicated these were the only illegitimate pollinations in which pollen tubes entered the ovary.

The technique of Stelly et al. (1984) for studies of megasporogenesis was modified for studies of early embryo development in *P. sagittata.* In order to establish a time course for normal embryo development, ovules were dissected from legitimately pollinated flowers at time intervals extending from 24 hr to 14 d after pollination. Ovules were then transferred through a graded ethanol series into several changes of water and stained for 2 d in Mayer's haemalum, a regressive haematoxylin stain (Sass, 1958). Ovules were destained from 1 to 3 d in 5% acetic acid depending on their staining intensity. Ovules were then transferred through a graded ethanol series into several changes of absolute alcohol, and through 2:1 and 1:2 absolute acohol : methyl salicylate into several changes of methyl salicylate. Ovules were mounted in methyl salicylate on glass well slides, and examined to determine the status of embryo development, using a Reichert Polyvar microscope.

Seventy-five ovaries of each of the illegitimate self-pollinations were collected after 10 d and processed as above. Observations of seed development indicated that it was possible to clearly distinguish aborted embryos from normal seed development at this time interval.

***Controlled pollination program***—Hand-pollinations were conducted under glasshouse conditions between 9:00 and 11:00 a.m. Fine netting was placed over open windows of the glasshouse to ensure a pollinator-free environment. Plants were chosen on the basis of their high inflorescence production and the occurrence of a minimum of four ramets of each genotype. These features ensured a high probability that all six genotypes would be flowering simultaneously.

In *P. sagittata* only pollinations between pollen and stigmas at the same height (legitimate pollinations) result in full seed set. All other pollen-pistil combinations (illegitimate pollinations) result in reduced seed set to varying degrees (Glover and Barrett, 1983). To investigate pollen tube growth in legitimate and illegitimate pollinations, two genotypes of each morph were selfed and crossed in all pollen-pistil combinations. Initial analysis of data on pollen tube growth by morph indicated no sig-

nificant differences in the behavior of self, intramorph, and intermorph pollen tubes for illegitimate pollinations with pollen from a given anther level. Therefore, self, intramorph, and intermorph pollinations with a particular pollen type were combined into a single category. This resulted in nine basic pollen-pistil combinations—L × l, L × m, L × s, M × l, M × m, M × s, S × l, S × m and S × s.

Pollen tube growth following the nine different pollen-pistil combinations was observed after three time intervals (2, 6, 48 hr). Pistils were collected after each time interval and fixed in 3:1, 95% ethanol to glacial acetic acid. Preliminary investigations indicated that 2 and 6 hr were appropriate time intervals to examine differential pollen tube behavior between legitimate and illegitimate pollinations (also see Glover and Barrett, 1983). The 48 hr time interval was chosen to observe final sites of pollen tube arrest, because all pollen tube growth had ceased by this time.

The four treatments for a given pollination type and time interval were conducted on the same day on a single recipient inflorescence, or two simultaneously flowering inflorescences of the same genotype. Flowers acting as maternal and paternal parents for each pollination treatment were selected to represent a range of positions within inflorescences on each day of pollination. All pollinations were conducted using fine forceps and a single anther from the male donor per stigma.

Flowers of the L and M morph can be pollinated without difficulty; however, pollinations of the S morph require special manipulation because of their concealed stigmas. Fine forceps were inserted between the upper and lower halves of the perianth tube at the base of the flower just below the stigma. The upper half of the perianth was then removed to expose the stigma for pollination. Preliminary trials with this technique indicated no adverse effects on pollen tube growth or seed set.

To quantify pollen tube growth several different measurements were made. Pistils were first fixed, cleared, and stained in aniline blue (see below). Measurements were then made on five pistils for each pollination type and genotype resulting in a total of 40 pistils per pollination treatment. At the 2 hr time interval, only the length of the longest pollen tube and number of pollen grains on the stigma could be quantified owing to the relative weakness of callose fluorescence.

At the 6 hr and 48 hr time intervals, increased callose deposition allowed quantification of the length of the longest pollen tube and the total number of pollen tubes at the top, middle, and base of the style, in the ovary, and contacting the obturator. Although fertilization was observed, it was not always possible to reliably detect and, therefore, was not included as a measurement. The number of pollen tubes entering sterile stylar canals was recorded at the midpoint of styles. Because pollen tubes were most clearly observed at 6 hr, several additional characteristics were measured at this time interval. The length of five callose plugs and width of five pollen tubes at the top of the style were recorded for each pistil. Preliminary observations indicated that these two parameters did not vary throughout the length of the style.

***Pollen tube staining***—Pistils were most effectively cleared by exposure to 2 M sodium hydroxide at 55 C for 8 hr. Solubilized tannins and raphides were then leached from the tissue during transfers through several overnight changes of water. For pollen tube staining, aniline blue (Polyscience, CI 42755) was prepared as a 0.1% solution in anhydrous 0.1 M tribasic potassium phosphate (pH 11.6) with 20% glycerin added to facilitate storage of slides without desiccation (Dumas and Knox, 1983). Pistils were mounted in a drop of stain, coverslips were gently lowered onto the tissue to prevent distortion of the ovary, and slides were left in the dark overnight to allow maximum fluorescence to develop. Slides were viewed on a Zeiss Universal Axioplan photomicroscope equipped with epi-fluorescence. A Zeiss No. 2 ultraviolet filter system consisting of exciter G-365, dichromatic beam splitter Ft-395, and barrier LP-420 was chosen to maximize excitation and emission of the aniline blue fluorochrome (Smith and McCully, 1978; Evans, Hoyne, and Stone, 1984).

Some dissected ovaries were stained for light microscopy with 5 mg of lacmoid and 5 mg of martius yellow dissolved in 15 ml of distilled water. The stain was filtered before use, and pistils were mounted in stain (Nebel, 1931).

***Scanning electron microscopy (SEM)***—To obtain more detailed observations of pollen tube growth, preparation of samples for SEM were made in the following manner. For SEM of the stigma surface, a series of pistils of each morph was pollinated either with one pollen type or a mix of the three pollen types. Pistils were then collected after 6 hr and fixed in 2% glutaraldehyde in phosphate buffer (pH 6.8) for 6 hr, and washed four times for 5 min in 0.05 M phosphate buffer. For examination of pollen tubes in the ovary, pistils were fixed 24 hr after pollination as described above. Once in buffer,

ovaries were placed under a Zeiss SV-8 stereomicroscope and dissected to expose the ovule using fine forceps and a microdissection blade.

After washing in buffer, pistils for stigmatic and ovarian observations were processed through a graded ethanol series into several changes of absolute ethanol. Tissue was critical-point dried using carbon dioxide as the intermediate fluid, mounted on metal stubs, and coated with gold-palladium for 5 min in a Hummer VI sputter-coater. Samples were then viewed on a Hitachi Model S570 scanning electron microscope set at 10 kV.

***Data analysis***—Data sets were tested for homogeneity of variances using Hartleys $F_{max}$-test (Sokal and Rohlf, 1981). Variables with heterogeneous variances were either log- or square root-transformed. After ANOVA means were compared with Scheffe's procedure at the 0.05 level (SAS version 6, 1987). For variables with heterogeneous variances, a Kruskal-Wallis test was performed after transformation (SAS version 6, 1987). Mean ranks were then compared using Dunn's multiple comparisons procedure at the 0.05 level (Hollander and Wolfe, 1973).

***Photography***—All light microscopy photographs of pollen tubes were taken on a Zeiss Universal Axioplan photomicroscope using Kodak 135 T-MAX 400 ASA film. For SEM Ilford 120 FP-4 roll film was used. Photographs of embryo development were taken on a Reichert Polyvar microscope using Kodak 135 Tech Pan film to enhance contrast.

## RESULTS

***Normal embryogenesis***—The normal stages of embryo and seed development are shown in Figs. 1–5 and are described below. At specific times after pollination, ovaries from all floral morphs exhibited close synchrony in stages of embryo and endosperm development. At 24 hr after pollination, zygotes or two-celled embryos were visible (Figs. 1, 2). By 3 d both of the cells shown in Fig. 2 had divided, one transversely and the other longitudinally, to form a four-celled proembryo (Fig. 3). By 6 d multicellular globular proembryos were evident (Fig. 4). At 10 d after pollination embryos had undergone considerable elongation and differentiation (Fig. 5). At this stage the epicotyl and hypocotyl of the cotyledon were clearly visible. In addition, the cotyledonary sheath enclosing the embryonic shoot apex and the rudimentary vascular supply were also evident. The rapid growth of endosperm at the cellular phase of development began to obscure the embryo at this time. At 14 d after pollination embryos had enlarged considerably and were close to maturity. Seeds matured 16–21 d after pollination.

***Embryo abortion***—Seventy-five ovaries were examined for status of embryo development for each illegitimate pollination where pollen tubes were known to enter the ovary. With the exception of M × l pollinations where seed set is high, the majority of ovaries showed no evidence of fertilization and appeared identical to ovaries of unpollinated flowers used as controls. In M × l pollinations 58 ovaries showed normal embryo development when compared with legitimate controls. Five embryos were aborted and the remainder were unfertilized. In S × m pollinations 21 ovaries showed normal embryo development, two were aborted, and the remainder were unfertilized. In S × l and L × m pollinations four and seven ovaries showed normal development, respectively. No abortions were observed in either pollination, and the remaining 71 and 68 ovaries, respectively, showed no evidence that fertilization had occurred.

Three examples of aborted embryos are illustrated in Figs. 6–8. Abortion of embryos always occurred at the globular stage of development. In Fig. 6 no endosperm development is visible, while in Fig. 7 only strands of endosperm are apparent (arrow). In Fig. 8 endosperm development has progressed further and is arrested at an intermediate stage of free nuclear division. Note the collapse of the walls of seeds in Figs. 6 and 7, and collapse of the embryo sac in Fig. 8. These observations indicate that embryo abortion is infrequent in *P. sagittata*. Postzygotic mechanisms cannot, therefore, account for the low levels of seed set obtained from illegitimate pollinations in which growth of pollen tubes into the ovary occurs.

***Pollen adhesion and germination***—Observations indicated the presence of numerous pollen grains on the stigma surface in the majority of pollinations regardless of whether they were legitimate or illegitimate. Measurements of the numbers of pollen grains on the stigma among the three time intervals indicated no significant differences between the 6 hr and 48 hr data. However, for all pollen-pistil combinations (except L × l) there were significantly fewer pollen grains on stigmas at the 2 hr time interval (Table 1). In some L × m (8) and L × s (13) pollinations at the 2 hr time interval, and in all pollinations involving the S morph

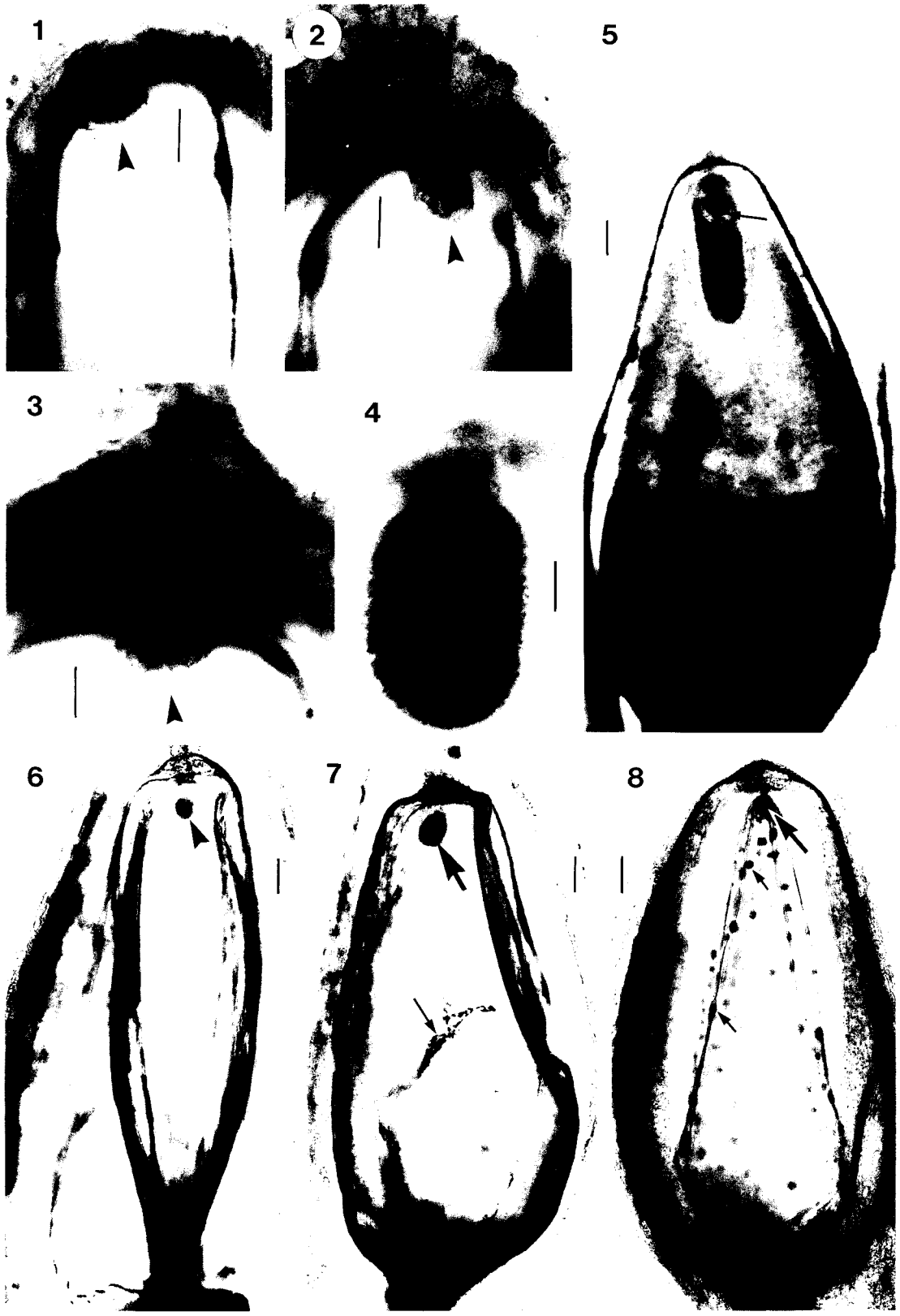
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TABLE 1. *Mean number of pollen grains on each stigma and standard deviation for nine pollen-pistil combinations and three time intervals after controlled pollination of* Pontederia sagittata. *After a Kruskal-Wallis test, ranks were compared using Dunn's multiple comparisons procedure. Letter superscripts refer to comparisons within a time interval; numeric superscripts refer to comparisons within a pollination treatment. Means sharing the same superscript are not significantly different ($P > 0.05$; $N = 40$). No pollen was present on S stigmas examined after 2 hr. Means for treatments involving s and m pollen have been divided by a constant (s: 4.52; m: 1.17) to correct for pollen production differences in the anther levels of* P. sagittata *(see Glover and Barrett, 1983)*

| Pollination treatment | Time interval | | |
|---|---|---|---|
| | 2 hr | 6 hr | 48 hr |
| L × l | $57.4^{A1} \pm 24.9$ | $67.2^{AB} \pm 18.2$ | $71.8^{AB} \pm 34.0$ |
| L × m | $20.0^{B1} \pm 24.5$ | $100.7^{A2} \pm 53.9$ | $101.5^{A2} \pm 39.9$ |
| L × s | $36.7^{B1} \pm 35.7$ | $66.1^{AB2} \pm 36.6$ | $50.0^{BC2} \pm 27.2$ |
| M × m | $41.5^{AB1} \pm 20.3$ | $97.8^{A2} \pm 48.9$ | $61.0^{BC2} \pm 32.9$ |
| M × l | $24.6^{B1} \pm 17.6$ | $46.8^{BC2} \pm 18.7$ | $65.3^{AB2} \pm 27.6$ |
| M × s | $19.5^{B1} \pm 10.7$ | $58.9^{ABC2} \pm 26.4$ | $57.9^{BC2} \pm 33.0$ |
| S × s | — | $23.8^{C1} \pm 11.4$ | $28.8^{D1} \pm 12.6$ |
| S × m | — | $29.4^{BC1} \pm 16.5$ | $44.0^{CD1} \pm 30.3$ |
| S × l | — | $19.9^{C1} \pm 15.8$ | $20.7^{D1} \pm 9.4$ |

at this time interval, stigmas were observed with no adhering pollen. These observations indicate that pollen grains had either failed to adhere and germinate or pollen tubes of germinated grains had not entered the stylar canal prior to collection and were lost during processing. The presence of smaller numbers of pollen grains on stigmas after 2 hr suggests that pollen germination take place over several hours but is completed by 6 hr.

Corrected values for the number of pollen grains on the stigma, taking into account differences in pollen production per anther level (see Glover and Barrett, 1983), do not indicate preferential adhesion of legitimate pollen over illegitimate pollen in any of the morphs (Table 1). Stigmas of the S morph capture significantly fewer pollen grains than stigmas of the L and M morph in both legitimate and illegitimate pollinations, regardless of time interval. For example, large numbers of s pollen grains were visible on stigmas of the L morph after L × s pollinations (Fig. 9), whereas in S × m pollinations (Fig. 11), and S × l (Figs. 10, 13) pollinations in particular, very few pollen grains were present. The observed differences suggest a reduced ability of pollen grains to adhere to the smaller stigmas of the S morph.

Pollen germination was high, with large numbers of pollen tubes growing into the stigma in all legitimate pollinations and most illegitimate pollinations (Fig. 12). Because of the large number of pollen grains present on stigmas, particularly in pollinations of the L and M morphs, it was nearly impossible to quantify pollen germination with any precision.

In L × s, L × m, and M × s pollinations at 6 and 48 hr, a larger percentage of ungerminated pollen grains was observed compared with legitimate pollinations of these morphs. In addition, some pollen tubes were observed growing randomly on the stigma surface exhibiting a lack of directional growth into the stigma (Fig. 14). Figure 15 illustrates the behavior of l, m, and s pollen grains on the stigma of the L morph. Note the lack of germination of s pollen grains.

Only one illegitimate pollination (M × l) consistently exhibited aberrations of pollen tube growth in the stigma. Some pollen tubes were wider than normal with callose plugs of increased thickness (Fig. 16, small arrow). Pollen tubes were often observed to swell at the tip region before ceasing growth (Fig. 16, large arrow). Paradoxically, the M × l cross is the most compatible illegitimate pollination in *P.*

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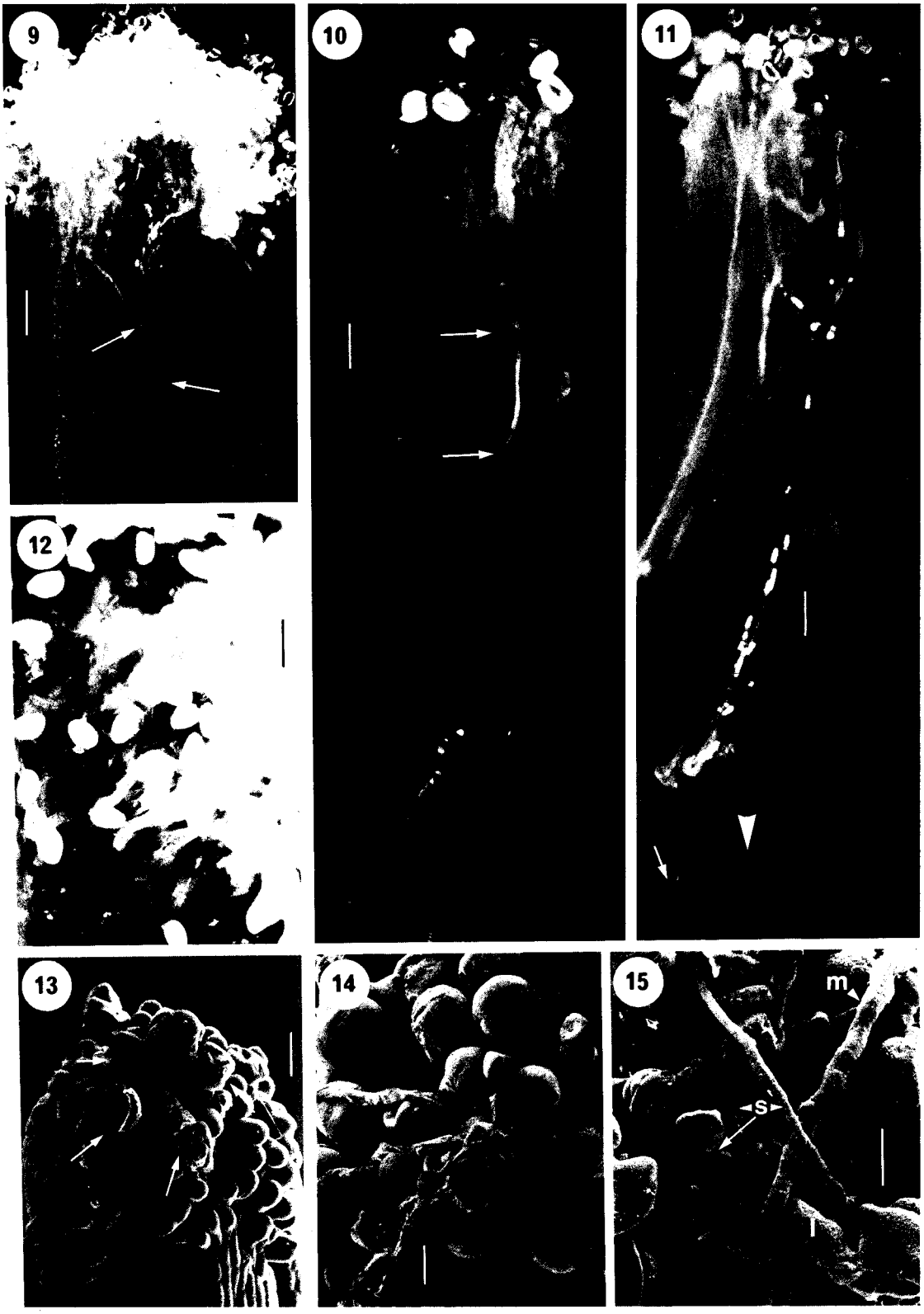
Figs. 1–8. Embryo development and abortion in *Pontederia sagittata.* LM micrographs. Figs. 1–5. Stages of embryo development. **1.** Zygote (arrowhead) 24 hr after pollination. Bar = 5 μm. **2.** Two-celled embryo (arrowhead) 2 d after pollination. Bar = 5 μm. **3.** Four-celled proembryo (arrowhead) 4 d after pollination. Bar = 5 μm. **4.** Globular stage embryo 6 d after pollination. Bar = 5 μm. **5.** Embryo 10 d after pollination. The cotyledonary sheath enclosing the shoot apex is clearly visible (arrow). Extensive cellular endosperm is visible below the embryo. Bar = 20 μm. Figs. 6–8. Aborted seeds collected 10 d after pollination. Note that the embryos (large arrowhead and large arrows) have aborted at the globular stage of development. Bars = 20 μm. **6.** No endosperm development has occurred. **7.** Strands of endosperm are visible (small arrow). **8.** Endosperm development has stopped at the free-nuclear stage (small arrows). Note the collapse of the walls of the seed in Figs. 6 and 7, and the collapse of the embryo sac in Fig. 8.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*sagittata*, setting an average of 73% seed (Glover and Barrett, 1983).

***Legitimate pollen tube growth in the style***— After entering the stylar canal, pollen tubes of all legitimate pollinations showed similar growth characteristics. Pollen tubes formed a tightly clumped mass as they grew down the style (Fig. 18). Pollen tubes were of uniform width within and between levels in the style (Fig. 17). Comparisons of pollen tube widths in legitimate pollinations at the 6 hr time interval indicated that pollen tubes of L pollen are significantly wider than pollen tubes of S pollen, with pollen tubes of M pollen being of intermediate width (Fig. 43) (compare s pollen tubes [Fig. 9] with m [Fig. 11] and l pollen tubes [Fig. 10]). Frequency of callose plugs did not appear to change through the length of the style in legitimate pollinations.

Pollen tube width and callose plug length increased in size from the 2 to 6 hr time interval but not at the 48 hr time interval. Pollen tubes at 2 hr exhibited less wall fluorescence than at later time intervals, indicating that callose continues to be deposited in pollen tubes in regions some distance back from the pollen tube tip. At 6 hr callose plug lengths in S × s pollinations were significantly shorter than those in M × m and L × l pollinations (Fig. 44).

Comparisons between the number of pollen grains deposited on the stigma (Table 1) and the number of pollen tubes at the top of the style (Fig. 41) indicate that pollen tubes from many pollen grains fail to reach the stylar canal. This pattern is particularly evident in L × s and M × s pollinations (see next section) but is also present in legitimate pollinations. In the L morph 35% of the legitimate pollen grains deposited on stigmas produced pollen tubes in the style vs. 29% in the M morph and 14% in the S morph at the 48 hr time interval.

Figure 41 shows the number of pollen tubes reaching each level in the style for the three pollination treatments of each morph at 48 hr. Despite the occurrence of large numbers of germinating pollen grains on the stigma surface, relatively few pollen tubes were observed in the style. In legitimate pollination of the L and M morphs approximately 20%–25% of the pollen tubes present at the top of the style failed to reach the midpoint of the style (Fig. 41). In contrast, in legitimate pollinations of the S morph 90% of the pollen tubes reach the midpoint of the style. Few additional pollen tubes ceased growth between the midpoint of the style and the ovary in all legitimate pollinations.

Legitimate pollen tubes that terminate growth in the upper part of the style exhibited several types of abnormal behavior. This was most commonly characterized by swelling of pollen tubes, absence of callose plugs (Figs. 22–26), and an increase in particulate callose within the pollen tube causing the entire pollen tube to fluoresce (Figs. 26–30). Loss of pollen tube directionality was also common, although this phenomenon varied considerably among styles (Figs. 22–24, 26). Coiling of pollen tubes was usually followed by cessation of growth shortly thereafter, although this did not involve bursting of pollen tube tips.

Abnormal behavior followed by the cessation of growth of large numbers of pollen tubes was correlated with the zone in which the trilobed stylar canal separates into three stylar canals with only one leading to a fertile carpel (Scribailo and Barrett, 1991). A significant reduction in the number of pollen tubes was visible below the point of stylar canal separation (Fig. 2). Since little pollen tube growth occurs in styles after 6 to 8 hr due to stylar senescence, pollen tubes present in the upper region of the style must have terminated growth. By the midpoint of the style the majority of remaining pollen tubes were found in the fertile stylar canal (Fig. 19, large arrow; Fig. 20).

An average of one-third of the total pollen

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Figs. 9–15. Pollen-pistil interactions in *Pontederia sagittata*. Figs. 9–12. LM micrographs. Figs. 13–15. SEM micrographs. **9.** Pollen tube growth in the style 6 hr after M × s pollination. Note the large number of pollen grains on the stigma surface and the small number of pollen tubes in the style (arrows). Bar = 20 μm. **10.** Pollen tube growth in the pistil 6 hr after S × l pollination. Note the two swollen pollen tubes in the style that have ceased to grow (arrows). Bar = 20 μm. **11.** Pollen tube growth in the pistil 6 hr after S × m pollination. Two pollen tubes are in contact with the obturator which has become distorted away from the micropyle of the ovule. Note the smaller size of pollen grains and their increased number on the stigma in comparison to Fig. 10. Bar = 20 μm. **12.** Pollen tubes growing into the stigma 6 hr after M × m pollination. Bar = 10 μm. **13.** Small numbers of pollen grains on the stigma of the S morph 6 hr after pollination with l pollen. Note the large size of the pollen grains (arrows) relative to the size of the stigmatic papillae. Bar = 20 μm. **14.** Short and mid pollen tubes showing a lack of directional growth into the stigma of the L morph 6 hr after pollination. Bar = 10 μm. **15.** Growth of the three pollen types on the stigma of the L morph. Note the increase in pollen tube diameter from the short (s) to mid (m) to long (l) pollen type (arrows). Bar = 10 μm.

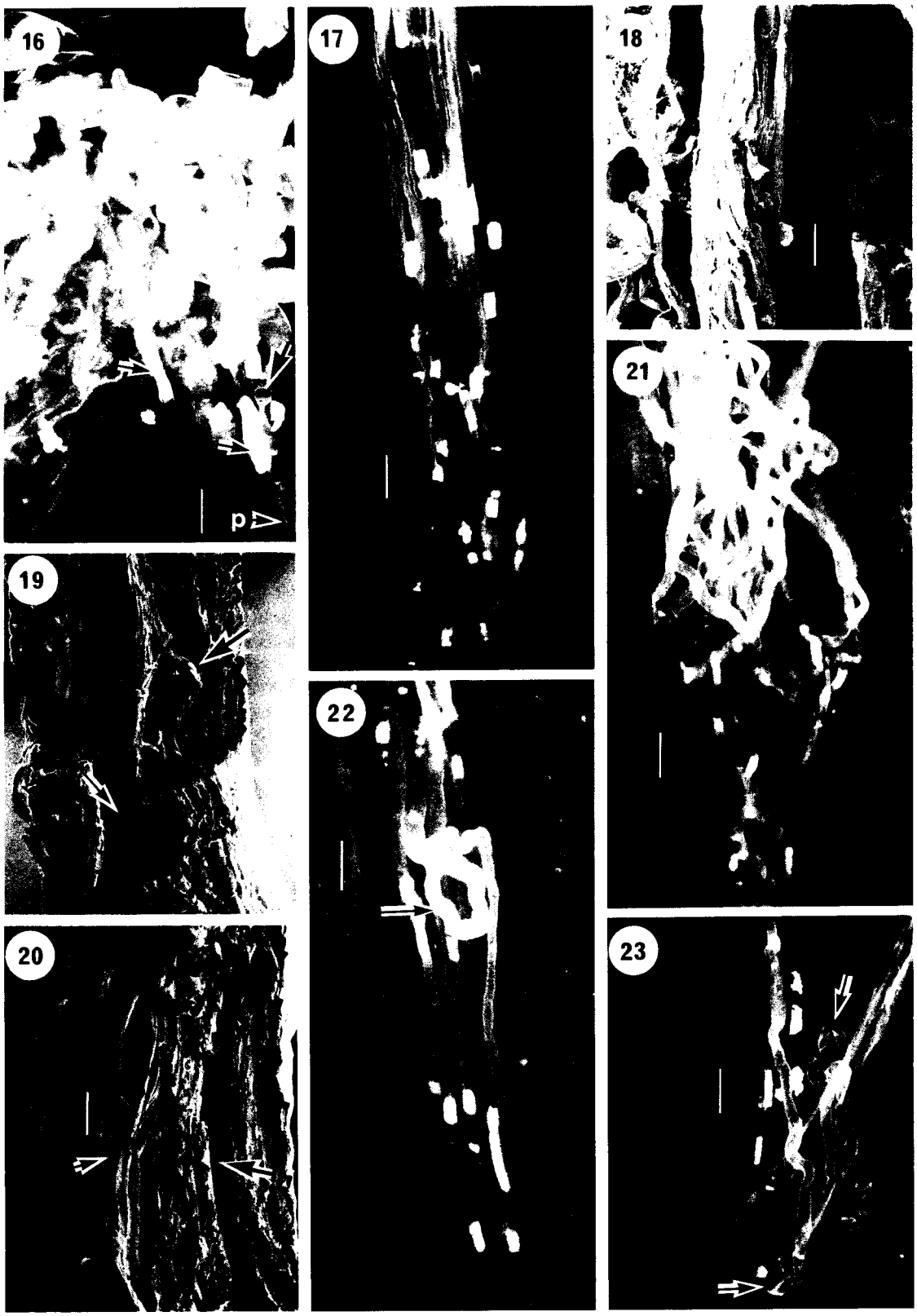
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TABLE 2. *Maximum number of pollen tubes that can be accommodated into the fertile stylar canal for each pollen-pistil combination in* Pontederia sagittata. *Values are based on calculated cross-sectional areas of pollen tubes using data for pollen tube diameter (± standard deviation). Canal cross-sectional areas (± standard deviation) are taken from the mid-level in the pistil using data from Scribailo and Barrett (1991). The mid-level in the style represents the zone in the pistil where the single stylar canal separates into three canals with only one canal (fertile) leading to the fertile carpel*

| Pollination treatment | Pollen tube diameter ($mm \times 10^{-3}$) | Pollen tube area ($mm^2 \times 10^{-5}$) | Fertile canal area ($mm^2 \times 10^{-3}$) | Number of pollen tubes |
|---|---|---|---|---|
| L × l | 8.25 ± 1.80 | 5.41 | 5.20 ± 1.11 | 96 |
| L × m | 7.25 ± 2.23 | 4.13 | 5.20 ± 1.11 | 127 |
| L × s | 3.77 ± 1.41 | 1.13 | 5.20 ± 1.11 | 460 |
| M × m | 8.86 ± 1.16 | 6.22 | 4.34 ± 0.89 | 70 |
| M × l | 10.27 ± 2.88 | 8.33 | 4.34 ± 0.89 | 52 |
| M × s | 5.69 ± 1.77 | 2.55 | 4.34 ± 0.89 | 170 |
| S × s | 5.27 ± 0.91 | 2.21 | 2.65 ± 0.50 | 120 |
| S × m | 8.15 ± 2.32 | 5.28 | 2.65 ± 0.50 | 50 |
| S × l | 10.38 ± 3.32 | 8.49 | 2.65 ± 0.50 | 31 |

tubes in the style should enter each stylar canal, assuming that the distribution of pollen grains on the stigma is random. Observations of relatively uniform pollen grain distribution on the three lobes of pollinated stigmas suggest that this assumption is likely a reasonable one.

Quantitative data indicate that considerably less than two-thirds of the total pollen tubes enter the two sterile stylar canals. This percentage did not vary significantly between the floral morphs, averaging 20.8% in the L morph, 25.9% in the M morph, and 27.2% in the S morph. These values are based on data pooled from both legitimate and illegitimate pollinations.

Calculations of the maximum number of pollen tubes that can be accommodated within the fertile stylar canal at the mid-level of the style are given in Table 2. These are based on stylar canal cross-sectional area data in Scribailo and Barrett (1991), pollen tube diameter measurements for each pollination type (Fig. 43), and an assumption that the total free area of the canal is available for pollen tube growth. Comparisons of these estimates for legitimate pollinations, with the observed numbers of pollen tubes at the middle of the style (Fig. 41), indicate a smaller number of pollen tubes are present in the style than complete packing would theoretically allow. This suggests that stylar clogging cannot explain the reduction in number of pollen tubes in styles of *P. sagittata.*

***Illegitimate pollen tube growth in the style*—** Number of pollen tubes increased from the 2 to 6 hr time interval in all illegitimate pollinations, but did not show a further increase from the 6 hr to 48 hr interval. With the exception of some pollen tubes in M × l pollintions, illegitimate pollinations did not display more aberrant pollen tube behavior than in legitimate pollinations.

Measurement of pollen tube diameter showed a stronger correlation with pollen type than with whether a pollination was legitimate or illegitimate (Fig. 43). Differences in pollen tube diameter between the pollen types are significant when data are pooled by pollen type across legitimate and illegitimate pollinations. Pollen tubes of s pollen are narrowest, l pollen tubes

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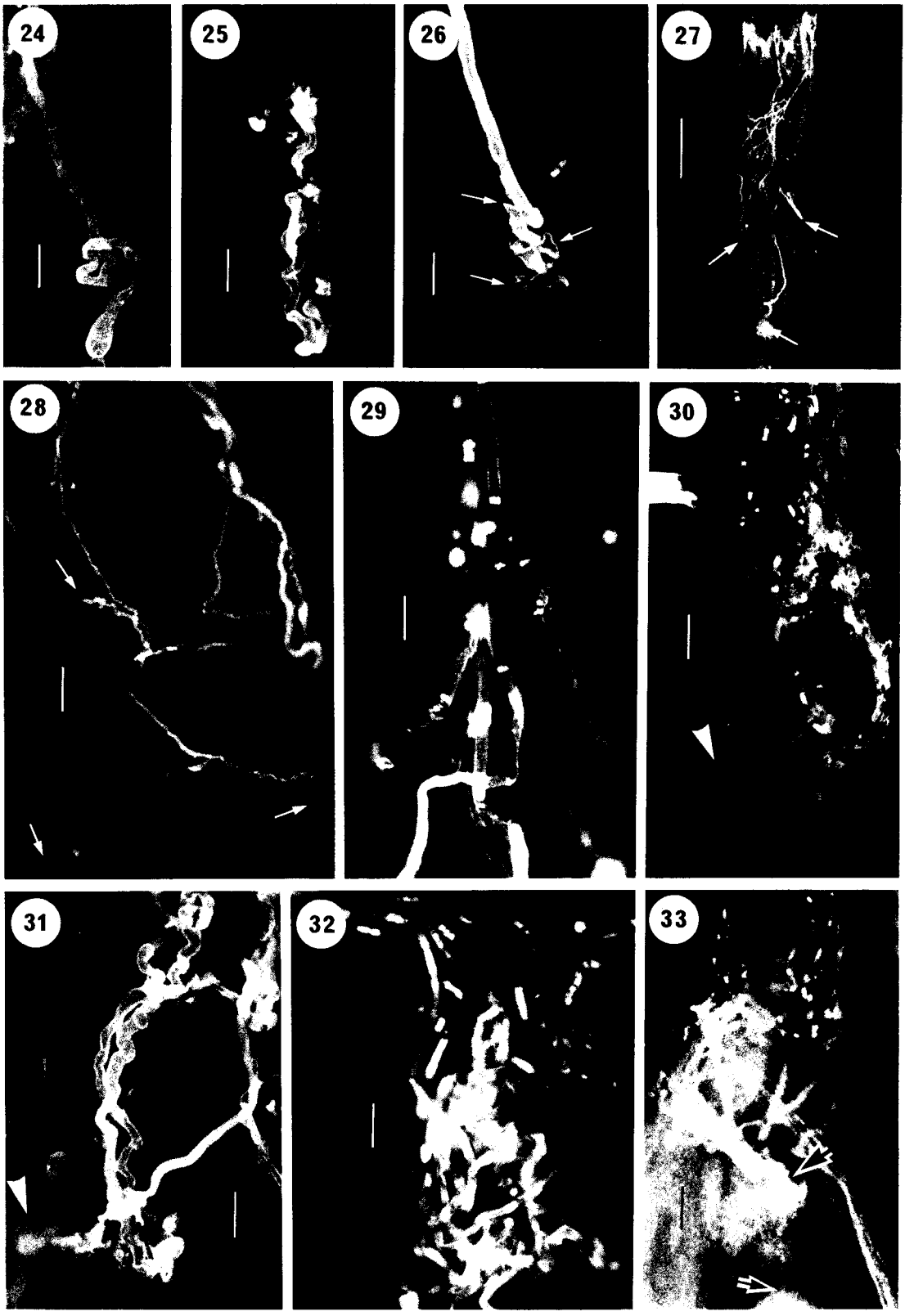
Figs. 16–23. Pollen tube growth in the style of *Pontederia sagittata.* Figs. 16–18, 21–23. **16.** Inhibition of l pollen tubes in the stigma of the M morph 6 hr after pollination. Note the thick callose plugs (small arrows), the swollen tip of the pollen tube to the right (large arrow), and the tip of a pollen tube that has ceased growth (p). Bar = 10 μm. **17.** M pollen tubes in the style of the L morph 6 hr after pollination. Note the uniform width of the pollen tubes and callose plugs laid down at intervals. Bar = 10 μm. **18.** Mid pollen tubes growing down the style of the S morph 6 hr after pollination. Note the tight clustering of pollen tubes. Bar = 5 μm. **19.** Pollen tube behavior in the style of a M × l pollination at the point of separation of the trilobed stylar canal into one fertile stylar canal and two sterile stylar canals. The majority of pollen tubes have passed into the fertile stylar canal (small arrow) while a small number have entered one of the sterile canals (large arrow). The other sterile stylar canal is not visible. Bar = 20 μm. **20.** Pollen tube growth in the style 6 hr after S × m pollination. Note the large number of pollen tubes in the fertile stylar canal to the left (large arrow) and the absence of pollen tubes in the sterile stylar canal (small arrow) to the right. Bar = 20 μm. **21-23.** Attrition of pollen tube numbers in the style 6 hr after L × m pollinations. Bars = 20 μm. Typical signs of aberrant behavior of pollen tubes associated with cessation of growth include absence of callose plugs, loss of directionality, and increased diameter. In addition, note the increased deposition of callose in Fig. 22 (arrow) and the absence of directional growth in Fig. 23 (arrows).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are widest, and m pollen tubes are of intermediate width (Fig. 43).

Considerable variation was observed in callose plug length between pollination types, although increasing thickness was not strongly associated with illegitimate pollinations (Fig. 44). In the S morph, pollen tubes from the three pollination types had the same callose plug lengths. In the L and M morphs, callose plugs of s pollen tubes were shortest, and those of M × l and L × m pollinations were longest. Pooling callose plug data by pollen type, the callose plugs of s pollen tubes are significantly narrower than those of l and m pollen tubes.

As in legitimate pollinations, the number of illegitimate pollen tubes at the top of the style represent only a small fraction of the potential number, given the number of germinating pollen grains present on the stigma. In the L morph pollen tube number at the top of the style for the 48 hr time interval (Table 1) (expressed as a percentage of uncorrected pollen grain number) decreased with pollen grain size from 15% in L × m pollinations to 7% in L × s pollinations. In the M morph the percentage of pollen tubes at the top of the style, in illegitimate M × l pollinations did not differ greatly from legitimate pollinations (20% vs. 29% respectively). In contrast, in M × s pollinations, the number of pollen tubes was only 8% of pollen grain number. Values for this parameter in illegitimate pollinations of the S morph were 22% in S × m pollinations and 44% in S × l pollinations.

In three illegitimate pollinations (L × m, L × s, M × s) the cessation of pollen tube growth occurred in characteristic regions of the style. In each case the illegitimate pollinations involved pollen of a size that was smaller than the legitimate size class of pollen for that style. The most striking feature of the data for pollen tube lengths is that in these illegitimate pollinations, lengths are not significantly different from those in the corresponding legitimate pollination using that pollen type (Fig. 42).

An obvious syndrome of pollen tube abnormalities was not associated with termination of growth. The sites of pollen tube arrest in these pollinations appear to represent the maximum distance to which pollen tubes were capable of growing. In L × s and M × s pollinations, pollen tubes penetrate the style (Figs. 27, 28; arrows) and travel a distance equivalent to that of a short pollen tube effecting fertilization during legitimate pollination. This distance is equivalent to approximately one-sixth the length of the style in the M morph, and $^1/_{12}$ the length of the style in the L morph. In L × m pollinations, pollen tubes are not significantly different in length from M × m pollinations and terminate growth at a point between the middle and base of the style.

In all illegitimate pollinations where pollen tubes grew to the base of the style or further, quantification of pollen tube numbers at different levels in the style indicated a large reduction in pollen tube number by the midpoint of the style (Fig. 41; L × m, M × l, S × l, S × m). Expressed as a percentage of pollen tubes at the top of the style for the 48 hr time interval, attrition rates for illegitimate pollen tubes in the L and M morph were significantly higher than in legitimate pollinations (20% for L × l vs. 47% for L × m; 25% for M × m vs. 58% for M × l). In the S morph 12% to 16% of pollen tubes failed to reach the midpoint of the style in both legitimate and illegitimate pollinations. Attrition in all pollinations was associated with the point of separation of the stylar canal into separate canals prior to the midpoint of the style. Only small reductions in the number of pollen tubes occurred between the midpoint of the style and the ovary in M × l, S × m, and S × l pollinations. The decline was comparable to that observed in legitimate pollinations.

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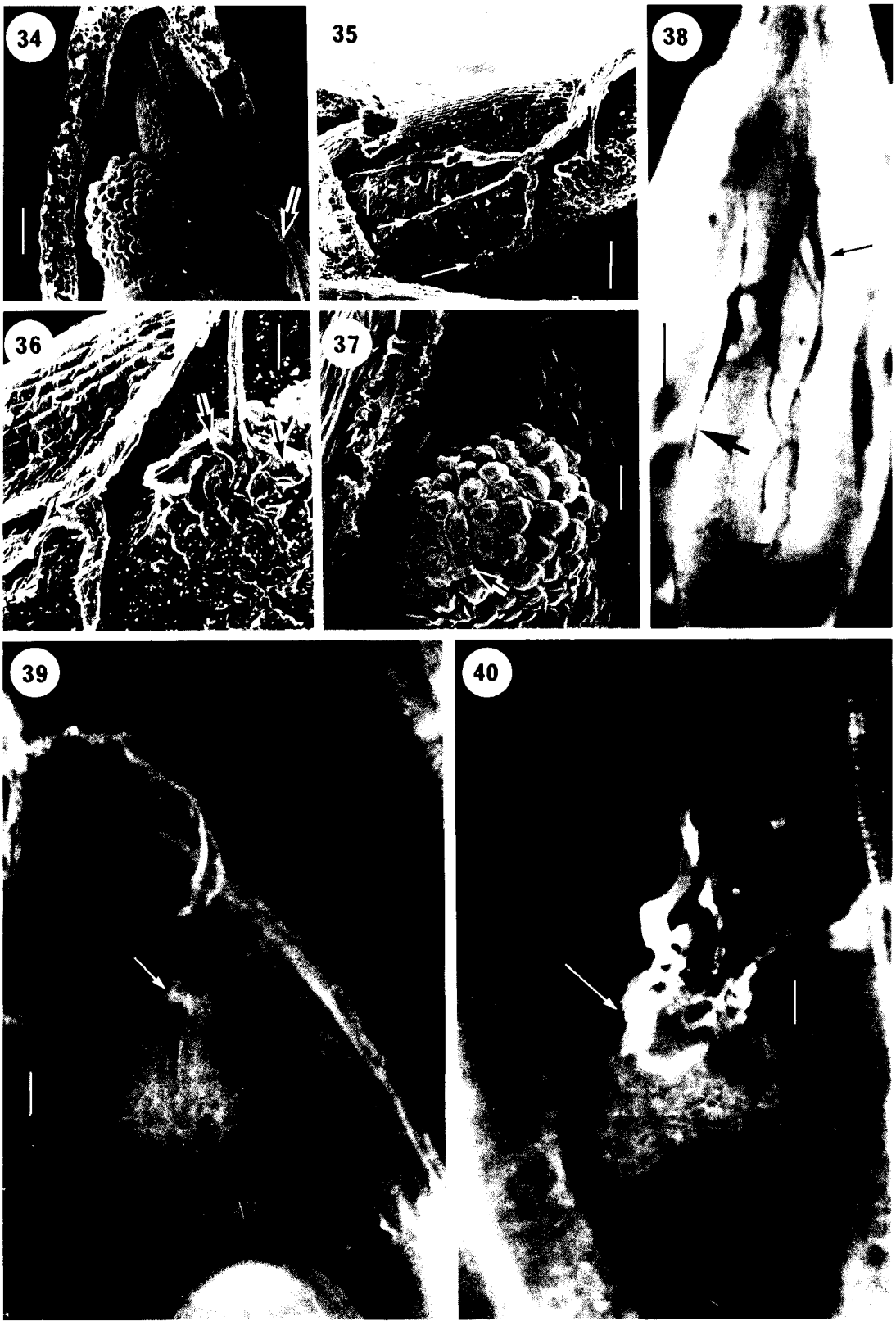
Figs. 24–33. Pollen tube growth in the style and ovary of *Pontederia sagittata*. Figs. 24–26. Bars = 10 μm. As in Figs. 21–23. Typical signs of aberrant behavior of pollen tubes associated with attrition of pollen tube numbers after L × m pollination. **24.** Swelling of the pollen tube tip. **25.** Twisting of the pollen tube. **26.** Branching of the pollen tube (arrows). **27.** Termination of pollen tube growth in the upper part of the style 6 hr after L × s pollination (arrows). Arrows indicate the tips of the pollen tubes. Bar = 100 μm. **28.** As in Fig. 27, but a higher magnification view of cessation of pollen tube growth (arrows). Bar = 10 μm. **29.** Pollen tube growth in the style at the point of entry into the ovary. Note the interspacing of pollen tubes, increased diameter, increase in particulate callose, and decrease in numbers of callose plugs toward the bottom of the micrograph. Bar = 10 μm. **30.** Pollen tube growth in the ovary 6 hr after legitimate pollination of the L morph. Note the abundance of pollen tubes contacting the obturator (arrowhead). Bar = 20 μm. **31.** As in Fig. 30, but note the increase in particulate callose in some of the pollen tubes. Pollen tubes are contacting the obturator at the lower left of the micrograph (arrowhead). Bar = 10 μm. **32.** Coiling of pollen tubes in the ovary 6 hr after M × m pollination. Note the loss of direction of growth in most of the pollen tubes. Bar = 10 μm. **33.** Fertilization 6 hr after M × m pollination (small arrow). Note the large number of pollen tubes contacting the obturator (large arrow), but only one pollen tube effecting entry of the micropyle. Bar = 20 μm.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***Legitimate pollen tube growth in the ovary*—** Pollen tubes were observed entering the ovary at the 2 hr time interval in legitimate pollinations of the L and M morph. At 6 hr, legitimate pollen tubes were observed in the ovary of the S morph. Number of pollen tubes did not increase at the 48 hr time interval for the three morphs. This observation is not unexpected since styles start to wilt by 6 hr after the beginning of anthesis. The compact mass of pollen tubes present in the fertile stylar canal (Fig. 18) tended to become more dispersed upon entry into the fertile carpel of the ovary (Figs. 29, 30). Pollen tubes in the sterile stylar canals were clearly visible in the two reduced carpels. Pollen tube width increased significantly in the ovary. Pollen tubes exhibited extensive coiling (Fig. 32) and a marked reduction in the number of callose plugs, particularly in close proximity to the obturator (Figs. 30, 31).

Although many pollen tubes were seen contacting the obturator (Figs. 30, 31, large arrow) only one per ovule was observed growing through the obturator to the micropyle (Fig. 33). Since there is no apparent opening in the obturator, pollen tubes must penetrate between the cell walls at the base of the papillate cells to enter the micropyle and effect fertilization.

In legitimate pollinations the majority of pollen tubes reaching the midpoint in the style were also present in the ovary (Fig. 41). Thus at the 48 hr time interval 68% of all pollen tubes reached the ovary in L × l pollinations vs. 64% in M × m and 79% in S × s pollinations. All pistils examined had at least some pollen tubes contacting the obturator. In L × l pollinations 52% of the total pollen tubes observed contacted the obturator vs. 47% in M × m and 61% in S × s pollinations.

***Illegitimate pollen tube growth in the ovary*—** Only three illegitimate pollinations (M × l, S × m, S × l) were observed with pollen tubes in the ovary. Pollen tube behavior in M × l pollinations was similar to that in legitimate pollinations. All pistils examined had pollen tubes contacting the obturator. Nevertheless, given that M × l pollinations set 73% and that a maximum of 7% of embryos might be expected to abort (five of 75), this indicates that fertilization does not occur 20% of the time.

In illegitimate pollinations of the S morph the number of pollen tubes in the ovary was significantly lower in S × l than in S × m pollinations (Fig. 41). This difference was due to significantly fewer pollen grains from l anthers adhering to stigmas (Table 1) and pollen tubes being present in the style (Fig. 41) in S × l pollinations.

Pollen tube behavior was distinctive in illegitimate pollinations of the S morph. In S × l pollinations 13% of pistils had pollen tubes contacting the obturator vs. 38% in S × m pollinations. In pollinations where no contact with the obturator occurred, pollen tubes bypassed the obturator continuing growth down the funiculus or ovule face and terminating growth at its base (Fig. 34, arrow; Fig. 38, large arrows). In pistils where obturator contact occurred, the majority of pollen tubes still bypassed the obturator as above but with only one to several pollen tubes reaching the obturator. In the latter cases pollen tubes often branched, flattening out against the papillae of the obturator (Fig. 35; Figs. 36, 37, arrows) and appeared unable to penetrate between the papillae and effect fertilization (Figs. 39, 40, arrows). The presence of pollen tubes growing to the base of the ovule accounts for the longer mean maximum pollen tube length in S × l and S × m pollinations when compared with S × s pollinations (Fig. 42).

Despite the observation of fewer pollen tube contacts of the obturator occurring in S × l vs. S × m pollinations, the decline in pollen tube number from the ovary to the obturator is steeper for S × m pollinations when compared with S × l pollinations (Fig. 41). This is because

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Figs. 34–40. Illegitimate pollen tube growth in the ovary of *Pontederia sagittata*. **34.** Pollen tube (arrow) behavior in the ovary 6 hr after S × l pollination. Note that pollen tubes bypass the obturator and continue growth down the funiculus. Bar = 50 μm. **35.** As in Fig. 34. The majority of pollen tubes have bypassed the obturator and terminated growth (small arrows), but two pollen tubes have made contact. Bar = 50 μm. **36.** Higher magnification view of pollen tubes on the obturator from Fig. 35. Bar = 20 μm. Note the extensive branching of the two pollen tubes (arrows) but lack of penetration of the obturator. **37.** Extensive branching of a single pollen tube on the obturator surface. The pollen tube tip is indicated with an arrow. Bar = 20 μm. **38.** As in Fig. 34. The ovule is viewed on the funicular side away from the obturator. The obturator is not visible but its location is marked by a small arrow. Note the extensive growth of pollen tubes (large arrows) on the surface of the funiculus almost to the base of the ovule. Bar = 50 μm. **39, 40.** Examples of pollen tube contact with the obturator (arrows) after S × m pollination. Pollen tubes have failed to penetrate the obturator and effect fertilization. Bars = 5 μm. Note the branching of pollen tubes before and after contact with the obturator in Fig. 40.

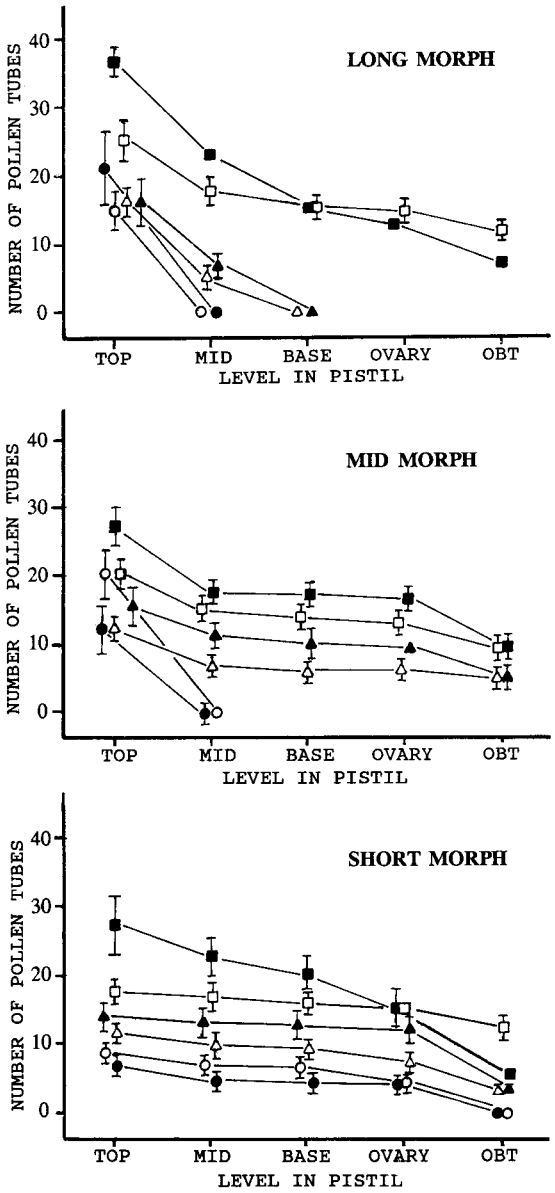
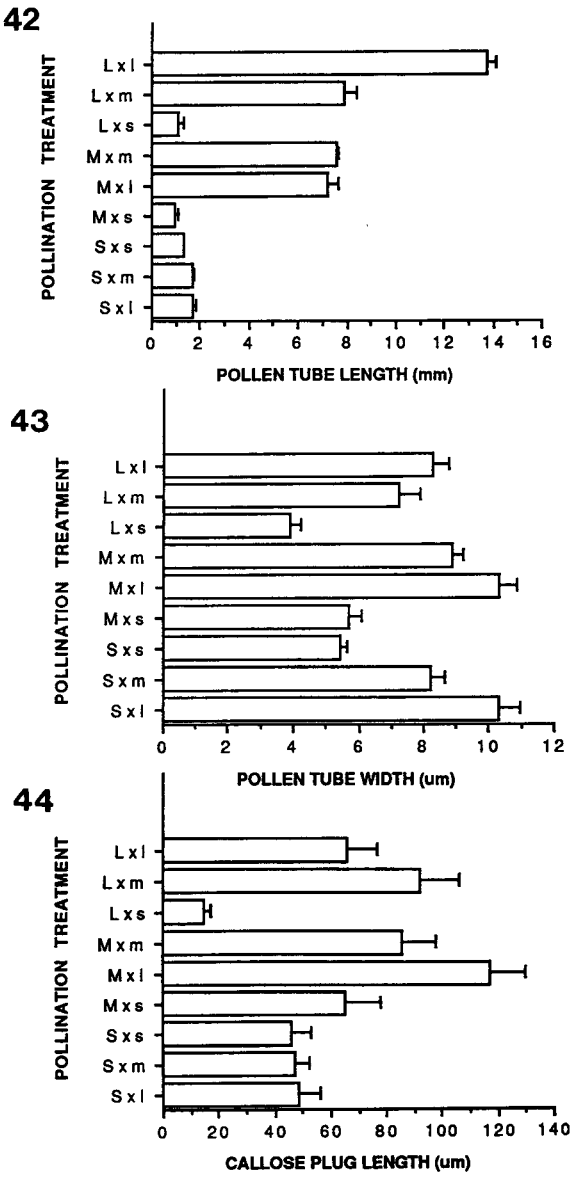



Fig. 41. Number of pollen tubes reaching each level in the pistil for the L, M, and S morphs of *Pontederia sagittata*. Means and 95% confidence intervals are shown for the three pollinations in each morph for pistils collected 6 hr (open symbols) and 48 hr (black symbols) after pollination. For each morph, legitimate pollinations are indicated by squares, the most compatible illegitimate pollinations are designated by triangles, and the most incompatible illegitimate pollinations are indicated by circles. OBT is an abbreviation for the obturator of the ovule. Distance on the X axis is not indicative of length. Each mean is based on measurements from 40 pistils.




Figs. 42–44. **42.** Mean length of the longest pollen tube (mm) and 95% confidence intervals for the nine pollination treatments collected 6 hr after pollination of *Pontederia sagittata*. Length of the longest pollen tube in the legitimate pollinations is the distance to the ovule. Means are based on measurements from 40 pistils. **43.** Pollen tube width ($\mu$m) and 95% confidence intervals for the nine pollination treatments collected 6 hr after pollination of *Pontederia sagittata*. Means are based on 200 measurements from 40 pistils. **44.** Callose plug length ($\mu$m) and 95% confidence intervals for the nine pollination treatments collected 6 hr after pollination of *Pontederia sagittata*. Means are based on 200 measurements from 40 pistils.

the majority of pollen tubes, which are present in greater numbers in the ovary of S × m pollinations, either stop growth prior to reaching the obturator or continue growth down the funiculus even where some obturator contact is present.

## DISCUSSION

The classical view of the evolutionary origin of heteromorphic self-incompatibility systems is that they have evolved from homomorphic sporophytic systems by loss of alleles and that

the same *S*-gene is present in both systems (Whitehouse, 1950; Crowe, 1964; Beach and Kress, 1980; Muenchow, 1981, 1982; Wyatt, 1983; Zavada, 1984). Consequently, it has also often been assumed that the mechanism of incompatibility is based on the interaction of molecular *S*-allele specificities in both systems (Richards, 1986). However, several lines of evidence (reviewed by Charlesworth, 1982; Gibbs, 1986; Barrett, 1988b; Lloyd and Webb, in press a) indicate independent evolutionary origins for heteromorphic and homomorphic incompatibility systems, thus casting doubt on the necessary involvement of similar physiological and molecular mechanisms in the two systems.

Studies of the functioning of self-incompatibility in homomorphic sporophytic systems have revealed a complex and refined mechanism of cell-cell recognition. Pollen adhesion and hydration, or early pollen tube growth, are inhibited on the stigma surface following an interaction between *S*-locus specific glycoproteins (SLSG) of the pollen and stigmatic papillae (Gaude and Dumas, 1987; Elleman et al., 1989). Immunolocalization studies of SLSGs show that they occur only in the papillae cells of the stigma (Kandasamy et al., 1989). Rapid callose deposition occurs at the point of contact of the stigmatic papillae and pollen grain, and in the pollen tube tip, causing termination of growth shortly after adhesion of the pollen (Knox, 1984; Roberts, Harrod, and Dickinson, 1984). If pollen tubes do penetrate the stigma, evidence indicates that there is no further obstacle to fertilization (O'Neill et al., 1984).

The major finding of this study is that a uniform stigmatic incompatibility rejection response of the type that occurs in taxa with homomorphic sporophytic self-incompatibility does not occur in *P. sagittata.* Instead, the region of the pistil where termination of pollen tube growth occurs varies depending on the particular style length and pollen size involved. Although some pollen tube behavior is characteristic of particular pollen-pistil combinations, the overall patterns of pollen tube growth are remarkably similar in both illegitimate and legitimate pollinations. These observations indicate that the general properties of incompatibility in *P. sagittata* are fundamentally different from those reported for homomorphic sporophytic systems and indicate a distinctive type of self-incompatibility system is present.

Although incompatibility is under sporophytic control in *P. sagittata,* the regions of the pistil where cessation of illegitimate pollen tube growth occurs are similar to those reported for several monocotyledonous genera with gametophytic self-incompatibility (Stout and Chandler, 1933; Sears, 1937; Brock, 1954; Brewbaker, 1957; Brewbaker and Gorrez, 1967; Brandham and Owens, 1978; Kenrick, Kaul, and Williams, 1986; Seavey and Bawa, 1986). These taxa exhibit late-acting self-incompatibility in the ovary, and it has been suggested that a number of features of the pollen tube pathway may contribute to the delayed incompatibility response (Brewbaker and Gorrez, 1967; Shivanna, 1980).

| POLLEN \ PISTIL | L | M | S |
|---|---|---|---|
| l | — | weak SI expression | failure to recognize ovular signal |
| m | insufficient storage reserves | — | failure to recognize ovular signal |
| s | insufficient storage reserves | insufficient storage reserves | — |

Fig. 45. Possible mechanisms to account for pollen tube behavior in the different pollen-pistil combinations in *Pontederia sagittata.*

Monocotyledonous stigmas are usually of the wet type, making specific cell-cell interaction and inhibition of illegitimate pollen at the adhesion and hydration stage less likely than when a dry stigma is present (Gaude and Dumas, 1987). In addition, the presence of unspecialized styles opening directly into a hollow secretory canal may offer little physical barrier to pollen tubes entering the stylar canal. Ovarian inhibition of pollen tubes may simply involve the replacement of stylar inhibition in taxa with unspecialized styles (Brewbaker and Gorrez, 1967). A lack of intimate contact between pollen tubes and maternal tissue afforded by a hollow style would contribute to this effect. The structural similarity of the pollen tube pathway in *P. sagittata* with that of other monocotyledons suggests that these features may have a similar effect on pollen tube behavior (Scribailo and Barrett, 1991).

Another feature of taxa with gametophytic self-incompatibility strongly correlated with pollen tube inhibition in the style and ovary is the possession of binucleate pollen (Brewbaker, 1957, 1967). Trinucleate pollen is associated with stigmatic inhibition of pollen or pollen tubes in sporophytic self-incompatibil-

ity systems. Although heterostylous taxa can have either binucleate or trinucleate pollen (Ganders, 1979), it is of interest that members of the Pontederiaceae have binucleate pollen and show signs of pollen tube arrest similar to those found in gametophytic systems. In species with binucleate pollen it has been suggested that paternal expression of self-incompatibility genes occurs at the time of generative cell division in the pollen tube (Pandey, 1979). This generally occurs after pollen tubes reach the style, with inhibition occurring some time later (Knox, 1984; Gaude and Dumas, 1987), thus increasing the likelihood of delayed inhibition sites.

In a companion paper we outlined the extent of heteromorphic characters in *P. sagittata* (Scribailo and Barrett, 1991) and compared our findings to those for other heterostylous taxa. Despite documentation of extensive floral heteromorphism, particularly in distylous taxa (see Gibbs, 1986; Richards, 1986), and hypotheses suggesting a direct involvement of these characters in the functioning of self-incompatibility (Dulberger, 1975a, b), the latter ideas have met only limited acceptance. This lack of acceptance may be associated with the commonly held view that the evolution of self-incompatibility precedes the origin of floral heteromorphism in heterostylous plants (Baker, 1966; Charlesworth and Charlesworth, 1979; Ganders, 1979). However, Lloyd and Webb (in press a, b) have recently suggested the reverse sequence with the stamen-style polymorphism evolving before the establishment of a self-incompatibility system. Although the details of their model are outside the scope of this paper, its relevance is that their work suggests that incompatibility in heterostylous plants may evolve strictly on the basis of interactions between heteromorphic characters, through gradual adjustment of pollen tube growth to the different stylar environments of the morphs. This contrasts with the view that heteromorphic incompatibility results from the mutual recognition of molecular specificities expressed as *S*-gene products in the pollen and pistil (see Richards, 1986) that are considered to control multiallelic incompatibility systems (Lewis, 1943; de Nettancourt, 1977).

Results from the present study of *P. sagittata* offer support for a role of heteromorphic characters in the functioning of incompatibility in tristylous plants. The most compelling evidence comes from comparisons of pollen tube length in legitimate and illegitimate pollinations with a particular pollen size. The fact that s and m pollen grains produce pollen tubes of the same length in both legitimate and illegitimate pollinations suggests that incompatibility in certain pollen-pistil combinations may simply be a function of storage reserves in the pollen. A similar pattern was also found by Anderson and Barrett (1986) in *P. cordata.*

Calculations of pollen grain volume using data on pollen size from Glover and Barrett (1983) and the equation for a parabaloid segment (the shape that most closely resembles pollen grains of *P. sagittata*) indicate that l pollen grains have 8.5 times the volume of s pollen grains, and m pollen grains have 4.2 times the volume of s pollen grains. The length of l pollen tubes in legitimate pollinations are 10.3 times, and m pollen tubes are 5.8 times the length of s pollen tubes, suggesting a possible functional relationship between the two parameters.

Additional data from the pollen tube growth study also offer support for the idea of a passive rather than an active mechanism of incompatibility in pollen-pistil combinations where pollen tubes terminate growth in the style. Measurements of both pollen tube diameter and callose plug length show a stronger correlation with pollen type than with whether a pollination is legitimate or illegitimate. Since rejection of incompatible pollen tubes is often associated with an increase in size of callose plugs and swelling of pollen tubes (Knox, 1984), the absence of a difference is also suggestive that an active incompatibility response may not be involved.

Not all illegitimate pollen tube growth in *P. sagittata* terminates growth in the style. The S morph pollinations with l and m pollen result in large numbers of pollen tubes that regularly grow to the base of the style and enter the ovary. Although the sites in which illegitimate pollen tube growth ceases differ in the S morph from what is observed in the L and M morphs, the mechanisms involved may not be qualitatively different. In each morph the general failure to set seed upon illegitimate pollination appears to result from a mismatch between pollen type and pistil length. This behavior bears some resemblance to what has been observed in cases of interspecific incompatibility in *Rhododendron* (Williams, Knox, and Rouse, 1982; Williams and Rouse, 1988, 1990). Such interactions are often termed incongruity (Hogenboom, 1975, 1984) and are believed to be based on maladjustments between maternal and paternal parents rather than from recognition of *S*-allele incompatibility specificities (Heslop-Harrison, 1987).

In their studies of interspecific pollination in *Rhododendron,* Williams and Rouse (1988, 1990) found that when the ratio of paternal to

maternal style length was less than 0.2, pollen tubes terminated growth in the upper region of the style. In contrast, when this ratio was greater than 6, pollen tubes often grew to the base of the ovary but rarely fertilized ovules. At intermediate values, variable seed set occurred. They further speculate that pollen tube growth may be limited by nutritional reserves within the pollen grain and restricted access to stylar reserves, and that pollen tubes of each species may be preprogrammed to produce a pollen tube length equivalent to the distance to the ovule. These findings bear some resemblance to those observed in illegitimate pollinations of *P. sagittata* and suggests similar kinds of interactions may be involved. Of relevance to these observations are recent studies in *Nicotiana* suggesting that maximum pollen tube length may be limited by protein synthesis capacity, since a finite amount of RNA is present in the pollen grain and no new synthesis occurs postpollination (Suss and Tupy, 1976; Tupy, Suss, and Rihova, 1986).

Based on our findings we propose the following model to account for the functioning of the self-incompatibility system in *P. sagittata* (Fig. 45). In illegitimate pollinations involving pollen smaller than the legitimate size class (L × m, L × s, M × s), pollen tubes fail to effect fertilization because they are incapable of growing the distance to the ovary. This could occur either because they have insufficient storage reserves to reach the ovules or through some other mechanism such as insufficient RNA synthesis capacity. In illegitimate pollinations where pollen tubes routinely enter the ovary the situation is more complex. It is hypothesized that in cases where pollen tubes fail to contact the obturator, pollen tubes may be incapable of responding to directional signals from the ovule.

The stage at which pollen tube competence is manifested may occur within some limited time period prior to fertilization. According to our model, pollen tubes from l pollen would rarely be competent to respond in pistils of the S morph because cessation of growth occurs in the ovary when pollen tubes have only attained 8% of the length they grow to effect fertilization in legitimate pollinations. In illegitimate pollinations of the S morph with m pollen, cessation of growth occurs when pollen tubes have reached 18% of this potential length. In contrast, in M × l pollinations pollen tubes have grown 52% of the length of legitimate pollen tubes and may therefore be competent to respond to an ovular signal, the majority of the time. This may account for the fact that this particular illegitimate pollination sets a high level (73%) of seed. Following this view, competence to respond to an ovular signal is more likely to occur when pollen tubes are closer to the length they normally achieve in legitimate pollinations.

Mechanisms of guidance of pollen tubes to the ovule have been the subject of considerable discussion. In a recent review, Heslop-Harrison and Heslop-Harrison (1986) questioned whether there is any good evidence for the involvement of chemotropic factors and suggested that demonstrating the identity of such factors is likely to be extremely difficult. Although no exudate was visible on the obturator of *P. sagittata* (Scribailo and Barrett, 1991) numerous cases are known where the ovule is secretory and such secretions have been implicated in pollen tube guidance (see Tilton, 1980a, b; Tilton and Horner, 1980; Arbeloa and Herrero, 1987; Herrero and Arbeloa, 1989). Evidence for the presence of a directional signal in *P. sagittata* comes from observation that the majority of pollen tubes enter the fertile stylar canal and that legitimate pollen tubes modify their direction of growth to contact the obturator and effect fertilization shortly after entering the ovary.

It is important to note that not all of our observations can be readily explained by the proposed model. Failure of many pollen grains to germinate on the stigma in certain illegitimate pollinations (L × s, L × m, M × s), aberrant pollen tube behavior in the stigma of M × l pollinations and cases of contact with the obturator in S × l, S × m, and M × l pollinations where pollen tubes fail to effect fertilization, are all observations indicating that incompatibility is more complex than our model would suggest. Although most of our observations can be explained without invoking the involvement of molecular SI specificities, it is certainly possible that some kind of active rejection mechanism of the kind found in homomorphic SI systems may be involved.

Observations of pollen tube growth in tristylous members of the Lythraceae and Oxalidaceae (Esser, 1953; Dulberger, 1970; Weller, 1975; Gibbs, 1986) share some similarities with patterns reported here. In these studies no major differences between legitimate and illegitimate pollen in its ability to germinate and enter the style were observed. However, in *Lythrum* and *Oxalis,* inhibition usually occurred in the stigmatic head (Gibbs, 1986) or upper part of the style (Kostoff, 1927; Esser, 1953; Dulberger, 1970; Weller, 1975), and illegitimate pollen tubes did not grow to the same length as legitimate pollen tubes. Both Esser (1953) and Weller (1975) reported that

pollen tubes inhibited in the style tended to show increased callose deposition and swelling of pollen tube tips. These observations suggest a more active mechanism of pollen tube rejection in these taxa than occurs in *Pontederia*.

Interactions between pollen grains and stigmatic papillae appear to be less important in inhibiting illegitimate pollination and pollen tube growth in *P. sagittata* than has been reported in several distylous taxa (e.g., Dulburger, 1974, 1975b, 1987; Schou, 1984; Schou and Mattsson, 1985). Some degree of differentiation was observed among the pollen types in adhesion and pollen germination in certain pollen-pistil combinations (e.g., particularly l pollen grains on S stigmas). This differentiation is likely a result of an interaction between pollen of different size and stigmas of varying degrees of wetness (Scribailo and Barrett, 1991).

Heteromorphic features of stigma and pollen are less well developed in *P. sagittata* than in distylous taxa where pronouced heteromorphisms of pollen exine and stigmatic papillae are almost ubiquitous (Ganders, 1979). While in *Pontederia* these heteromorphic characters are either absent or weakly developed, the degree of pollen size heteromorphism is greater than reported in any other heterostylous plant (Darwin, 1877; Glover and Barrett, 1983). The latter observation is of interest since it may reflect a strong functional relationship between pollen size and pollen tube length in *Pontederia*.

Some aspects of pollen load data collected in natural populations of *Pontederia* species (Glover and Barrett, 1986; Wolfe and Barrett, 1989) may be explained by observations of pollen-stigma interactions made in this study. In general, open-pollinated pollen loads of the S morph are consistently lower in number than in the L and M morph. The reduced size and "dry" nature of the S stigma combined with its smaller papillae may reduce the likelihood of adhesion of larger illegitimate pollen grains. In contrast, exudate accumulation on the larger "wet" stigmas of the L morph may contribute to larger pollen loads that are consistently composed of a high proportion of legitimate pollen.

Pollen and stigma heteromorphisms in *P. sagittata* may serve to reduce levels of illegitimate pollination, pollen germination, and pollen tube growth, and, in common with stylar and ovarian mechanisms of incompatibility, appear to exert their influence largely in a quantitative fashion. The incompatibility system in *Pontederia* can be thought of as a series of barriers which, in combination, favor legitimate fertilizations over illegitimate fertilizations. The variable expression of incompatibility in *Pontederia* spp. (Barrett, 1977; Glover and Barrett, 1983; Barrett and Anderson, 1985) indicates that a variety of genetic and environmental influences may modify the effectiveness of these barriers to illegitimate pollen tube growth. Interestingly, where these barriers are weakly developed, as in self-compatible tristylous *Eichhornia* spp., floral heteromorphisms, particularly those involving pollen size, are weakly expressed (Barrett, 1988a). This further suggests that floral heteromorphisms play an important role in governing incompatibility responses in heterostylous plants.

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