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Directed Movement of Latex Particles in the Gynoecia of Three Species of Flowering Plants

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The secretory matrix of the stylar-transmitting tract of angiosperms has been characterized as a nutrient medium for the growth of pollen tubes, acting to guide tubes to the ovules. When nonliving particles (latex beads) were artificially introduced onto the transmitting tracts of styles of *Hemerocallis flava*, *Raphanus raphanistrum*, and *Vicia faba*, they were translocated to the ovary at rates similar to those of pollen tubes. Direct observations were made on the movement of individual beads along the secretory epidermis in the style and ovary of *Vicia faba*. The transmitting tract may play an active role in extending tube tips to their destination in the ovary.

POLLINATION INVOLVES THE PROcesses of pollen capture by the stigma, hydration, and pollen tube growth through the gynoecial tissues to place the sperm cells they carry in the embryo sac in the ovule. The pollen tube grows by tip extension, restricting the constant amount of cytoplasm there by production of a callose wall or plug that periodically sequesters the living part of the cell at the tube tip. A mechanism for tip growth in pollen has been proposed that accounts for the clearly demonstrated capability of pollen tube growth in vitro (*1*). In this model, internal osmotic pressure is the force behind tip extension; however, in vitro pollen tubes typically do not grow as fast or as long as they do in vivo.

Beginning just below the surface cells of the stigma, an extracellular matrix of secretions defines the path of pollen tube growth to the ovule in the ovary (*2*). In open styles, the matrix covers the surface of the inner epidermal cells, and in closed styles, the matrix occurs in the intercellular spaces of the transmitting tract. Pollen tube growth is typically restricted to this secretory matrix. Attempts to characterize the chemical components of the stylar medium have revealed a variety of substances, with high molecular weight compounds being polysaccharide mucilages (pectic compounds and proteoglycans) and complex proteins (glycoproteins and lipoproteins) (*3, 4*). Arabinogalactans appear frequently in stigma and stylar secretions, and it is assumed that they have a role in at least adhesion of the pollen grain to the stigma (*5*).

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To our knowledge, a role has not been proposed for the stylar matrix in the pollination process other than one of a nutritional fluid and, hence, a pollen tube guide. Here, we examine the possibility that the stylar matrix may play an active role in facilitating directed pollen tube growth. We applied inert particles the size of a pollen tube tip to the transmitting tracts of three species. The growth of pollen tubes and the movement of latex beads were followed in separate styles.

In *Vicia faba*, emasculations 2 days before anthesis were necessary to avoid self-pollination; the other two species are self-incompatible. Pollinations of *Hemerocallis flava* were done in situ. In this variety, growth of pollen tubes to the ovary is rare, so experiments were done on the upper half of the 120-mm-long style. Flowers of *Raphanus raphanistrum* and *V. faba* were excised and pedicels were placed in a 2% agar medium; the flowers were hand-pollinated and placed under lights. Red-dyed monodispersed latex polystyrene beads (2.5% solid latex, Polysciences) with a mean diameter of 6 μm were applied from a syringe in a 2-μl drop containing thousands of beads. In *H. flava*, beads were applied to the stigma directly, in situ. For both *R. raphanistrum* and *V. faba*, flowers were placed in vitro for bead applications, as done for the pollinations. In both, the stigma and part of the style were excised (Fig. 1, B and C) before bead application, because direct application to the stigma resulted in no bead movement into the style.

Pollinated samples from all species were fixed, at various times after pollination, from 2.5 to 25 hours, in a 3 to 1 mixture of ethanol to glacial acetic acid and then left overnight. Tissue was then washed in 70% ethanol for 20 minutes and softened in 1*M* NaOH at 70°F. The samples were squashed in aniline blue and viewed on a Zeiss fluorescence microscope, equipped with Zeiss filter set 487705. Many pollen tubes grow initially on the stigma, but only a subset of these reach the ovary to effect fertilization. The distance that the longest pollen tubes had traveled was measured with an ocular micrometer to get an accurate measure of the pollen tube growth rates that effect fertilization. In *H. flava*, the red beads could be seen through the semitransparent tissue of the style moving in a front that was composed of hundreds of beads. Measurements of this movement were taken in situ approximately every hour. At various times (2 to 10 hours) after bead application in *R. raphanistrum* and *V. faba*, gynoecia were wiped clean with a tissue to remove any beads on the outer surface and dissected to expose the transmitting tract. The samples were placed in a depression slide containing 2% agar and were viewed under a light microscope. Only a small subset of the applied beads would be seen on the transmitting tract at this time. The distance the farthest beads had traveled was measured with an ocular micrometer. Less than 20 beads were translocated to the region of the

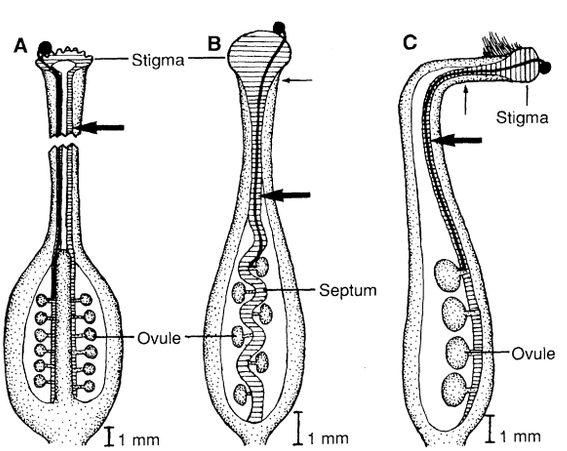

Fig. 1. Diagram of longitudinal sections of gynoecia, showing the path pollen tubes travel in compatible pollinations of (**A**) *H. flava*, (**B**) *R. raphanistrum*, and (**C**) *V. faba*. The transmitting tract is represented by cross-hatching. Large arrows indicate areas of tract shown in Fig. 2, A, C, and E. Small arrows in (B) and (C) indicate where the gynoecia were cut for bead applications.

ovules. Time-lapse photographs were taken of beads traveling on the exposed transmitting tract of *V. faba*.

After timed pollinations or application of beads, gynoecia were hand-dissected to expose the transmitting tract and quickly immersed into liquid nitrogen that had been supercooled under vacuum. The frozen samples were sublimated and sputter-coated with gold and then transferred to and viewed in a Philips 515 scanning electron microscope at −170°C. After pollination or application of beads, samples of *V. faba* were fixed in 2.5% glutaraldehyde in 0.025*M* phosphate buffer, *p*H 7, dehydrated in an alcohol series, embedded in glycol methacrylate, and sectioned at 5 μm. Sections were stained with toluidine blue for observation with the light microscope or with aniline blue for observation with a Zeiss fluorescence microscope. Kodak 2415 Technical Pan film was used for light micrographs and Ilford Pan F film for fluorescence.

When 6-μm latex beads were applied to the open stigma of daylily, *H. flava* (open style), and to the cut stylar ends of the gynoecia of *R. raphanistrum* (closed style) and *V. faba* (intermediate type) (Fig. 1), they were observed traveling on the transmitting tract to the ovary (Fig. 2, A, C, E, G, J, and K) and, in a few cases, into the micropyles (*Vicia* and *Raphanus*). The mean rates of pollen tube growth and those of bead movement in the style were not significantly different (Table 1). To preclude gravity as a factor in bead movement, several bead trials on *H. flava* were performed, in situ, by placing flowers in a horizontal position, thereby inverting the curved style. This had no effect on the rate of bead movement. The movement of individual beads could be directly observed in *V. faba* and *R. raphanistrum* by placing a portion of the transmitting tract on agar and viewing it under the microscope. The beads were observed traveling unidirectionally in the style, toward the ovules. The unidirectionality and the speed of bead movement preclude diffusion as a mechanism of translocation (*6, 7*).

In all three species, bead translocation mimicked the normal patterns of in situ pollen tube growth (Fig. 2). In *H. flava*, the red-dyed beads were applied to the stigma surface and could be observed, in situ, moving as a front down the semitransparent, hollow style, as do pollen tubes. As the beads traveled, they were appressed to the secretory epidermis beneath the cuticle that lines the hollow style, the normal site of pollen tube growth (Fig. 2, A and B). In *R. raphanistrum*, the beads traveled intercellularly, as do pollen tubes (*8*), in the secretory matrix to the ovules (Fig. 2, C and D). In *V. faba*, the beads traveled only on the localized transmitting tract that normally supports pollen tubes in the hollow portion of the style and in the ovary (*9*) (Fig. 1C and Fig. 2, E to K). The beads closely adhered to the secretory cell surfaces (Fig. 2G), as did the pollen tubes (Fig. 2, H and I). Time-lapse photographs (Fig. 2, J and K) document direct observation of bead movement in *V. faba*, although rates were reduced by about tenfold once dissection of the ovary occurred. Movement of beads was negligible if applied to gynoecia of *V. faba* 1 day before anthesis (when the transmitting tract will not support pollen tube growth) or after self-pollination.

There is extensive literature on the theory of chemotropic factors in the style that guide the tubes to the ovary, but no substance has yet been isolated (*10, 11*). Although pollen tubes grow outside the stylar medium, their behavior in vitro fails to mimic that of pollen tubes in vivo, since in vitro pollen tubes typically do not grow as fast or as long as they do in vivo. Pollen tube growth functions in the physical movement of the vegetative cell and the two sperm cells

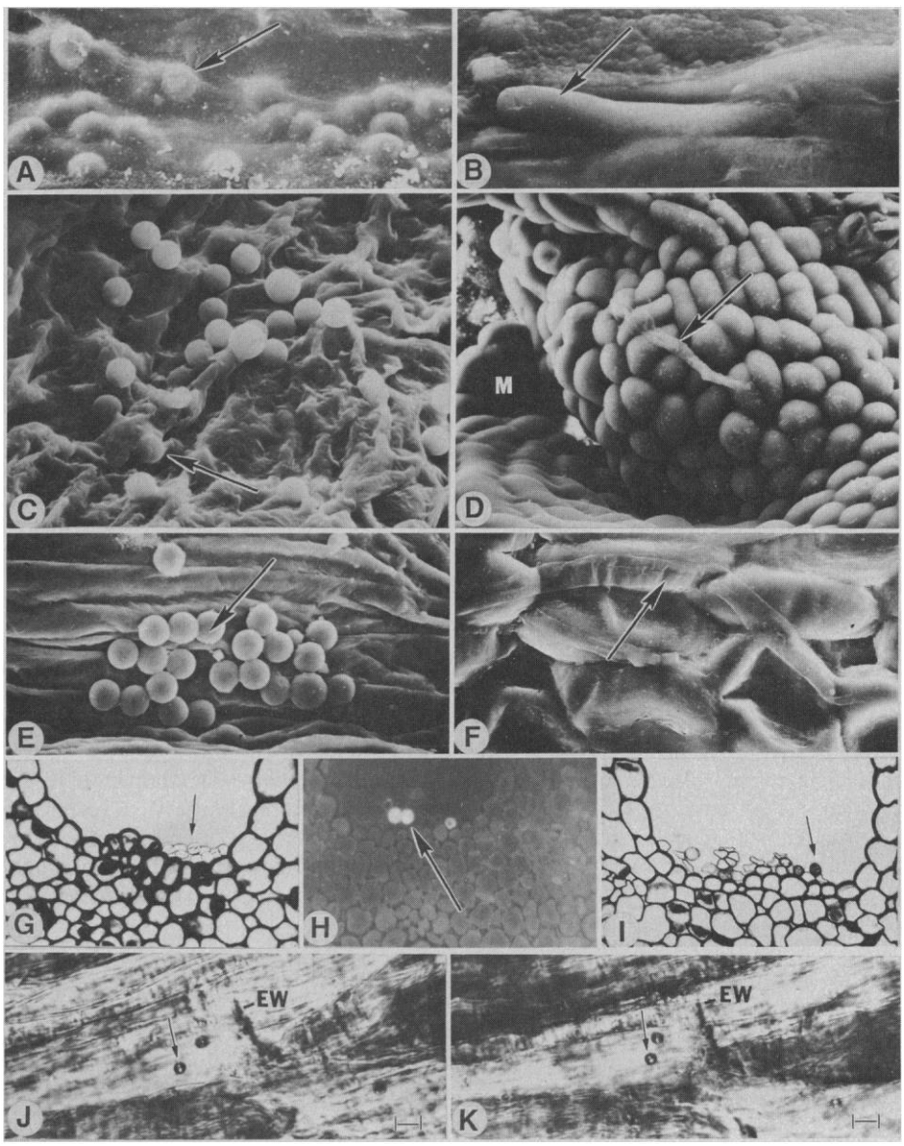

Fig. 2. Scanning electron micrographs and plastic-embedded sections of beads and pollen tubes in the transmitting tract (TT) and a fresh preparation of TT with beads. (**A** and **B**) *Hemerocallis flava*. (A) Surface view of TT with beads (arrow) beneath the cuticle that overlies the extracellular matrix of secretions (×690). (B) Pollen tubes (arrow) on TT (×690). (**C** and **D**) *Raphanus raphanistrum*. (C) Internal view of dissected septum with beads (arrow) embedded in secretions (×690). (D) Pollen tube (arrow) on TT outgrowth of funiculus near micropyle (M) (×344). (**E** to **K**) *Vicia faba*. (E) Surface view of TT with beads (arrow) (×690). (F) Pollen tubes (arrow) on surface of TT (×344). (G to I) Cross sections of *V. faba* showing beads and pollen tubes on TT secretions. (G) Beads (arrow) (×158). (H) Pollen tubes stained with aniline blue under fluorescence (arrow) (×158). (I) Pollen tubes (arrow) (×200). (J and K) Bead (arrow) on TT of *V. faba*. (J) Time zero after dissection of gynoecium 6 hours after application of beads to cut stigma end. (K) Fifteen minutes later; EW, cell end wall. Bars, 10 μm.

Table 1. Bead movement and pollen tube growth rates (in micrometers per minute ± SD). Values represent mean rates of those beads and pollen tubes that traveled the farthest; n is number of gynoecia observed. Bead and pollen tube rates show no significant differences at $P < 0.02$, Mann-Whitney U test.

Species	Beads	Pollen tubes
H. flava	59.5 ± 17.6 (n = 7)	57.2 ± 23.9 (n = 5)
R. raphanistrum	20.7 ± 10.8 (n = 15)	16.1 ± 7.3 (n = 20)
V. faba	10.8 ± 3.2 (n = 6)	15.4 ± 4.8 (n = 12)

through the gynoecium to the ovules. Because there is no appreciable increase in cytoplasmic volume at the tip as the tube grows (*12*), the tip itself is essentially analogous to a moving cell, although older regions of the tube wall keep a connection to the pollen grain on the stigma surface. Cell movement in animals during embryogenesis occurs in an extracellular matrix composed of glycoproteins and proteoglycans (*13*). Researchers have demonstrated that nonliving particles can be transported along these matrices to distant locations (*14, 15*). The proposal in animal systems is that the cells themselves are not solely responsible for their movement but are facilitated by the matrix; hence, the phrase matrix-driven translocation. The secretory matrix of the gynoecium may actually interact with the pollen tube as it forms at the tip and may guide the tube toward the ovule. This could explain the directionality and rates of pollen tube growth seen in vivo, which are not seen in vitro. To date, research has focused mainly on a search for recognition molecules in self-incompatibility systems rather than on mechanisms for compatible tube growth. The molecules that have been isolated in self-incompatible systems are thought to interact somehow with the tube to stop growth; however, these molecules may also have a role associated with compatible pollen tube growth. Because self-compatibility is thought to be the ancestral condition in the angiosperms, additional research on the interactions of the pollen tube with the stigma and style in compatible crosses may provide insights into the mechanism of pollination and how it became modified in the evolution of self-incompatibility. The agent responsible for bead movement is not known, but these results describe a new kind of motility operating in plants.

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6. Calculating diffusion rates: The following equation was used to determine the time needed for a 6-μm sphere to diffuse the longest distances beads had traveled in each species (*7*)

$$t = 3\pi\eta x^2/kT$$

where k is Boltzmann's constant, 1.38×10^{-16} erg/K, and T is temperature in kelvins. These experiments occurred at room temperature, 300 K; η is the viscosity of the medium. We used the η for water to represent the secretions, that is, 1 cP or 0.01 g cm^{-1} s^{-1}. Sphere radius, r, was 3 μm, and x^2 (the distance squared) varied with each species. An example of how this equation was used is as follows: In *H. flava*, the longest observed distance the beads traveled was 31 mm; by the above equation, it would take 208 years for a 6-μm particle to diffuse that distance.
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Commitment of Neural Crest Cells to the Sensory Neuron Lineage

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Clonal cultures and monoclonal antibodies against a lineage-specific epitope, stage-specific embryonic antigen-1 (SSEA-1) were used to analyze the commitment of quail neural crest cells to the sensory neuron pathway. There were two distinct populations of sensory cells at the time of gangliogenesis. Postmitotic neuroblasts that remained in close association with the neural tube coexisted with a large number of pluripotent cells that formed the leading edge of the emigrating cells and gave rise to sensory and autonomic neuroblasts and to melanocytes. The data suggest a dual origin of spinal sensory neuroblasts and a predominantly late divergence of the autonomic and sensory lineages.

AUTONOMIC NEURONS, AS WELL AS spinal and some cranial primary sensory neurons, are derived from the neural crest (*1–3*). However, the mechanisms that regulate the formation of the autonomic and sensory nervous systems are poorly understood. We used clonal cultures and antibodies against SSEA-1 to probe the spinal sensory neuron lineage in the quail neural crest. We found a temporally and spatially dual origin of spinal sensory neuroblasts. The data indicate that the predominant mechanism is segregation of the adrenergic and sensory lineages after formation of the sensory ganglia is initiated. In addition, however, there are a number of early-differ-

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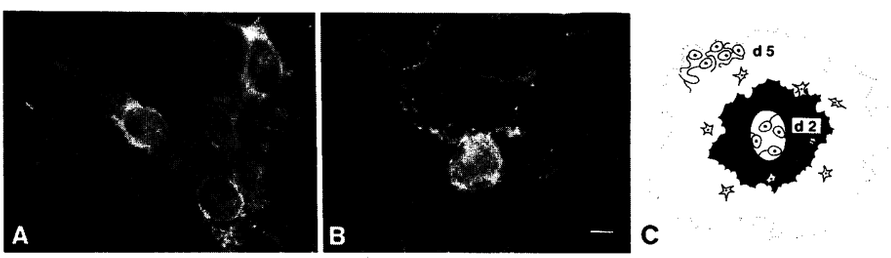

Fig. 1. Two subpopulations of SSEA-1–immunoreactive (*27*) cells in primary neural crest cell cultures. (**A**) Centrally located SSEA-1^+ sensory neuroblasts on day 14 that were attached to a neural tube remnant. (**B**) Peripherally located SSEA-1–immunoreactive cell on day 21. (**C**) Schematic drawing of a primary neural crest cell culture. Centrally located sensory neuroblasts appeared on day 2 (E4) and were surrounded by a ring of pigment cells (darkly shaded area), which were located on top of unpigmented cells and appeared on days 3 to 4. Multipolar adrenergic neuroblasts (TH^+ and DBH^+) first appeared on days 3 to 4 at both sides of the margin of the area containing pigmented cells. A second population of SSEA-1^+ cells appeared in peripheral areas. Occasional SSEA-1^+ cells were observed as early as on culture day 5 (E7), large numbers by day 8.