

POLLEN TUBE TRANSMITTING TISSUE: PLACE OF COMPETITION OF MALE GAMETOPHYTES

Claudia Erbar¹

Heidelberg Institute of Plant Sciences (HIP), Biodiversity and Plant Systematics, University of Heidelberg,
Im Neuenheimer Feld 345, 69120 Heidelberg, Germany

In angiosperm reproduction, pollen tubes elongate from the stigma through the stylar transmitting tissue to the ovary to deliver the male gametes for fertilization. This article reviews different forms of pollen tube transmitting tracts within the style as well as possibilities for bridging the gap from the transmitting tissue to the ovule. The pollen tube transmitting tissue is the place of possible pollen tube competition. Often the number of pollen grains deposited onto the stigma or stigmata of a flower exceeds the number of ovules in the ovary of the pollinated flower. In the styles of most taxa investigated, a reduction in number of the pollen tubes occurs from the stigma to the ovary (pollen tube attrition): the pollen tubes compete for entry into the embryo sac of the ovules within the ovary. A prediction of the extent of pollen tube competition can be partially equated to the P-S/O ratio. This ratio relates the total number of pollen grains (P) deposited onto the stigmata (S) at the end of the female phase of anthesis (stigma receptivity) to the number of ovules (O) present in the ovary. Some quantitative data on pollen load on the stigma or stigmata and resulting pollen tube competition will be demonstrated especially in the choricarpous gynoeceium of *Geum rivale* (Rosaceae) and in the coenocarpous gynoeceium of *Cichorium intybus* (Asteraceae). Data from this study are compared with data of other taxa obtained from the literature.

Keywords: pollen tube transmitting tissue, pollen tube competition, quantitative aspects of pollination, P/O ratio, P-S/O ratio.

Introduction

The breeding system of an angiosperm is multidimensional and covers, among other aspects, sequential processes such as pollen delivery, pollination, pollen germination on the stigma, pollen tube growth down the style, fertilization of the ovules, seed development, and dispersal of the diaspores. Out of this broad field, this article mainly deals with selected aspects related to pollen tube growth and competition.

Pollen grains (male gametophytes), after arriving on the stigma of a compatible flower, germinate, producing a pollen tube in which the sperm cells are transported to the female gametophyte via the pollen tube transmitting tissue, the specially differentiated tract between stigma and ovary. Once in the ovary, the critical gap from the gynoeceial tissue toward the ovule is bridged in several ways, such as by secretions or by morphological structures. The first part of the article reviews different histological modifications of the pollen tube transmitting tissue, including discussion on the bridging of the gap between the pollen tube transmitting tissue and the ovule.

The pollen tube transmitting tissue not only provides a convenient pathway for the pollen tubes from below the stigma toward the ovules but is also the site of pollen tube competition and attrition. One can observe that pollen tubes germinating from several pollen grains deposited onto a stigma show different growth rates within the pollen tube transmitting tissue:

often numerous pollen tubes are found just below the stigmata; this number decreases toward the ovules (see figs. 6–9 in Smith-Huerta 1997; fig. 156 in Leins 2000; fig. 9 in Sonnberger 2002), and only the fastest-growing compatible pollen tubes achieve fertilization of their sperm cells with the egg cells within the embryo sacs. This selection on the male gametophytes operating between deposition of the pollen grains and fertilization easily screens for the best genomes (Mulcahy 1979) and influences the resulting sporophytic generation positively, as already postulated by Correns (1928).

In the second part of this article, in the context of possible pollen tube competition, *Geum rivale* (Rosaceae) with a choricarpous gynoeceium and *Cichorium intybus* (Asteraceae) with a coenocarpous gynoeceium are discussed as examples for answering the following questions: How many pollen grains have been deposited onto the stigma at the end of female anthesis after several visits of insects? Does the possibility exist for pollen tube competition (one form of prezygotic selection) in these two systems?

Pollen Tube Transmitting Tissue: Pathway for Pollen Tube Growth

Pollen grains arrive on the stigma, and after germination on the receptive tissue, they grow by the shortest route into the pollen tube transmitting tissue (fig. 1 in Mulcahy and Mulcahy 1987 [*Geranium maculatum*] or fig. 23 [*Nigella damascena*])

¹ E-mail erle@urz.uni-heidelberg.de.

and fig. 47 [*Geranium sanguineum*] in Erbar 1998). In general, there are two forms of pollen tube transmitting tissue (for a more detailed classification, see Hanf 1936 and Endress 1994). In the first case, the pollen tube transmitting tissue is mainly formed by a secretory epidermis, such that the pollen tubes grow in a more or less ample secretion that fills the stylar

canal, as, e.g., in *Campanula* (fig. 1a). In the second case, pollen tube transmitting tissue several cell layers thick lies below the epidermis, and the pollen tubes find their path within this tissue. This form is characteristic of, e.g., Asteraceae (fig. 1b). The cell walls of the transmitting tissue appear swollen from a pectinous secretion that forms a thick intercellular ma-

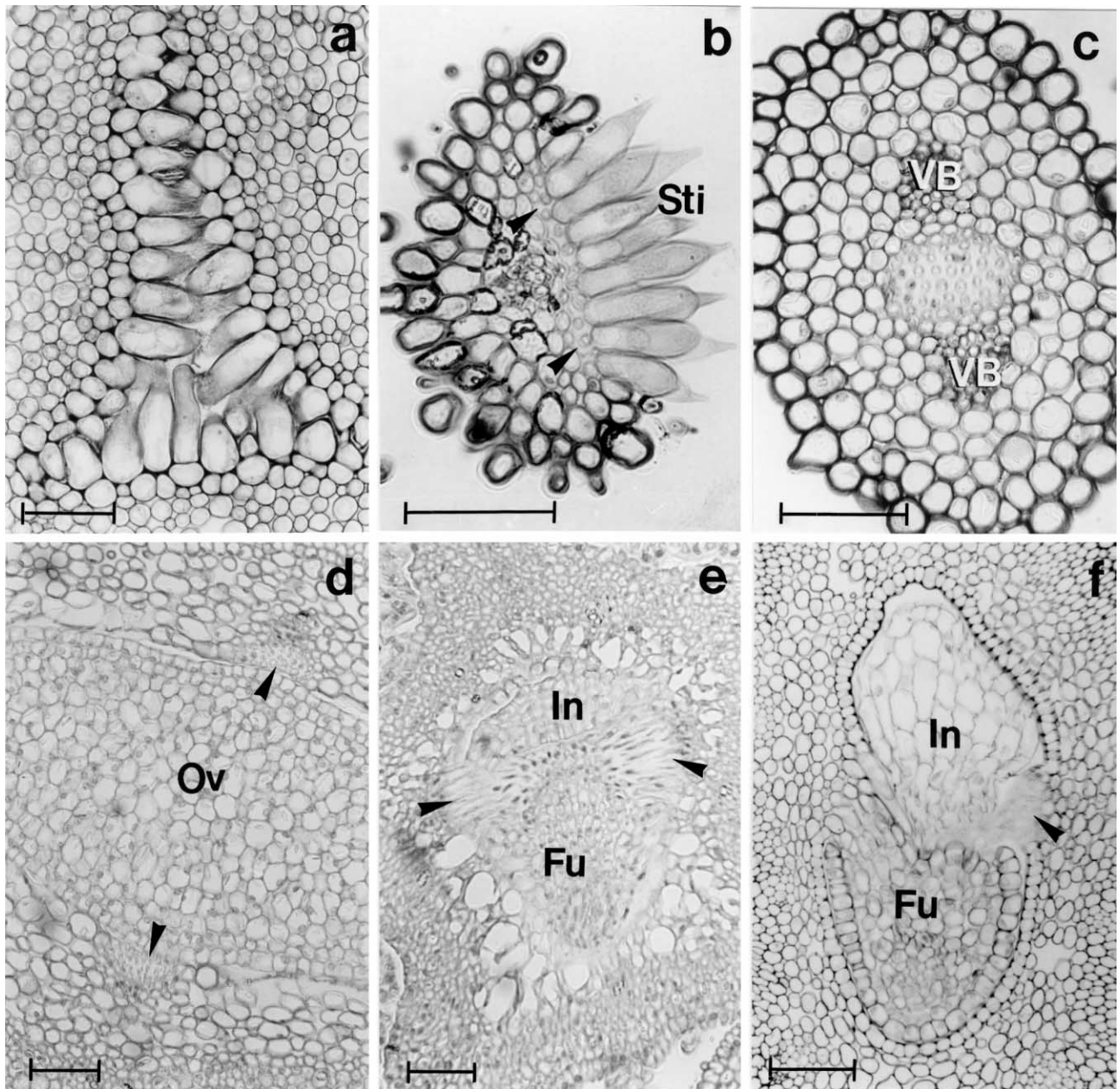


Fig. 1 Pollen tube transmitting tissue. Cross sections of adult styles and ovaries. *a*, *Campanula rotundifolia* (Campanulaceae). Stylar canal with a secretory epidermis. *b–e*, *Cichorium intybus* (Asteraceae-Cichorioideae). *b*, Stylar branch with band of pollen tube transmitting tissue (arrows) beneath the stigmatic tissue. *c*, Central strand of transmitting tissue in the common style. *d*, Midregion of the ovary. The transmitting tissue runs in the region of the two reduced septal ledges (arrows). *e*, In the zone directly beneath the micropyle, the transmitting tissue (arrows) of the two septal ledges fuses. *f*, *Bupthalmum salicifolium* (Asteraceae-Asteroideae). At the bottom of the ovary, the transmitting tissue of the septal ledges (arrow) comes into close contact with a similar transmitting tissue on the funiculus; section somewhat oblique. *Fu* = funiculus, *In* = integument, *Ov* = ovule, *Sti* = stigma, *VB* = vascular bundle. Bars = 50 μ m. *a* from Erbar (1995); *b–e* from Erbar and Enghofer (2001).

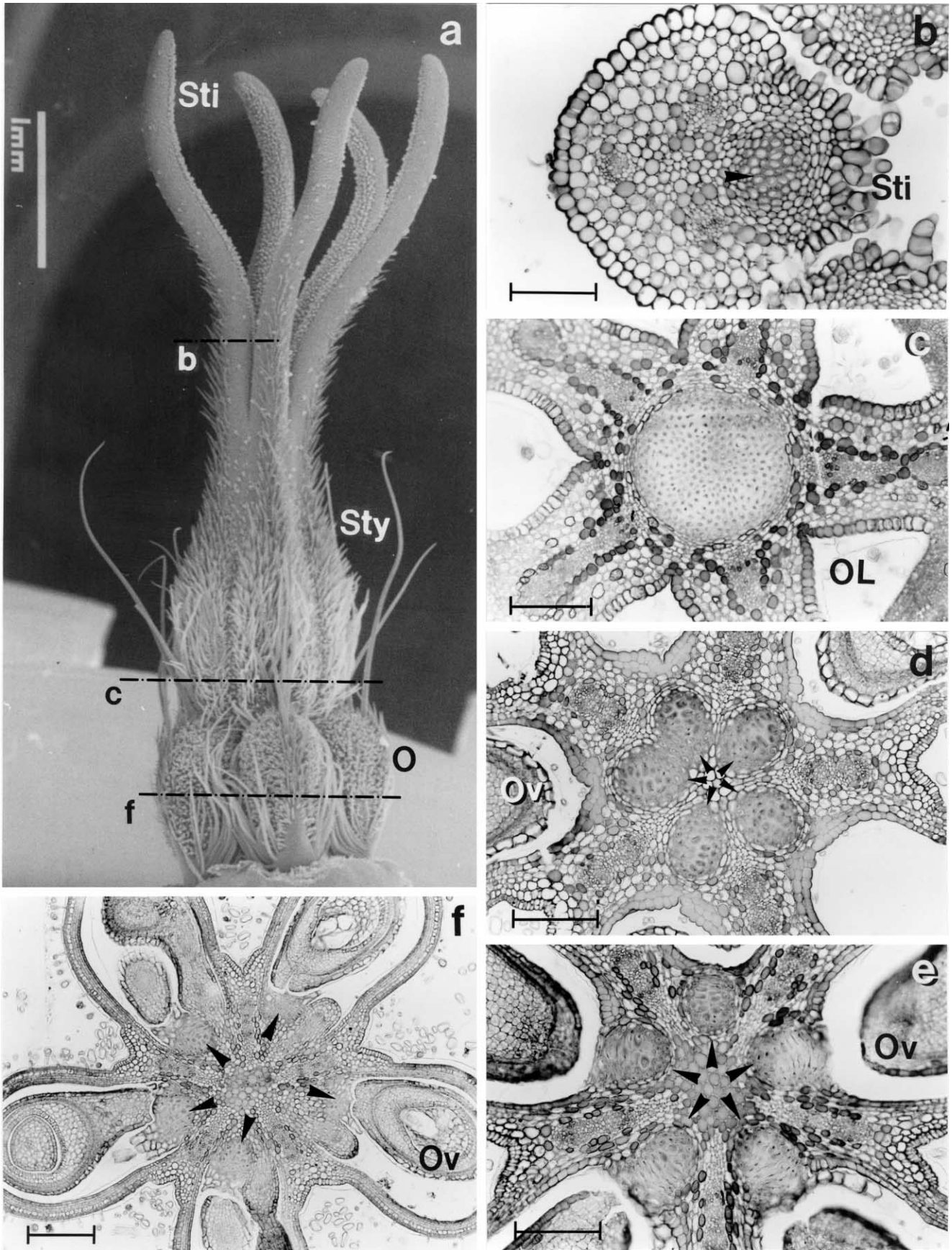


Fig. 2

trix (Kroh and Munting 1967; Sassen 1974). The growing pollen tubes pass through the mucilaginous part of the cell wall and intercellular matrix, respectively (Sassen 1974; Fahn 1982).

The transmitting tissue provides not only guidance and an adequate environment for the growth of pollen tubes, namely, in the form of pollen tube nutrition, but perhaps it also provides the medium for the passage of electrical and other signals between stigma and ovary. Whereas the latter function is only hinted at (Knox 1984; Heslop-Harrison 1987; Malhó 1998; Wilhelmi and Preuss 1999; Shimizu and Okada 2000), the nutritional role of the transmitting tissue is proven (Cheung et al. 1995; Wu et al. 1995). The major constituents of the pectinic secretion in the intercellular spaces are carbohydrates and proteins (Knox 1984). In addition, the existence of some pistil-specific glycoproteins has been shown (see review of Herrera and Hormaza 1996). One of these proteins, the transmitting tissue-specific glycoprotein (TTS protein; Cheung et al. 1995), attracts pollen tubes and stimulates their growth; it adheres to the pollen tubes and is incorporated into pollen tube walls. The nutritional function of this protein is shown by deglycosylation of the TTS protein through the pollen tubes (Wu et al. 1995).

The pollen tubes grow over a long distance from the stigma toward the ovary, near the ovules in a secretion or in a specially differentiated tissue (Sassen 1974). A critical region for the pollen tube is the transition from the carpel tissue to the ovule. The course of the pollen tube transmitting tissue and the passage of the pollen tube in this tissue are demonstrated exemplarily in *Geranium* (Hofmeister 1864; Erbar 1998), a typical case of a coenocarpous-septate gynoecium with five separate, relatively long stylar arms (fig. 2a). In the separate stylar arms, the transmitting tissue lies as a single tract that is closely associated with the stigmatic area (fig. 2b). In the coenocarpous region of the style, the five separate tracts fuse into one central tract (fig. 2c). Just above the insertion area of the funiculi, this thick central tract separates into five tracts, and the five tracts run into each of the five fertile locules (fig. 2d, 2e). The pollen tube transmitting tissue extends to the placenta, and due to the curvature of the anatropous ovule, the micropyle faces the transmitting tissue (fig. 2f), thus bringing the pollen tube in close proximity to the entrance of the ovule.

As demonstrated in *Geranium*, in gynoecia with separate stylar arms, the individual pollen tube transmitting tissues fuse with each other, at least for a short distance, before eventually separating again, in the case of septate ovaries. At this level of the gynoecium (where the transmitting tissue tracts unite), another important function of the pollen tube transmitting tissue is apparent. By the common part of the pollen tube transmitting tissue, a common inner gynoecial space is

achieved, the so-called compitum (Carr and Carr 1961). In this common region, where the inner surfaces of all carpels communicate, the even distribution of pollen tubes is facilitated; a pollen grain, via the growth of the pollen tube in the compitum, may reach any ovule in any ovary locule, regardless on which stigma the pollen grain has germinated (Endress 1982, 1994; Erbar 1998).

Geranium shows one of the simplest cases in facilitating the transition of the pollen tubes from the ovarian tissue to the ovule, in that the micropyle faces the papillate surface of the placenta (fig. 2f). In other cases, the critical gap from the ovarian tissue toward the ovule is bridged by secretions or morphological structures. The pollen tube migration from the pollen tube transmitting tissue near the ovules is relatively simple in cases such as *Monodora* (Annonaceae), where the whole ovary lumen is filled by a secretion (fig. 3a). In other cases, such as in Euphorbiaceae (Endress 1994) and some Liliaceae (Tilton and Horner 1980), a proliferation of the placental region, a so-called obturator, is present to bridge this gap between transmitting tissue and ovule. Often, like in *Euphorbia characias* (fig. 3b), the obturator extends as a plug into the micropyle, and thus the path from style to embryo sac in the ovule is bridged. In species with a central placenta, the top of the placental column may be connected directly with the transmitting tissue. In *Thesium rostratum* (Santalaceae), the summit of the placental column, which bears three upwardly orientated ovules, reaches into the stylar canal and thus gains direct contact with the transmitting tissue of the style (fig. 3c, 3d). A free extension of the placental summit is also found in *Myrsine* (Myrsinaceae), whereas in *Aegiceria* (Myrsinaceae) the bridge for pollen tubes between stylar canal and a central placenta is provided via a secretion (Endress 1994).

Until now only a few studies (Tilton 1980; Yan et al. 1991) have shown that the functional counterpart of the transmitting tissue of the ovary is the epidermis of the micropylar region, which is apparently also secretory. A secretory micropylar region is demonstrated in Asteraceae, in the barnadesioid *Arnaldoa macbrideana* (fig. 4f), the cichorioid *Cichorium intybus*, the asteroid *Buphthalmum salicifolium* (C. Erbar, personal observation), and *Helianthus* (Yan et al. 1991). We have shown recently that in *A. macbrideana*, a member of Barnadesioideae, the subfamily assumed to be primitive within the Asteraceae, pollen tube transmitting tissue runs along the surface of the long funiculus of the single basal ovule (figs. 4e–4g) and thus connects the pollen tube transmitting tissue of the ovary with the micropyle (Erbar and Leins 2000). In *C. intybus* the single ovule arises, as in the other Asteraceae, from a very short septum at the base of the bicarpellate ovary. In the otherwise unilocular ovary, only reduced (sometimes much reduced) septal ledges can be found in transverse position. The

Fig. 2 *Geranium sanguineum* (Geraniaceae). a, Adult gynoecium, SEM image. The letters b, c, and f in a refer to the position of the corresponding cross sections. b–f, Cross sections through the ovary illustrating the course of pollen tube transmitting tissue (arrows). b, At the level of a free stylar branch; the transmitting tissue lies as a single tract closely under the stigmatic area. c, At the level of the lower common part of the style and sterile part of the ovary locules, respectively; the five separate tracts have fused into one central tract. d, e, At the level of the fertile ovary locules just above the insertion area of the funiculi; the thick central tract separates again, and the five tracts run into each fertile locule. f, At the level of the funiculi; the pollen tube transmitting tissue extends to the placenta. O = ovary, OL = ovary locule, Ov = ovule, Sti = stigma, Sty = style. Bars = 100 μ m in b–e, 200 μ m in f. b–f from Erbar (1998), modified.

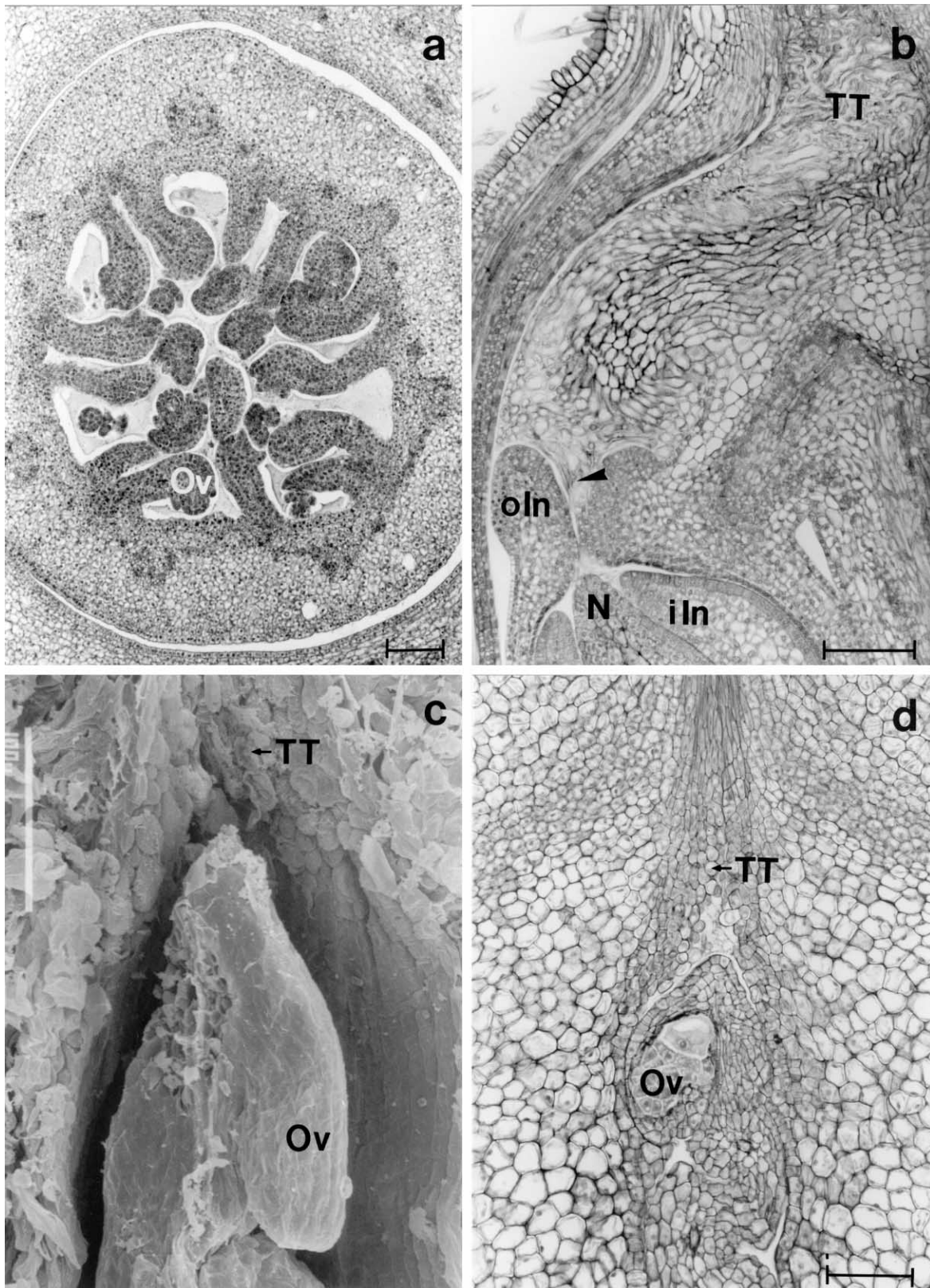


Fig. 3 Different possibilities for bridging the gap between pollen tube transmitting tissue of the carpel and the ovule: *a*, cross section; *b*, *d*, longitudinal section; *c*, SEM image. *a*, *Monodora crispata* (Annonaceae); whole ovary lumen filled by a secretion. *b*, *Euphorbia characias* (Euphorbiaceae); obturator (arrow) extends as a plug into the micropyle. *c*, *d*, *Thesium rostratum* (Santalaceae); top of central placenta getting direct contact to the transmitting tissue of the style. *iIn* = inner integument; *N* = nucellus; *Ov* = ovule; *oIn* = outer integument; *TT* = pollen tube transmitting tissue. Bars = 100 μ m. *a* from Leins and Erbar (1982); *c*, *d* from Erbar and Leins (2001).

transmitting tissue, which lies as a band beneath the stigmatic tissue of the free stylar arms (fig. 1b) and forms a central strand in the common style (fig. 1c), separates in the ovary and runs into the region of the two reduced septal ledges (fig. 1d). In *Arnaldoa* these septal ledges are prominent in the upper region of the ovary and are covered by pollen tube transmitting tissue (fig. 4b). Toward the point of ovule insertion, the ledges become less prominent (fig. 4c, 4d), and at the base of the ovary the two septal ledges fuse into a short septum from which the ovule arises. At the bottom of the ovary (fig. 4f, 4g), the ovarian transmitting tissue comes into close contact with the transmitting tissue on the funiculus. The funiculus is of a conspicuous length in *Arnaldoa* (fig. 4a). The pollen tube transmitting tissue runs in two lateral lines along the funiculus (fig. 4e) up to the micropyle. Beneath the entrance to the micropyle, they fuse into one (bipartite) band that finally enters the micropyle (fig. 4d). A similar course of the transmitting tissue can also be found in other members of the Asteraceae, e.g., in *C. intybus* (fig. 1e), subfamily Cichorioideae, and *B. salicifolium* (fig. 1f), subfamily Asteroideae, each with only a short funiculus.

In order to demonstrate that the pollen tubes do indeed travel through the transmitting tissue, serial sections of the ovary of *C. intybus* were treated with decolorized aniline blue and then observed under the fluorescence microscope. The callose of the pollen tubes glows within the transmitting tissue (fig. 5b–5d). It is noteworthy that in this species the number of pollen tubes decreases from upper style toward the ovary (fig. 5a), as is commonly observed in other species (see below), but more than one pollen tube can be found near the micropyle (fig. 5e).

Pollen Load on Stigma and Pollen Tube Competition

The pollen tube transmitting tissue is the main place of pollen tube competition. Often the number of pollen grains deposited onto the stigma or stigmata of a flower exceeds the number of ovules in the corresponding ovary. The pollen tubes compete for access to ovules. Through gametophytic competition, certain pollen tubes are favored over others on the basis of genetic differences. Only the fastest-growing pollen tubes achieve fertilization, and this prezygotic selection has positive effect on the offspring (Correns 1928; Mulcahy and Mulcahy 1975, 1987; Mulcahy 1979; Mulcahy et al. 1983; Schlichting et al. 1987; Winsor et al. 1987, 2000; Bertin 1990; Snow and Spira 1991; Hormaza and Herrero 1992; Palmer and Zimmermann 1994; Stephenson et al. 1995; Johannsson and Stephenson 1997; Niesenbaum 1999). The “fitness” of the male gametophytes is clearly transferred to the resulting sporophyte

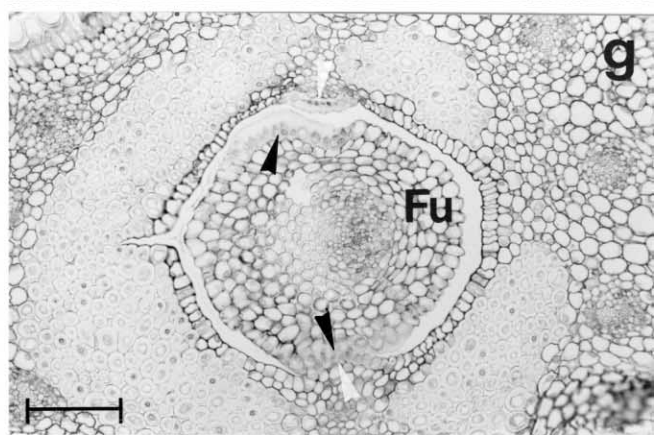
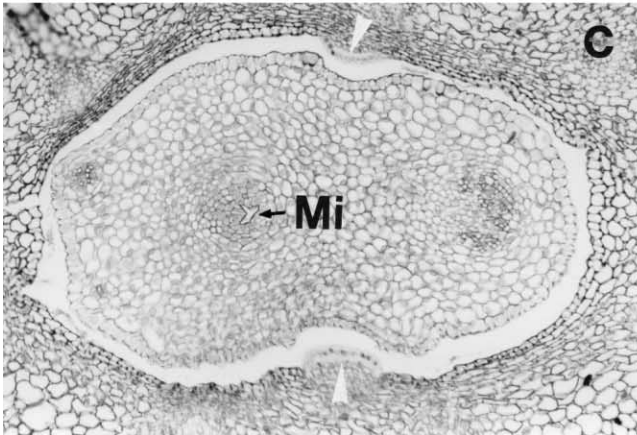
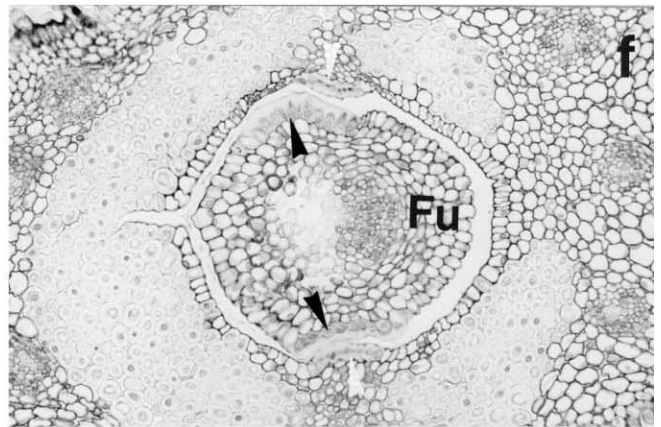
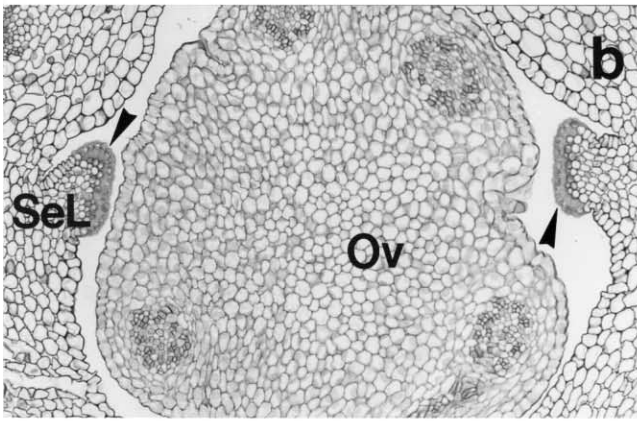
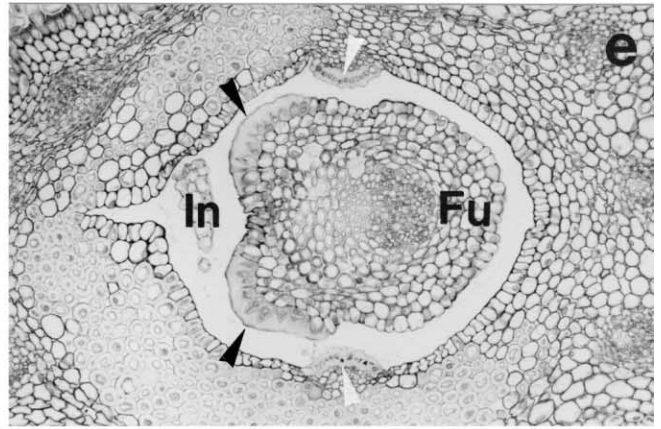
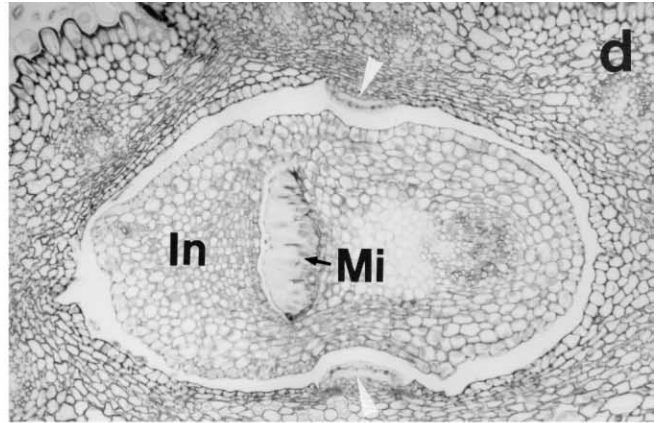
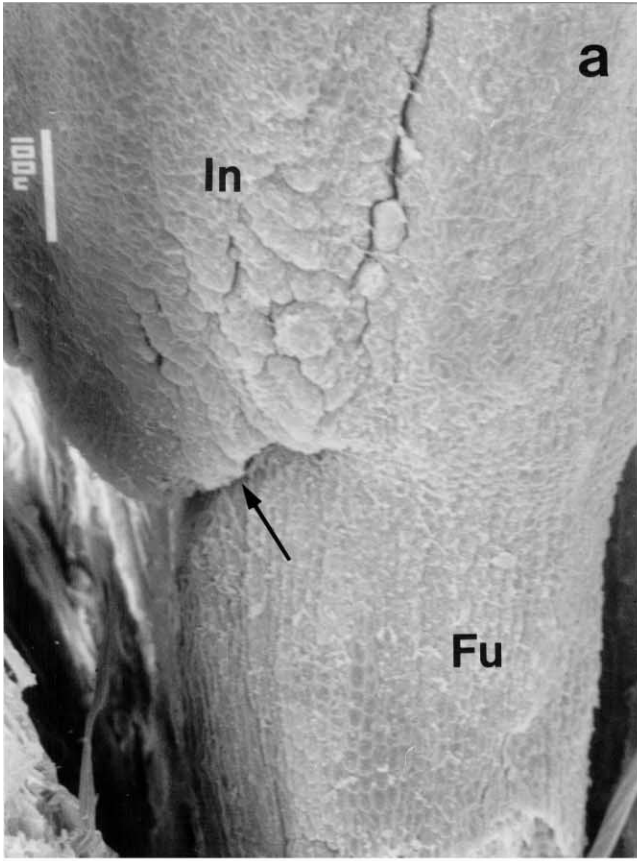
because more than 60% of the genes expressed in the sporophytic phase of the life cycle are also expressed in the male gametophyte (Tanksley et al. 1981; Willing and Mascarenhas 1984; Mulcahy and Mulcahy 1987). Plants have little direct control over the number or the genotypes of the pollen grains deposited onto their stigmata. This, however, does not mean that they have no control over the genetic quality of their progeny. The quality of the offspring can be regulated by pre- and postzygotic mechanisms. The prezygotic selection includes pollen tube competition, and the postzygotic selection includes selective seed or fruit abortion and seedling competition. Under the conditions of pollen tube competition, offspring vigor is enhanced in several traits such as germination, seedling survival, root growth, number of flowers and fruits, number of seeds per fruit, and seed weight (Hormaza and Herrero 1992; Palmer and Zimmermann 1994; Stephenson et al. 1995; Johannsson and Stephenson 1997; Kalla and Ashman 2002).

There are, however, some differing opinions as to the importance of pollen tube competition, especially to the positive effect that pollen tube competition exerts on the resulting offspring. Charlesworth (1988) and Walsh and Charlesworth (1992) argue that high pollen loads may stimulate the maternal plant to devote more resources to those flowers with a high pollen load, causing them to potentially produce better-quality offspring, quite apart from any effect of male gametophyte competition. In addition, Walsh and Charlesworth (1992) maintain that if the pollen grains that grow more rapidly than others have an advantage related to better access to ovules, genes for accelerated growth should spread in a population. Consequently, there should be little extant genetic variation in growth rates.

Flower-pollinator interactions are often complex (see net of correlations in Leins 2000). Spatial and temporal variation in pollinator behavior seems to preclude broad generalizations. However, the opportunity for pollen tube competition appears to be rather different, based on measurements of pollen deposition rate and seed set. In natural populations, pollen deposition onto styles in different flowers of a single plant may range from zero to amounts far exceeding that required for full seed set (Winsor et al. 1987).

One may ask, How many pollen grains actually arrive on the stigmata? *Cichorium intybus* (Asteraceae) was investigated exemplarily with regard to the quantitative aspects of pollen transfer (Erbar and Enghofer 2001). The average number of pollen grains produced in one capitulum is 34,300 (median number of 14 flowers; anthers of a flower contain, on average, 2451 ± 626 pollen grains). The most frequent visitors are the sweat bees *Halictus maculatus* and *Halictus rubicundus* and the hoverflies *Episyrphus balteatus* and *Sphaerophoria scripta*. After the visit of a single capitulum, *H. maculatus* and *H.*

Fig. 4 *Arnaldoa macbrideana* (Asteraceae-Barnadesioideae). a, SEM image; b–g, cross sections. a, Part of older ovule. Arrow points to the entrance of the micropyle. b, Just above midregion of the ovary; prominent septal ledges covered by pollen tube transmitting tissue (arrows). c–g, Cross sections of a series from the midregion of the ovary (c) to the base (g) showing the course of pollen tube transmitting tissue on the septal ledges of the ovary (white arrows) and on the funiculus of the ovule (black arrows). c, d, At the level of the micropyle. e, At the level of the funiculus. f, g, At the ovary base; septum with ovule base; the pollen tube transmitting tissues of the ovary (white arrows) and ovule (black arrows) come into contact. *Fu* = funiculus; *In* = integument; *Mi* = micropyle; *Ov* = ovule; *SeL* = septal ledge. Distance c to d 60 μm ; d to e 156 μm ; e to f 84 μm ; f to g 12 μm . Bars = 100 μm . From Erbar and Leins (2000), modified.



rubicundus carry off 652 (± 237) and 1329 (± 270) pollen grains, respectively, thus taking away 2% and 4%, respectively, of the whole pollen production of a capitulum. When approaching a capitulum, the pollen load on the insects is lower (334 ± 151 and 1089 ± 302 pollen grains, respectively). Due to the high visitation activity of these pollinators, the pollen load on the stigma or stigmata at the end of anthesis is high; 47.8 (± 26.5) pollen grains are deposited onto the stigmata of an individual flower.²

Due to the high pollen load (48 pollen grains per stigma or stigmata) in *Cichorium* flowers, it is likely that pollen tube competition exists, as the germinating pollen tubes compete for fertilization of the single ovule within the coenocarpous gynoecium (fig. 5a). In the uniovulate carpels of the choricarpous gynoeciums of *Geum rivale* (Rosaceae) and *Fragaria virginiana* (Rosaceae), pollen tubes also compete for entry into the single ovule. In *G. rivale*, on average, 18.6 (± 13.3 ; maximal load 52, minimal load 2) pollen tubes start the race on the stigma (Erbar et al. 2001). In *F. virginiana* high pollen loads averaged 26.0 \pm 1.0 pollen per style, whereas low loads averaged 4.2 \pm 0.2 pollen per style (Ashman 2000; Kalla and Ashman 2002).

Pollen attrition (fig. 5a), the reduction of the number of pollen tubes from the stigma to the ovary (Cruzan 1989), has been observed in many coenocarpous gynoecia (Snow 1986b; Cruzan 1986, 1989; Plitmann 1993; Smith-Huerta 1997; Erbar and Enghofer 2001; Sonnberger 2002). In *G. rivale* (Rosaceae) we recently presented quantitative data on pollen tube competition in a choricarpous gynoecium (Erbar et al. 2001; fig. 6a). The strongest attrition (from 18.6 [± 13.3 , maximum 52] pollen tubes) to 8.7 (± 3.7 , maximum 18) pollen tubes occurs in a very short zone beneath the stigma (cf. fig. 6b, 6c). The number is further reduced to 3.5 (± 1.4 , maximum 8; fig. 6d) in the middle of the distal stylar part and to 2.5 (± 0.7 , maximum 4) just above the double hairpin bend. Only 1.6 (± 0.6 , maximum 3) pollen tubes overcome the double hairpin bend (fig. 6e), 1.3 (± 0.5 , maximum 3) pollen tubes reaching the ovary.³

² The maximal load was 187; the minimal load was seven pollen grains ($n = 141$). These data are obtained in fine weather. In bad weather, the pollen load on the stigma is lower: 2.9 (± 2.4 pollen grains; maximum 10, minimum 0, $n = 121$). For more details see Erbar and Enghofer 2001.

³ The double hairpin bend in the style, characteristic of many species in *Geum*, is the result of two simultaneously occurring but oppositely directed individual curvatures. At fruit maturity, necrotic cells in the turning point of the double hairpin bend determine the zone of demolition of the upper region of the style. The detachment of the distal pilose stylar part is caused by wind. In this way the accrescent persistent proximal stylar part gets a hooked stiff tip (hooked achenes promoting epizoochory). For further details, see Erbar et al. (2001).

To analyze reproductive systems, usually the pollen to ovule (P/O) ratio *sensu* Cruden (1976, 1977) is calculated, i.e., the ratio of the amount of pollen to the number of ovules in the flower. From this quantitative measurement, conclusions can be drawn about the proportion of selfing or outcrossing. According to Cruden (1977), the P/O ratios decrease from xenogamous to autogamous species (P/O ≈ 5 , cleistogamy; ≈ 30 , obligate autogamy; ≈ 170 , facultative autogamy; ≈ 800 , facultative xenogamy; ≈ 6000 , xenogamy). This correlation has been confirmed many times, but nevertheless sometimes considerable deviations have been found. For example, in species in which aggregated pollen is transferred, such as in Mimosaceae (Kenrick and Knox 1982), Asclepiadaceae (Wyatt et al. 2000), and Orchidaceae (Nazarov and Gerlach 1997), the P/O ratio is extremely low. Another example is the xenogamous *C. intybus*, in which the P/O ratio is ca. 2450 and thus is clearly lower than the 6000 postulated by Cruden (1977). However, Cruden (1977) also stated that the more efficient the transfer of pollen, the lower the P/O ratio should be.

Some preliminary results indicate that pollinators have a selective influence on the P/O ratio, as the amount of pollen they remove from a flower may be greater or less than the amount they deposit on the following flower they visit. The type of pollinator also influences the P/O ratio. For example, Ramirez and Seres (1994) showed for tropical monocots that the P/O ratio is lower in flowers pollinated by bees, bats, and birds than by beetles and flies. Another example is *C. intybus* (Asteraceae), which is visited by sweat bees and hoverflies. These pollinators remove 2%–4% of the whole pollen production of a capitulum per visit, and they return with only 1%–3% of their pollen load. Therefore, it appears that pollen may have been lost by grooming and during the flight. The brushing mechanism of secondary pollen presentation in *Cichorium* is one of several mechanisms that functions to reduce pollen removal by individual pollinators during a single visit (Erbar and Leins 1995; Leins 2000). In comparison, in *Erythronium grandiflorum*, bees remove more than 80% of the pollen available in a flower during a single visit; however, only 0.6% of the pollen leaving an *Erythronium* flower arrives on the stigmata of other plants (Harder and Thomson 1989). Similarly, butterflies deposit only ca. 0.5% of the pollen removed from *Pblox* flowers onto stigmata of subsequently visited plants (Levin and Berube 1972). It appears that the proportion of removed pollen that reaches stigmata diminishes as the amount of pollen removed increases (Harder and Thomson 1989).

The P/O ratio is a conservative indicator of breeding systems and reflects the likelihood of sufficient pollen grains reaching each stigma: the more efficient the transfer of pollen, the lower the P/O ratio (Cruden 1977). The P/O ratio, however, does

Fig. 5 *Cichorium intybus* (Asteraceae). Fluorescence microscopic images: a, squashed style; b–d cross sections through coenocarpous gynoecium at different levels; e, longitudinal section. a, Decrease in pollen tube number (some indicated by arrows) in the style from top to bottom; arrows point to callose plugs in the pollen tubes. b, In the central strandlike transmitting tissue of the style (cf. fig. 1c) cross-sectioned pollen tubes are visible as bright circles (some indicated by arrows). c, Also in the transmitting tissue of the septal ledges (cf. fig. 1d) glowing pollen tubes (some indicated by arrows) can be found, although less than in the style. d, Pollen tubes (arrows) in the transmitting tissue at the base of the ovary (cf. fig. 1e). e, Some pollen tubes (arrows) can be found in front of the micropyle. Ov = ovule; VB = vascular bundle. Bars = 50 μm , except a = 100 μm . From Erbar and Enghofer (2001), modified.

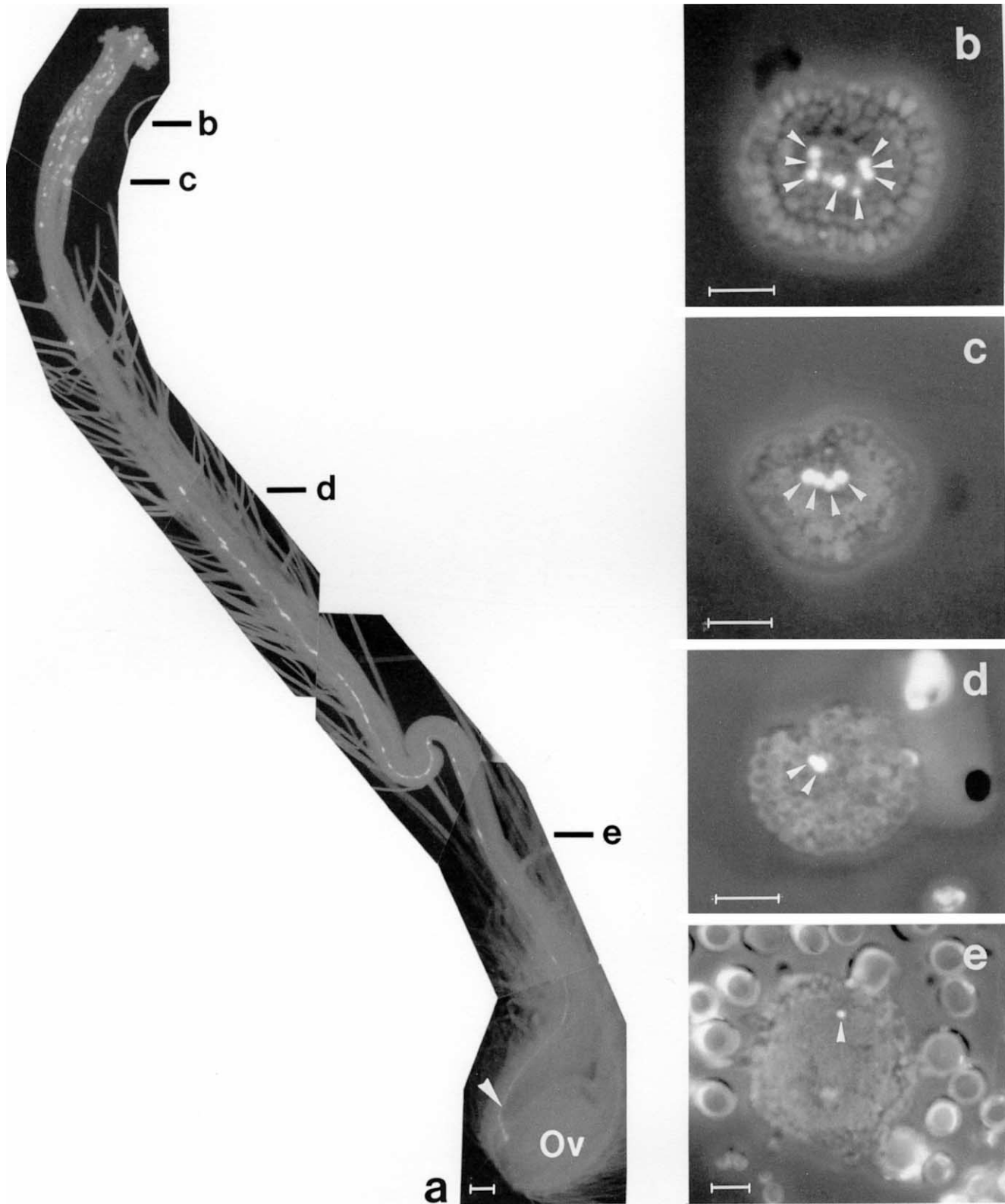


Fig. 6 *Geum rivale* (Rosaceae). Fluorescence microscopic images demonstrating pollen tube attrition in an individual carpel of choricarpous gynoecium. *a*, Squashed carpel. Pollen tube attrition from stylar apex to base; only one pollen tube (arrow) reaches the ovule (*Ov*). *b–e*, Cross sections of the same style at levels indicated by corresponding letters in *a*. *b*, Apical stylar area with eight glowing pollen tubes (arrows). *c*, 495 μm deeper, only four pollen tubes (arrows) are visible. *d*, At the middle of the distal stylar part, two pollen tubes (arrows) are visible. *e*, At the middle of the proximal stylar part, only one pollen tube (arrow) is found. Bars = 50 μm , except *a* = 100 μm . From Erbar et al. (2001), modified.

not specify how many pollen grains have been deposited onto the stigma at the end of anthesis that compete for fertilization of the ovules. We introduced another ratio, the P-S/O ratio, which relates the total number of pollen grains that are deposited onto the stigma or stigmata at the end of female anthesis to the number of ovules in either in the individual carpels of a choricarpous gynoecium or in the common ovary of a coenocarpous gynoecium (Erbar and Enghofer 2001).

This P-S/O ratio provides—to a certain degree—information on the dimensions of pollen tube competition, which is the basis of a prezygotic selection (see above). The P-S/O ratio corresponds to the pollen load on the stigma in cases such as *Geum* and *Cichorium* where there is only one ovule in either the carpel or the coenocarpous gynoecium. Therefore, the P-S/O ratio is, on average, 48 in *C. intybus* and 19 in *G. rivale*.⁴ Thus the number of deposited pollen grains clearly exceeds the number of ovules in the corresponding ovary. From the literature, only very little data can be calculated to make a comparison, because one needs information regarding the pollen load as well as the ovule number. In *Cucurbita foetidissima* (Cucurbitaceae), one can calculate a P-S/O ratio of ca. 18 (Winsor et al. 2000); in North American and European species of *Epilobium* (Ongraceae), the P-S/O ratio lies between 6.5 and 1.17 (Snow 1986b; Müller 2000). In orchids, the number of pollen grains deposited per ovule ranges from 1.2 to 3.1 (Nazarov and Gerlach 1997).

Another ratio can be found in the literature, the pollen : seed ratio (Snow 1986b), which indicates that, as a rule, more than one pollen grain is required for the production of a single ripe seed. In *Mirabilis jalapa* (Nyctaginaceae), four pollen grains per seed are needed; in *Viola nephrophylla* (Violaceae), six (Cruden 1976); in *Epilobium canum*, three (Snow 1986b); in *Campanula americana* (Campanulaceae), three to six (Richardson and Stephenson 1991); and in *C. foetidissima*, four (Winsor et al. 2000). There is also information detailing the minimum load of pollen grains required on a stigma in order to trigger seed set; in many cases, a sufficient number of pollen grains on the stigma is required to stimulate pollen tube growth (Cruzan 1986; Cruden 2000), perhaps functioning to facilitate a synchronous start. Whereas the pollen : seed ratio is ca. 2 in *Passiflora vitifolia* (Passifloraceae), only a load >25 pollen grains leads to seed set (Snow 1986a). These data of the pollen : seed ratio also indicate an induced pollen tube competition. In addition, a so-called pioneer or mentor effect can be observed (Schemske and Fenster 1983; Visser 1983, 1986); early pollen deposition may stimulate the growth of later-deposited pollen grains (Snow 1986a).

With regard to the selective ripening of fruits, pollen tube competition also plays an important role. Fruits with only a few ripening seeds resulting from low pollen competition (only a few pollen tubes) are more easily aborted than are fruits with more seeds, which have resulted from intensive competition between the microgametophytes (Lee 1984; Winsor et al. 1987). However, pollen tube competition is not necessarily the most important selective stage in the life cycle of plants

(Schlichting et al. 1990), but some influence on sporophytic fitness cannot be ignored (Hormaza and Herrero 1992; Palmer and Zimmermann 1994; Stephenson et al. 1995; Johannsson and Stephenson 1997; Winsor et al. 2000; Kalla and Ashman 2002).

Besides chance dispersal of pollen grains by different vectors, the timing of pollen arrival also may determine which pollen tubes are successful. Although pollen grains arrive sporadically, as shown in *Geranium maculatum*, variance in pollen tube growth rates was sufficiently large to allow pollen from later pollinations to surpass the slowest pollen tubes of an earlier pollination (Mulcahy et al. 1983). In *C. intybus*, some of the disadvantages of a nonsynchronous deposition of pollen grains are compensated by the fact that later-arriving pollen grains are deposited on the stylar branches closer to the ovary due to the outward curvature of the branches that takes place during the female phase of anthesis. Thus pollen grains arriving in a series of pollinator visits can nevertheless compete in a fair race (Erbar and Enghofer 2001), and pollen tube competition occurs not only between pollen grains that reach the stigma simultaneously but also between pollen tubes resulting from separate pollination events. The intensity of pollen tube selection also depends on the length of the style; much longer styles may intensify competition by allowing small differences in pollen tube growth rate to be expressed (Correns 1928; Mulcahy and Mulcahy 1975; McKenna and Mulcahy 1983; Snow 1986a).

The role of the maternal sporophyte in preventing the growth of incompatible pollen in self-incompatible species is well known (de Nettancourt 1977; Heslop-Harrison 1983; Sage et al. 1994). After germination and early stages of pollen tube growth, further growth depends on both the pollen and the stylar tissue. For this reason the male gametophyte does not function in an entirely passive environment, and pollen tube growth is thus not merely a reflection of pollen genotype. Instead pollen tube growth is also influenced by the genotype of the sporophyte (Hülkamp et al. 1995).

The facts that pollen tube growth in the style is heterotrophic and that this growth is at the expense of the reserves of the transmitting tissue suggest that the availability of these reserves may affect the number of pollen tubes growing within the style (Herrero and Hormaza 1996). Besides this physiological parameter, the number of pollen tubes found in a style may be limited by physical constraints. A progressive reduction in the width of the transmitting tissue from the stigma to the ovary has been repeatedly observed (Herrero and Hormaza 1996; Hormaza and Herrero 1996; Smith-Huerta 1997). Though the pollen tubes are guided through diploid stylar tissue, it is known that the female gametophyte is also necessary for the guidance of the ovule, at least during the last phase of the pollen tube's journey to the female gametes (Hülkamp et al. 1995; Wilhelm and Preuss 1996, 1999; Ray et al. 1997; Shimizu and Okada 2000).

Finally, *Spigelia anthelmia* (Spigeliaceae, Gentianales), an annual tropical weed, is presented as an example demonstrating unusual prezygotic selection of the fittest male gametophytes; in the autogamous flowers, the upper part of the style detaches from the lower part at a histologically differentiated area (Erbar and Leins 1999). This detachment, which occurs shortly after the onset of anthesis, may be interpreted as a

⁴ It has to be taken into consideration that the viability of pollen grains decreases with time (Dafni and Firmage 2000). In *C. intybus*, we can assume that at the time of pollination, ca. 70% of the pollen grains are viable so that the pollen load contains, on average, 33 viable pollen grains (Erbar and Enghofer 2001).

possible prezygotic selection, which counteracts possible inbreeding depression following a decrease in genetic variability through high self-fertilization. In *S. anthelmia*, many germinated pollen grains remain on the upper part of the style after its detachment from the lower part (fig. 27 in Erbar and Leins 1999); these pollen grains are the “losers” that could not keep up with the competition. The fastest-growing pollen tubes, however, have reached the lower stylar part before detachment of the upper part and penetrate the ovules. Obviously, *S. anthelmia* accepts a lower seed set in favor of higher fitness of the descendants; although sufficient pollen grains are deposited onto the stigma, as a rule, not all of the ovules develop into ripe seeds. However, maternal fitness or influence has not so

far been considered. *Spigelia anthelmia*, the only species of the genus found outside America, is a successful pioneer plant and weed, not only in tropical and subtropical America but also in tropical Africa and Asia. Its success may perhaps be due to its “strategy of controlled autogamy” (Erbar and Leins 1999, p. 401).

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