Changes in the Ovary Related to Pollen Tube Guidance

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Pollen tube growth in the ovary is examined, paying particular attention to how changes in the sporophytic tissues of the ovary and ovule relate to pollen tube guidance. Once pollen tubes reach the ovary they have to "surf" along the placenta, reach the ovule exostome, enter the micropyle, traverse the nucellus and enter the embryo sac via a synergid. In peach, as in other Prunus species, pollen tube growth in the ovary is not straight and accelerations and decelerations occur along this path. Likewise, while some pollen tubes follow a definite route and achieve fertilization, others lose their course and navigate in a chaotic way. Developmental changes in the ovary and ovule appear to be responsible for this different pollen tube behaviour. Pollen tubes first stop at the obturator, a placental protuberance that lines the pollen tube pathway towards the ovule. Pollen tube growth is not resumed until this structure enters a secretory phase. A close examination of the integumentary domain at the ovule shows that secretion is also required at that point for successful pollen tube penetration. The external integument cells at the exostome, that look no different to their neighbouring cells at anthesis, enter a secretory phase in older ovules; once again pollen tube penetration is concomitant with production of this secretion. A similar situation occurs in the internal integument cells that line the micropylar canal since pollen tubes also require a secretion to traverse this area. However, the production of these secretions does not occur in all ovules, only in those that retain their starch at the time of pollen tube arrival and, thus, are competent to enter the secretory phase.

Key words: Prunus, Prunus persica, peach, ovary, ovule, obturator, pollen tube, fertilization.

INTRODUCTION

The pistil appears to be well designed to encourage pollen-pistil interactions, and a number of mechanisms supporting this general strategy have been described both in the stigma and in the style. These tissues host incompatibility systems (Heslop-Harrison, 1983; Nashrallah et al., 1994; Dickinson, 1995; Dodds, Clarke and Newbigin, 1996), and they also play an active role in compatible matings (Knox, 1984; Heslop-Harrison and Heslop-Harrison, 1985; Linskens, 1986; Herrero, 1992a; Hormaza and Herrero, 1999). However, much less work has been done on the ovary, although information gathered so far indicates that an intense pollen-pistil interaction could also occur there. The ovary appears to be the site for intraspecific (Seavey and Bawa, 1986; Sage, Bertin and Williams, 1994) and also interspecific (Williams, Knox and Rouse, 1982) incompatibility in some species; and it could also play an important role in compatible matings (Herrero and Hormaza, 1996). The paucity of information on the male-female interaction in the ovary may be related to the fact that this region is far more difficult to investigate since a number of concentric wrappings envelop the female gametophyte. However, these wrappings are not hermetic but instead have little 'gates' that provide an entry point. The pollen tube has first to find these gates and, then, be able to traverse them to finally reach the female gametophyte. Thus, the female gametophyte is lodged in the embryo sac, that is hosted by the nucellus, which in turn is wrapped by the integuments forming the ovule, that is nested in the ovary. Fertilization is not a straightforward process and, while in some species the pollen tube moves swiftly to the ovule, in others a lag phase occurs between the arrival of pollen tubes at the base of the style and fertilization (Herrero and Arbeloa, 1989). However, the reasons behind this lag phase or the processes occurring during this time are, as yet, unknown. In some species, regulation of pollen tube entry into the ovary has been reported (Arbeloa and Herrero, 1987; Martinez-Pullé and Herrero, 1995). Work with mutants has shown there to be a genetically-based control of ovule penetration operating both at the gametophytic (Hülskamp, Schneitz and Pruit, 1995; Ray, Park and Ray, 1997; Drews, Lee and Christensen, 1998) and at the sporophytic (Wilhelmi and Preuss, 1996) level. However, the mechanisms regulating this control are unknown.

In this work pollen tube growth in the ovary is examined, paying special attention to how changes in the ovary and the ovule relate to pollen tube guidance. While mutant analysis emphasizes the fact that the female gametophyte clearly plays a part in pollen tube attraction (Hülskamp et al., 1995; Ray et al., 1997), much remains to be discovered about how this control is exerted. The information gathered here examines how changes in the sporophytic tissues of the ovary and ovule relate to pollen tube guidance. Although most of the work reviewed here refers to fruit trees, in which the reproductive process occurs at a slow pace allowing ample opportunity to observe changes, the conservation of most of the structures and processes described in different species suggests that the mechanisms described here could be widespread in higher plants.

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POLLEN TUBE PATHWAY IN THE OVARY

Once pollen grains have germinated on the stigma, they send their pollen tubes down the style, where they finally encounter the ovary. The pollen tubes 'surf' their way along the placenta to find the ovule, enter the ovule through the exostome, travel down the micropylar canal to the nucellus, grow across the nucellus and finally penetrate the embryo sac, via a synergid.

While pollen tube growth in the stigma and style appears to be a more or less straightforward process, much remains to be learnt about the driving forces responsible for the pollen tubes taking the correct path in the ovary. Recently, some clues about the nature of some of the components involved in directing pollen tube growth in the stigma have been revealed (Wolter-Arts, Lush and Mariani, 1998). Pollen germination on the stigma occurs in an autotrophic way at the expense of the pollen grain reserves (Herrero and Dickinson, 1981), but upon penetration of the style pollen tube growth is heterotrophic, and takes place at the expense of the transmitting tissue stylar reserves (Herrero and Dickinson, 1979). Thus the 'frontline' of the growing pollen tube tips in the style marks the contrast between stylar tissue rich in nutritional reserves in front of the pollen tubes, and a deprived tissue, devoid of reserves, behind this 'frontline'. Since growing pollen tubes require nutritional reserves of the style to build up their cell walls, it is not unexpected that the pollen tubes follow the path where reserves are available. Recently, a number of pistil-specific glycoproteins were identified (Goldman et al., 1992; Du et al., 1994; Cheung, Wang and Wu, 1995; Sommer-Knudsen, Clarke and Bacic, 1996). Some of these have a nutritional role, since they can be deglycosylated by in vitro growing pollen tubes (Wu, Wang and Cheung, 1995) and are incorporated into actively growing pollen tubes (Lind et al., 1996). This could imply that these glycoproteins support pollen tube growth (Cheung, 1995; Mathó, 1998; Franklin-Tong, 1999).

Upon arrival at the placenta, the path for the growing pollen tubes is much less clearly defined. The placenta appears to have a smooth surface covered by secretions (Sage et al., 1994) and no defined route available for the pollen tube. In peach, the pollen tubes 'wander' around in this area (Herrero, 1992b). However, the absence of marked paths around the placenta contrasts with the precision required to find the ovule entrance. In kiwi, as in other multi-ovulated species, the pollen tubes abandon the placenta to grow on the funiculus of the ovule (González, Coque and Herrero, 1996). Surprisingly, instead of growing on the first ovule they meet, they often bypass a number of ovules before adhering to the one of their choice. A clear explanation of this phenomenon is not yet available.

Once the pollen tube has targeted a particular ovule, subsequent pollen tube growth is by no means straight. Finding the right entrance provides the next challenge and, in peach, it has been observed that pollen tubes navigate around the exostome in a chaotic way. Once the pollen tube enters the exostome it has to traverse the narrow micropylar canal, where it meets the nucellus. Again the lack of clear direction at this point contrasts with the subsequent precision involved in targeting the degenerated synergid. The pollen tube grows across the nucellus and invariably alights precisely at this cell.

These wanderings during pollen tube growth in the ovary reflect growth kinetics that are clearly not constant, but involve both accelerations and decelerations in growth rate (Herrero and Arbeloa, 1989). The contrast between wandering and precision targeting along the pollen tube pathway led us to investigate whether changes in the different tissues the pollen tube has to traverse could be responsible for this differential behaviour.

OVARY GUIDANCE TO POLLEN TUBE GROWTH

Once the pollen tubes have grown through the style and alighted at the ovarian locule, they target the ovules. Different strategies have been adopted by different species to support growth in this transitional territory. Most of these strategies share the common developmental characteristic of a 'carpet' of secretory cells that bridge the space between the base of the style and the ovule exostome. Thus, in many multi-ovulated species the whole placenta is covered by secretory cells (González et al., 1996). In Nicotiana, the secretion is formed by arabinogalactans (Gane, Clarke and Bacic, 1995). One of these secretory structures is the obturator. In spite of the early description of the obturator (Juel, 1918) and its observation in a number of unrelated species (Tilton and Horner, 1980), very little is known about its function. However, it invariably appears to support pollen tube growth on its way to the ovule, and it could be speculated that it represents a further adaptation of a secretory placenta. Thus, by forming a little protuberance between the ovule exostome and the flat placenta, it bridges the gap between the placenta and the ovule entrance, thereby facilitating pollen tube growth. In peach flowers, when the pollen tube arrives at the obturator the cells of the obturator surface are full of starch and devoid of secretion. The pollen tubes are arrested at the obturator for a number of days until this structure enters a secretory phase accompanied by the vanishing of starch and accumulation of callose in the obturator (Arbeloa and Herrero, 1987). Pollen tube growth is only resumed concomitantly with the production of this secretion. This is illustrated in Fig. 1. Thus, in this species, the obturator acts as a drawbridge, connecting the base of the style with the ovule. In kiwi, a multi-ovulated species, small obturators line the placenta, with one facing each ovule. Interestingly, in this species where reproductive performance is strong (yielding over 1000 seeds per fruit) the obturators enter the secretory phase immediately after anthesis, and the pollen tubes travel swiftly over this structure and enter the ovules (González et al., 1996).

In other species, different structures fulfilling a similar role have been described. In Zea mays, papillary hairs line the ovary entrance and may play a role in conducting the pollen tubes from the base of the style to the ovule. Interestingly, upon pollen tube passage, these hairs lose turgidity and prevent other pollen tubes from entering the ovary (Heslop-Harrison, Heslop-Harrison and Reger,
A similar strategy has been described in pistachio, a chalazogamous species, in which the pollen tube enters the ovule via the chalaza instead of following the normal path through the micropyle (Martinez-Pallé and Herrero, 1998). In this species a structure, the ponticulus, fulfills a similar role to the obturator by physically connecting the ovule to the base of the style, thereby facilitating pollen tube access to the ovule (Martinez-Pallé and Herrero, 1995). Once the pollen tubes have passed through the ponticulus, the ovule again becomes isolated from the style by a gap produced by the development of the ovary cavity.

All these mechanisms appear to share a common strategy that involves extending a bridge from the base of the style to the ovule entrance. This bridge, which appears to be well established from anthesis in some species, acts as a drawbridge in others by establishing a connection at a particular time and closing it later. As secretion appears to be required for pollen tube growth, this might enable control of pollen tube passage by simple modification of the time when this secretion is produced.

**OVULE GUIDANCE TO POLLEN TUBE GROWTH**

Once the pollen tube has travelled along the placental surface, it faces the ovule entrance at the exostome. Ovule penetration appears to be a well-controlled process. Analysis of mutants has provided evidence for the existence of genetic control of this process, both at the gametophytic (Hülskamp et al., 1995; Ray et al., 1997) and at the sporophytic (Wilhelm and Preuss, 1996) level. However, very little is known about these mechanisms.

In some ovules in Prunus, pollen tubes wander around the ovule exostome while in others the pollen tube readily penetrates the ovule. To determine whether differences in pollen tube behaviour are related to changes in the ovule, the development of the ovule has been examined in detail during the pollination process. In this species, the ovule is crassinucleated and biegmnic with a Polygonum type embryo sac. Two ovules are present at the ovary but only one develops into a seed; the other degenerates, usually prior to fertilization (Arbeloa and Herrero, 1991). Starch appears to play a primary role in determining the fate of each ovule. At anthesis both ovules have very similar amounts of starch, but subsequently one of them experiences a sudden decrease in starch level, followed by callose deposition at the chalaza. This leads to the isolation and degeneration of this ovule, while the other ovule continues development using up its starch reserves (Rodrigo and Herrero, 1998).

In peach, the integumentary domain is formed by the inner integument, that surrounds the nucellus, and by the outer integument that envelopes the inner integument. The integuments do not fully envelop the nucellus, but there is an opening at the exostome formed by the external integument, as shown in Fig. 2. At anthesis the cells of the exostome do not appear any different to their neighbouring cells. However, later on, and only in some ovules, these cells enter a secretory phase (Fig. 3), followed by callose deposition. Investigation into the presence of starch in this area revealed that only those ovules that still retain their starch at the exostome at the time of pollen tube arrival are able to produce this secretion. These ovules are the ones approached by a pollen tube. Thus, this seems to play an important role in determining pollen tube guidance.

Once pollen tubes have entered the exostome, they face the micropylar canal that is lined by cells of the internal integument. Again, at anthesis, these cells do not look any different to their neighbouring cells, as shown in Fig. 4. However, only in some ovules do these cells enter a secretory phase, and the secretion can be observed inside the micropyle (Fig. 5). The production of secretion is followed by pollen tube penetration (Fig. 6). To understand why this secretion does not appear in all ovules, temporal studies of starch localization in ovules were carried out. Results showed that the secretion is only produced in ovules that still contain starch in the cells lining the micropylar canal at this time and, hence, are competent to enter this secretory phase. Interestingly, the presence of secretion in the micropyle has been described in a number of unrelated species (Chao, 1971; Tilton, 1980; Heslop-Harrison et al., 1985; Reger, Chaubal and Presser, 1992; Fransen-Verheijen and Willemse, 1993; Sage et al., 1994). However, the origin of this secretion is not clear.

The fact that different structures along the pollen tube pathway play an important role in the control of pollen tube penetration of the ovule has been further emphasized by the study of ovule developmental mutants (Gasser, Broadhvest and Hauser, 1998), and with the use of transformed plants with antisense constructs for ACC oxidase (De Martinis and Mariani, 1999). In both cases, plants with incomplete ovule development fail to attract pollen tubes (De Martinis and Mariani, 1999). All these studies suggest that a particular developmental stage must be attained for the ovule to become competent to receive the pollen tube. In peach, two independent controls occur at the integumentary domain. One is exerted by the external integument that lines the exostome, and the other by the internal integument that lines the micropylar canal. For successful pollen tube penetration, the production of secretion in these two domains is necessary. This secretion appears to ‘blaze the trail’ for pollen tube penetration.

**SIGNIFICANCE OF THE INTRAOVARIAN MALE–FEMALE INTERACTION**

It is clear that an intense intraovarian male–female interaction occurs prior to ovule penetration. Both male and female counterparts appear to be mutually affected by each other, and the ovary, like the stigma and the style, also appears to be especially well designed to encourage pollen–pistil interactions. Sporophytic control in the ovary appears to be regulated at two points: by the tissues of the ovary itself and by the sporophytic tissues of the ovule. Developmental stage appears to play a major part in this regulation. It is clear that the pistil has to attain a certain degree of development to support pollen tube growth (Kandasamy, Nasrallah and Nasrallah, 1994). However, this development is not completed upon flower opening, but continues throughout the flower’s lifespan.
Fig. 1. Pollen tube (arrow) growing on a peach obturator (ob) on its way from the base of the style towards the ovule; stained with a mixture (30:1) of 0.1% aniline blue in 0.1 M K$_3$PO$_4$ and 0.07% calcofluor. ×480.

Fig. 2. Exostome in a peach ovule (arrows); stained with 0.07% calcofluor. ×480.

Fig. 3. Secretion (*) inside the exostome of a peach ovule. 2 μm JB4 plastic resin section stained with 0.07% calcofluor and 0.001% auramine in 0.005 M phosphate buffer. ×1200.

Fig. 4. Micropylar canal (arrows) lined by the internal integument (in) and facing the nucellus (nu), in a peach flower at anthesis. 2 μm JB4 plastic resin section stained with 0.07% calcofluor and 0.001% auramine in 0.005 M phosphate buffer. ×480.

Fig. 5. Secreting cells (sc) at the micropylar canal of a peach flower 24 d after pollination. 2 μm JB4 plastic resin section stained with 0.07% calcofluor. ×1200.

Fig. 6. Pollen tube (arrow) in the micropylar canal and reaching the nucellus (nu), in a peach flower 24 d after pollination. 2 μm JB4 plastic resin section stained with 0.07% calcofluor. ×480.
The developmental changes experienced by the different pistillar structures appear to play a major part in conditioning pollen tube behaviour and guidance.

Ovary control appears to be exerted by the placental surface linking the base of the style with the ovule. The pollen tube has to grow on the placental surface and relies on the secretion produced by this structure. Although it remains to be determined whether this secretion shares a common composition with the intercellular substance in the style, interestingly, characterization of arabinogalactans in the pistil (Gane et al., 1995) revealed that they are present both in the style and on the placental surface. A number of glycoproteins have been characterized in the style of different species (Goldman et al., 1992; Du et al., 1994; Cheung et al., 1995; Sommer-Knudsen et al., 1996). The ubiquitous presence of these glycoproteins in the style makes it difficult to assign a precise role for them. However, the mechanisms described here in the ovary of peach provide an ‘on’ and ‘off’ system, with a precise time and location for the production of a secretion. This appears to be essential for pollen tube guidance. The elucidation of the molecules comprising this secretion should provide evidence for the nature of the chemotropic signals required for pollen tube guidance.

The ovule itself also controls pollen tube growth. While other parts of the ovule have yet to be investigated, it is clear that the integuments play a definite role in regulating pollen tube access to the ovule. In peach, this control appears to be exerted by the production of secretions both at the exostome by the external integument, and at the micropylar canal by the internal integument. These secretions are again produced at a particular time and stage of development, and are required for the pollen tube to penetrate the ovule.

It would be interesting to establish how the biochemical changes experienced by these structures, upon pollen tube passage, affect other incoming pollen tubes. It is well known that, in spite of the high number of pollen grains that may land on the stigma of a flower and the high number of ovules that are present in some species, fertilization occurs in an orderly manner, with each ovule being penetrated by a single pollen tube. However, the nature of the signalling cues sent by a penetrated ovule to other pollen tubes to deter them from approaching or entering is still far from clear. Changes taking place following pollen tube passage, e.g. the lack of turgidity in the hairs of Zea mays (Heslop-Harrison et al., 1985), could well play a part in regulating further access of pollen tubes to that particular ovule.

Although it is not known whether the interactions described here exist in other species, the fact that the structures supporting these mechanisms are conserved in distantly related species suggests that this may well be the case. The structures described here are highly conserved in angiosperms, although a clear function has not yet been allocated to them. However, the possible existence of mechanisms, similar to those described here, could explain why these structures are evolutionarily conserved. Likewise secretions, both in the ovule (Chao, 1971; Tilton, 1980; Heslop-Harrison et al., 1985; Reger, Chauval and Presser, 1992; Franssen-Verheijen and Willems, 1993; Sage et al., 1994) and in the placenta (Singh and Walles, 1992), have been described in a number of different species. Surprisingly, the secretions recorded in the ovule resemble the secretion observed in the naked ovules of gymnosperms (Owens, Takaso and Runions, 1998), as well as in extant angiosperms (see Sage et al., 1994 for a review). It is tempting to speculate that this may be a highly conserved characteristic that has survived with the acquisition of the pistil, thereby conferring angiosperms with added systems of control. Whether this is the case or, alternatively, we are facing convergent evolutionary processes, ovular secretions appear to play a primary role in the control of pollen tube access to the ovule both in angiosperms and gymnosperms.

Nevertheless, the real implications of this pollen tube–ovary interaction remain unknown. It would be interesting to evaluate how these mechanisms relate to ovary incompatibility systems, both at the interspecific (Williams et al., 1982) and at the intraspecific level (Seavey and Bawa, 1986; Sage et al., 1994). Likewise the occurrence of mate choice in plants has been much discussed (Willson and Burley, 1983; Marshall and Folsom, 1991). In this sense, the interaction between the pistil and the growing male gametophytes might result in non-random fertilization (Hormaza and Herrero, 1994) producing changes in the subsequent generation with significant evolutionary implications (Mulcahy, 1979). It would be worthwhile evaluating how the mechanisms described here can be modified in order to favour fertilization by a particular genotype and how these mechanisms relate to fertilization success. In peach, a clear link appears to exist between the production of secretion in the obturator, exostome and in the micropylar canal and successful pollen tube growth in these structures, indicating that secretion is a prerequisite for pollen tube penetration of the ovule. Failure to produce these secretions leads to chaotic and undirected pollen tube growth. However, more information is required to establish why a proportion of the ovules fail to produce these secretions. The fact that starch is necessary for the production of this secretion may be part of the answer and work is in progress to evaluate the implications of starch reserves in the reproductive process (Rodrigo and Herrero, 1998).

Both ovary and ovule control of pollen tube growth appear to share a common nexus and this is the production of secretions required for guiding pollen tube growth. In some species these secretions appear to be present right from the time of anthesis, while in others they are produced at a specific time, when a particular developmental stage is reached. This temporal factor confers the ovary with an added system of regulating pollen tube behaviour, since small differences in the timing of ovary and ovule development play a part in determining pollen tube behaviour. Taken together, the information available clearly suggests that the tortuous pollen tube trip along the ovary is greatly affected by developmental changes taking place both in the ovary and ovule. The pollen tube in turn, also affects both ovary and ovule development (O’Neil, 1997). As a result of these two-way interactions, the pollen tube finds its way towards the embryo sac and fertilization occurs in a well-controlled and orderly fashion.
The elucidation of these mechanisms in other species and their implications in the control of fertilization should shed light on how plants regulate this final private, largely hidden and poorly understood phase of male–female interaction and its consequences for the next generation.

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LITERATURE CITED


