Male and female synchrony and the regulation of mating in flowering plants

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Successful mating clearly requires synchronous development of the male and female sexual organs. Evidence is accumulating that this synchrony of development also persists after pollination, with both pollen and pistil following complex, but highly integrated developmental pathways. The timing of the male–female interaction is crucial for the pistil, which, far from being a mature passive structure, is engaged in a continuing programme of development: only being receptive to the advances of the pollen for a relatively short window of time. This developmental programme is most conspicuous in the ovary, and this review focuses on the interaction between the male and female tissues in this structure. The review first considers pollen tube development in the ovary, concentrating of the mechanisms by which its growth is modulated at various control points associated with structures within the ovary. Second, alterations to this ‘normal’ developmental programme are reviewed and considered in the context of a breakdown of developmental synchrony. Finally, the consequences of male–female developmental synchrony and asynchrony are explored. Clearly, a synchronous male–female relationship leads to a successful fertilization. However, lack of synchrony also occurs, and could emerge as a powerful tool to investigate the regulation of mating.

Keywords: pollen tube; ovary; ovule; megagametophyte; fertilization

1. INTRODUCTION

Reproductive success in any organism demands coordinated male and female reproductive development, and it follows that complete failure of this coordination leads directly to reproductive isolation. However, slight differences in developmental synchrony may affect the direction of gene flow, rather than preventing it; for example, slight asynchrony in development is held to promote outbreeding in a range of species (Sedgley & Griffin 1989). Importantly, a more subtle, but none the less essential requirement for developmental synchrony also occurs after pollination, once the microgametophyte has commenced development within the pistil.

Fertilization in plants, as in animals, is preceded by a complex and sometimes extended interaction between the male and female participants: a process believed to have evolved to ensure matching of appropriate mating partners. Motility in animals plays a clear part in this ‘courtship’, but, in plants, perhaps because of their sessile nature, this interaction occurs within the flower. Pistils of different species have evolved myriad morphologies and physiologies for this purpose. Following germination of the pollen grain on the stigma, a structure clearly adapted for the capture and the promotion of early development of the grain, the pollen tube grows first into the style and thence into the ovary. This phase of growth maintains the separation of the male and female gametes, and provides the opportunity for an extensive molecular conversation

between the male and female protagonists (Knox 1984; Herrero 1992; Cheung & Wu 2001; Franklin-Tong 2002). Although much is now known of events in the style (Cheung 1996) the events in the ovary, before fertilization, remain largely unknown. The female gamete itself is enveloped in several concentric layers, the nucellus and integuments, which develop consecutively around the embryo sac. The pollen tube, on its journey to the female gamete, has to traverse these barriers through specific entrance points, which effectively act as gates regulating access to the egg (Herrero 2001). The timing of these events is crucial for, far from been a passive structure, the pistil continues to develop along a pathway that can both favour and discourage growth of the pollen tube. This is particularly apparent in the ovary, which owing to the basipetal maturation of the pistil (Herrero & Arbeloa 1989), develops last. Of course, the pollen tube itself is following its own developmental pathway as it passes through the stigma and into the ovary, and the synchrony of these parallel pathways is clearly a key to successful fertilization and seed formation.

Evidence is reviewed here of instances where this synchrony is particularly essential between male and female development during growth of the pollen tube within the ovary. Consideration is also given to the consequences to reproductive success, and thence gene flow, when this male–female developmental synchrony breaks down.

2. SUCCESSFUL MATING IS PRECEDED BY A SYNCHRONOUS MALE–FEMALE RELATIONSHIP

Despite the massive variety of floral morphologies, pollen tube development in the pistil remains a highly con-

served process. The tube invariably follows the same pathway, germinating in the stigma, growing through the style and entering the ovary. Research so far has identified the pistil as a largely passive structure through which the pollen tubes grow. Evidence is, however, accumulating that the pistil is a highly active structure undergoing continual development, and which plays a crucial part in regulating pollen tube growth (Herrero & Hormaza 1996).

(a) *Pollen tube pathway in the ovary*

Upon arrival at the base of the style, the pollen tube moves onto the surface of the placenta. Whereas growth in the style follows a pathway principally determined by the availability of reserves (Herrero & Dickinson 1979), in the ovary the pollen tube faces a different environment, open and, apparently, with no clearly defined pathway ahead. In several unrelated species, the tube, as it approaches the ovule over the placental surface, encounters the obturator. This structure normally takes the form of a small protusion on the placental surface facing the entrance to the ovule. Although this structure was described a century ago (Juel 1918), little attention has been given to it since. However, in the few descriptions in the literature, the pollen tube is always reported to travel over its surface towards the ovule (Tilton & Horner 1980).

Once at the ovule, all pollen tubes, whether or not they have encountered an obturator, enter through the exostome, travel through the micropyle, penetrate the nucellus and finally enter the embryo sac. Penetration of the embryo sac occurs through one of the two synergids. In porogamous species, in which pollen tubes enter directly from the micropyle, the synergids together with the egg cell, are located directly in the pathway of the pollen tube. In chalazogamous species, however, where the pollen tube penetrates the ovule at the opposite pole, at the chalaza, the pollen tube tip travels around the periphery of the embryo sac to the micropylar pole, where again it enters one of the synergids (Martinez-Pallé & Herrero 1998).

(b) *Ovary development*

As the pollen tube extends into the ovary, conspicuous changes overcome several structures that interact with the developing microgametophyte. Significantly, these events appear to occur in phase with changes in pollen tube development. Although the data remain necessarily preliminary, such changes have so far been recorded at the placenta and obturator, and also in the sporophytic tissues of the ovule in the exostome, micropyle and nucellus. Further, changes also occur within the megagametophyte itself.

Secretions on the placental surface have been described in several unrelated species (reviewed in Sage *et al.* 1994); however, work on peaches showed that the obturator also produced a secretion that was required for pollen tube extension (Arbeloa & Herrero 1987). Evidence for this came from experiments which showed that, upon arrival at the base of the style, pollen tubes stopped growth for some 5 days and only resumed extension on production of this secretion. Thus, the obturator acts as a bridge facilitating the passage of the pollen tube in its journey towards the ovule, but only for a defined period, and at a specific stage of development. In *Zea mays* a similar mechanism

was also described. Here, papillar hairs cover the ovary entrance which, as the pollen tube passes through, lose turgidity and prevent other pollen tubes from entering the ovary (Heslop-Harrison *et al.* 1985). In *Pistacia*, a similar strategy is adopted by a different structure: the ponticulus, which develops coincidentally with the appearance of the pollen tube at the ovary, and provides a bridge across the gap between the ovule and the base of the style (Martinez-Pallé & Herrero 1995).

Several changes also appear to be related to ovule penetration. For example, in the peach, the cells bordering the exostome enter a secretory phase. Without this secretion, pollen tubes will not enter the micropyle. Similarly, the cells bordering the micropylar canal also produce a secretion as the pollen tubes pass through this region (Herrero 2000). Ovular secretions have also been described for a range of different species (Chao 1971; Tilton 1980; Sage *et al.* 1994). The origin of this secretion remains unclear: in some instances it appears to arise from the sporophytic cells that border the micropyle (Herrero 2000), whereas in others it appears to arise from the synergids themselves (Franssen-Verheijen & Willemse 1993).

Finally, the synergids are emerging as playing a pivotal role in the reproductive process (Higashiyama 2002). In all species studied, synergid degeneration occurs on pollen tube penetration (Jensen 1965; Russell 1996), and recent work with ablation experiments clearly demonstrated that the synergids are required for pollen tube penetration into the embryo sac (Higashiyama *et al.* 2001). Further, embryo sacs with degenerated synergids fail to attract pollen tubes (Higashiyama *et al.* 1998). As the synergid degenerates, an actin corona has been reported to develop in mature embryo sacs (Huang & Russell 1994), which has been shown to play a central part in the delivery of the male gametes to the egg cell and polar nucleus (Russell 1996; Fu *et al.* 2000).

3. ALTERATIONS IN POLLEN TUBE DEVELOPMENT AND MALE-FEMALE ASYNCHRONY

Establishing a relationship between pollen tube growth and developmental changes in the pistil has proved possible by observing pollen tube behaviour during aberrant pistil development. From this work it is clear that interactions between the male and female tissues differ dramatically at different locations within the pistil, but also that these mechanisms are strikingly conserved between species.

(a) *Aberrations in pollen tube development*

Alterations to normal pollen tube behaviour have been observed in a wide range of species over a period of many years, frequently without any clear explanation. However, the recent identification of developmental mutants, which have distorted pollen tube growth (Hulskamp *et al.* 1995; Wihelmi & Preuss 1996; Shimizu & Okada 2000), and accumulating data that changes in ovary development impact on pollen tube growth (Herrero 2000, 2001), provide a new framework in which to view these observations.

In general, aberrant pollen tube behaviour within the ovary falls into three categories: pollen tube wandering, pollen tube branching and chalazogamy. Whereas the pollen tube normally grows steadily through the style towards

the ovary, aberrant tube development has been recorded for several species on arrival at the ovary (Maheshwari 1950). In some instances the pollen tube wanders without any obvious direction, often describing a circular path. In others, pollen tube branching occurs at this stage, as though the pollen tube tip had difficulties deciding on the course it should take. Some of these reports referred to these branched tubes as haustorial structures, and they were interpreted as having a nutritional role (Johri 1992). This behaviour has also been reported in interspecific crosses and has been related to interspecific incompatibility (Williams *et al.* 1982). However, pollen tube wandering and branching has also been reported in intraspecific crosses, and has been linked to self-incompatibility occurring within the ovary (Seavey & Bawa 1986). Although all these studies attempt to associate aberrant behaviour with aspects of incompatibility and incongruity, the fact remains that pollen tubes wander and branch in the ovary following normal compatible matings (Herrero 2000, 2001). Here, these phenomena appear to be associated with a lack of maturity of the female tissue: as normal pollen tube growth resumed concomitantly with the production of secretion at particular points along the pollen tube pathway within the ovary. It thus suggests that the different female structures encountered by the growing pollen tube have to attain particular developmental states before they are competent to 'encourage' the passage of the pollen tubes. Of course, both intraspecific and/or interspecific incompatibility mechanisms may cause these interactions to become out of phase, and thus prevent fertilization. Because mutants of *Arabidopsis* displaying distorted pollen tube growth in the ovary are currently available, it is likely that the genes involved in these interactions will soon be identified (Hulskamp *et al.* 1995; Wihelmi & Preuss 1996; Palanivelu *et al.* 2001; Shimizu & Okada 2000).

In most plants, the pollen tube follows a highly conserved path, entering the ovule through the micropyle, a narrow canal formed by the integuments. However, in several species, pollen tubes follow an alternative pathway and penetrate the ovule through the chalaza, at the opposite end of the micropyle. The discovery of chalazogamy at the beginning of the last century led to the suggestion that a new classification of angiosperms should be devised, depending on the site of pollen tube penetration. However, chalazogamy occurs in otherwise unrelated species and, further, chalazogamy occurs together with the normal porogamous penetration in closely related species, or even within the same species. Recently, the observation that, within the same species, the pollen tube follows the normal path through the micropyle when the micropyle is fully developed, but displays chalazogamous penetration in immature micropyles (Luza & Polito 1991) has led to the proposal that chalazogamy is an alternative pathway for pollen tube growth, when the micropyle is non-receptive. Interestingly, close examination of early drawings of chalazogamous penetration in different species has confirmed an underdeveloped micropyle in many of the ovules studied (Martinez-Pallé & Herrero 1998).

(b) *Male-female asynchrony during ovary development*

The concept that differences in pistil development may lead to altered pollen tube behaviour is based on three

main lines of evidence: studies of development, work with mutants and ablation experiments. This interrelationship between male and female tissues has now been described for several different points along the pollen tube pathway in the ovary. Work on peaches (Herrero 2000) has identified several control points or gates within the ovary, which regulate access of the pollen tube to the female gametophyte. These control points are the obturator, exostome, micropyle and eventually, the nucellar tissue itself. On the placental surface of the peach, alterations in pollen tube growth were first seen associated with the obturator (Arbeloa & Herrero 1987), which strongly suggested that a secretion by this structure was required for the pollen tube to progress. Further, in plants where a delayed penetration of the ovule was recorded (Herrero & Arbeloa 1989), it was later discovered that the delay coincided precisely with the time required for the ovules to produce a secretion at the exostome and micropyle (Herrero 2000).

Although 'developmental' timing is clearly important in pollen-pistil relationships, work with mutants has also highlighted that changes in female development can also impact on pollen tube growth. For example, the first observations on defective pollen tube guidance were done on mutants that failed to develop an embryo sac (Hulskamp *et al.* 1995), although it was not clear whether this was a direct consequence of the absence of the embryo sac, or resulted from secondary effects via sporophytic tissues (Hulskamp *et al.* 1995; Drews *et al.* 1998). The crucial role of the embryo sac in pollen tube guidance was clearly demonstrated in plants with a reciprocal chromosome translocation that generated flowers in which half of the ovules possessed no embryo sac. On pollination only half of the ovules—those with a fully developed embryo sac—were penetrated by a pollen tube (Ray *et al.* 1997). The same effect was also achieved with meiotic mutants possessing 50% aborted embryo sacs (Couteau *et al.* 1999). More recently, the roles that the different cell types of the embryo sac have in 'managing' the final stages of pollen tube growth have been demonstrated by ablation experiments (Higashiyama *et al.* 2001). The synergids clearly play a central part in this process (Higashiyama 2002), and the two actin coronas that develop in these cells are obviously essential for sperm delivery and double fertilization (Huang & Russell 1994). Interestingly, mutants with an altered corona also show differences in pollen tube penetration (Huang & Sheridan 1998), suggesting that a fully developed embryo sac and, in particular, a functional egg cell apparatus (Higashiyama *et al.* 2001) is required for successful pollen tube penetration.

Whether indirectly or directly, the sporophyte must also have a key role in the male-female interactions that characterize the later stages of pollen tube growth. Distorted pollen tube growth in the placenta has been reported in sporophytic mutants of *Arabidopsis* (Wihelmi & Preuss 1996), and the study of a range of ovule development mutants has identified the sporophyte as regulating interactions with the growing pollen tube (Gasser *et al.* 1998). Finally, the use of transformed plants containing ACC oxidase antisense constructs has also highlighted the fact that underdeveloped ovules fail to attract pollen tubes (De Martinis & Mariani 1999). Taken together, these data all confirm that for the ovule to be attractive to the pollen tube it must first attain a particular

developmental state. It is anticipated that a combination of new studies on mutants with defective pollen tube guidance resulting from delayed development of the female gametophyte (Shimizu & Okada 2000), and an increased understanding of the genetic control of female gametophyte development and function (Gary *et al.* 2002), will allow the molecular genetic analysis of these complex but highly significant interactions.

A delicate interplay between the developing pollen tube and both sporophytic tissues and the female gametophyte is thus required for ovular penetration. Because pollen tube arrest at different 'control points' has been described both in interspecific (Williams *et al.* 1982) and intraspecific (Seavey & Bawa 1986; Sage *et al.* 1994) pollinations, this suggests evolution may have exploited these interactions to regulate matings both within and between species.

4. PERSPECTIVES

Although further work is necessary to determine the trigger for these responses in the pistil, the fact that some of them (Arbeloa & Herrero 1987; Herrero 2000) are developmentally regulated indicates that development and timing play a critical part in controlling the reproductive process. Synchrony of development between the male and female partners is obviously essential for reproductive success. It follows that experiments where this synchrony is disrupted will provide valuable data on the key events of the mating process.

(a) *The benefits of synchrony*

Although a synchronous male–female relationship clearly leads to successful fertilization, the question remains as to how this synchrony is achieved. One possibility is that changes in the pistil required for pollen tube growth are triggered by either the pollination event or the growing pollen tube. However, evidence is accumulating that whereas some events certainly are triggered by pollination, others are developmentally regulated. Pollination has been shown to induce several changes in the pistil (Linskens 1986); in the stigma, pollination induces the production of stigmatic secretion in several species (Sedgley & Scholefield 1980), whereas in the style pollination stimulates secretion in cells of the transmitting tissue. Interestingly, starch accumulated in the transmitting tissue is depleted as the pollen tubes travel past, but remains unaltered in unpollinated flowers, suggesting that starch metabolism is also stimulated by the presence of the pollen tube (Herrero & Dickinson 1979). However, in the ovary, secretion at the obturator is not affected by the pollen tubes and occurs at the same developmental time-point in pollinated and unpollinated flowers (Arbeloa & Herrero 1987).

Whether developmentally regulated or triggered by the pollen tube, these events within the pistil constitute a complex programme, which, for successful pollination, must be followed in the 'correct' order. Whether these mechanisms are linked together, or simply occur independently of one another, remains to be determined. Certainly the events occurring in the sporophytic tissues can be regarded as components of a normal developmental pathway, but the relationship between the megagametophyte, the surrounding sporophyte and the pollen tube is far from

clear. Indeed, in some species such as in the peach (Arbeloa & Herrero 1991), the female gametophyte does not even exist at the time when the sporophytic machinery necessary for fertilization is established. There is little information on the lifespan of the megagametophyte, but observations on *Prunus* species (M. Herrero, unpublished results) show that it is surprisingly short. Moreover, it remains in a process of continuous development throughout its existence, with fertilization only occurring in a specific stage of development. The narrowness of this window for successful fertilization is also highlighted by recent work that shows that only ovules with at least one undegenerated synergid are able to attract a pollen tube (Higashiyama *et al.* 2001). Further, the fact that fertilization will only occur when the two gametes are at the same stage of the cell cycle (Friedman 1999), also reduces this window of receptivity, and stresses the requirement for a male–female synchrony.

(b) *Asynchrony as a tool for investigating the control of mating*

Although lack of developmental synchrony between the male and female must normally lead to sterility, slight asynchrony is not always lethal. Delayed fertilization is a characteristic of gymnosperms, but is also common in many angiosperms (Willson & Burley 1983). Indeed, despite the fact that the reproductive process is highly conserved, the time required by different species to accomplish fertilization is very variable, and ranges from a few hours in *Arabidopsis* (Faure *et al.* 2002) to months in *Corylus* or *Quercus* (Willson & Burley 1983). No clear explanation has, so far, been provided for this observation. In the peach, the pollen tube has to wait at several points along its pathway within the ovary, waiting for particular developmental stages to be attained (Herrero 2000). It is not unreasonable to propose that a delayed development of the pistil may also account for delayed fertilization in other species, and further studies in the species listed above are clearly required.

Importantly, the reproductive process is sensitive to environmental conditions. For instance, temperature—one of the easiest parameters to study—has been shown to affect the speed of pollen tube growth (Lewis 1942). But temperature also affects pistil development and degeneration. Warm temperatures speed up pollen tube growth, but also accelerate pistil degeneration. Cool temperatures have the opposite effect (Sanzol & Herrero 2001). This different response of the male and female tissues must act to compensate for the effect of temperature, and may have assisted in adaptation to a wider range of temperatures. This vulnerability of both the male and the female to environmental stresses, together with the short receptive period of the megagametophyte may have resulted in the evolution of a phase of tight synchrony between male and female development, perhaps necessary to maximize reproductive success.

These differences in male–female synchrony occurring in nature may simply be a function of the remarkable capacity of the pollen tube to 'wait'. Alternatively, this asynchrony may be of more significance, for it has been proposed that delayed fertilization may increase the time for mate choice and selective fertilization (Willson & Burley 1983). It also follows that as differences between the

male and female in the programming of reproductive maturity become more extreme, genetic isolation will result. As with spatial isolation, this could lead to rapid speciation.

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REFERENCES

- Arbeloa, A. & Herrero, M. 1987 The significance of the obturator in the control of pollen tube entry into the ovary in peach (*Prunus persica*). *Ann. Bot.* **60**, 681–685.
- Arbeloa, A. & Herrero, M. 1991 Development of the ovular structures in peach (*Prunus persica* (L.) Batsch). *New Phytol.* **118**, 527–534.
- Chao, C. Y. 1971 A periodic acid-Schiff's substance related to the directional growth of pollen tube into embryo sac in *Paspalum* ovules. *Am. J. Bot.* **58**, 649–654.
- Cheung, A. Y. 1996 Pollen–pistil interactions during pollen-tube growth. *Trends Pl. Sci.* **1**, 45–51.
- Cheung, A. Y. & Wu, H. M. 2001 Plant biology: pollen tube guidance right on target. *Science* **293**, 1441–1442.
- Couteau, F., Belzile, F., Horlow, C., Grandjean, O., Vezon, D. & Doutriaux, M. P. 1999 Random chromosome segregation without meiotic arrest in both male and female meiocytes of a *dmc1* mutant of *Arabidopsis*. *Pl. Cell* **11**, 1623–1634.
- De Martinis, D. & Mariani, C. 1999 Silencing gene expression of the ethylene-forming enzyme results in a reversible inhibition of ovule development in transgenic tobacco plants. *Pl. Cell* **11**, 1061–1072.
- Drews, G. N., Lee, D. & Christensen, C. A. 1998 Genetic analysis of female gametophyte development. *Pl. Cell* **10**, 5–17.
- Faure, J.-E., Rotman, N., Fortuné, P. & Dumas, C. 2002 Fertilization in *Arabidopsis thaliana* wild-type: developmental stages and time-course. *Pl. J.* **30**, 481–488.
- Franklin-Tong, V. E. 2002 The difficult question of sex: the mating game. *Curr. Opin. Pl. Biol.* **5**, 14–18.
- Franssen-Verheijen, M. A. W. & Willemsse, M. T. M. 1993 Micropylar exudate in *Gasteria* (Aloaceae) and its possible function in pollen tube growth. *Am. J. Bot.* **80**, 253–262.
- Friedman, W. E. 1999 Expression of cell cycle in sperm of *Arabidopsis*: implications for understanding patterns of gametogenesis and fertilization in plants and other eukaryotes. *Development* **126**, 1065–1075.
- Fu, Y., Yuan, M., Huang, B.-Q., Yang, H.-Y., Zee, S.-Y. & O'Brien, T. P. 2000 Changes in actin organization in the living egg apparatus of *Torenia fournieri* during fertilization. *Sex. Pl. Reprod.* **12**, 315–322.
- Gary, N., Drews, L. & Yadegari, R. 2002 Development and function of the angiosperm female gametophyte. *A. Rev. Genet.* **36**, 99–124.
- Gasser, C. S., Broadhvest, J. & Hauser, B. A. 1998 Genetic analysis of ovule development. *A. Rev. Pl. Physiol. Pl. Mol. Biol.* **49**, 1–24.
- Herrero, M. 1992 From pollination to fertilization in fruit trees. *Pl. Growth Reg.* **11**, 27–32.
- Herrero, M. 2000 Changes in the ovary related to pollen tube guidance. *Ann. Bot.* **85**(Suppl. A), 79–85.
- Herrero, M. 2001 Ovary signals for directional pollen tube growth. *Sex. Pl. Reprod.* **14**, 3–7.
- Herrero, M. & Arbeloa, A. 1989 Influence of the pistil on pollen tube kinetics in peach (*Prunus persica*). *Am. J. Bot.* **76**, 1441–1447.
- Herrero, M. & Dickinson, H. G. 1979 Pollen–pistil incompatibility in *Petunia hybrida*: changes in the pistil following compatible and incompatible intraspecific crosses. *J. Cell Sci.* **36**, 1–18.
- Herrero, M. & Hormaza, J. I. 1996 Pistil strategies controlling pollen tube growth. *Sex. Pl. Reprod.* **9**, 343–347.
- Heslop-Harrison, J., Heslop-Harrison, Y. & Reger, B. J. 1985 The pollen–stigma interaction in the grasses. 7. Pollen tube guidance and the regulation of tube number in *Zea mays* L. *Acta Bot. Neerl.* **34**, 193–211.
- Higashiyama, T. 2002 The synergid cell: attractor and acceptor of the pollen tube for double fertilization. *J. Pl. Res.* **115**, 149–160.
- Higashiyama, T., Kuroiwa, H., Kawano, S. & Kuroiwa, T. 1998 Guidance *in vitro* of the pollen tube to the naked embryo sac of *Torenia fournieri*. *Pl. Cell* **10**, 2019–2031.
- Higashiyama, T., Yabe, S., Sasaki, N., Nishimura, Y., Miyagishima, S., Kuroiwa, H. & Kuroiwa, T. 2001 Pollen tube attraction by the synergid cell. *Science* **293**, 1480–1483.
- Huang, B. Q. & Russell, S. D. 1994 Fertilization in *Nicotiana tabacum*: cytoskeletal modifications in the embryo sac during synergid degeneration. *Planta* **194**, 200–214.
- Huang, B. Q. & Sheridan, W. E. 1998 Actin coronas in normal and indeterminate gametophyte 1 embryo sacs of maize. *Sex. Pl. Reprod.* **11**, 257–264.
- Hülkamp, M., Schneitz, K. & Pruitt, R. E. 1995 Genetic evidence for a long-range activity that directs pollen tube guidance in *Arabidopsis*. *Pl. Cell* **7**, 57–64.
- Jensen, W. A. 1965 The ultrastructure and histochemistry of the synergids of cotton. *Am. J. Bot.* **52**, 238–256.
- Johri, B. M. 1992 Haustorial role of pollen tubes. *Ann. Bot.* **70**, 471–475.
- Juel, H. O. 1918 Beiträge zur Blütenanatomie und zur systematik der Rosaceen. *Kungliga Svenska Vetenskapsakademien Handlingar* **58**, 5.
- Knox, R. B. 1984 Pollen–pistil interactions. In *Cellular interactions. Encyclopedia of plant physiology, new series 17* (ed. H. F. Linskens & J. Heslop-Harrison), pp. 508–608. Berlin: Springer.
- Linskens, H. F. 1986 Recognition during the progamic phase. In *Biology of reproduction and cell motility in plants and animals* (ed. M. Cresti & R. Dallai), pp. 21–31. Siena, Italy: University of Siena.
- Lewis, D. 1942 The physiology of incompatibility in plants. I. Effect of temperature. *Proc. R. Soc. Lond. B* **131**, 13–26.
- Luza, J. G. & Polito, V. S. 1991 Porogamy and chalazogamy in walnut (*Juglans regia* L.). *Bot. Gaz.* **15**, 100–106.
- Maheshwari, P. 1950 *An introduction to the embryology of Angiosperms*. New York: McGraw-Hill.
- Martínez-Pallé, E. & Herrero, M. 1995 The ponticulus: a structure bridging pollen tube access to the ovule in *Pistacia vera*. *Sex. Pl. Reprod.* **8**, 217–222.
- Martínez-Pallé, E. & Herrero, M. 1998 Pollen tube pathway in chalazogamous *Pistacia vera* L. *Int. J. Pl. Sci.* **159**, 566–574.
- Palanivelu, R., Wihelmi, L. K. & Preuss, D. 2001 POP2, a gene required for guidance of *Arabidopsis* pollen tubes is similar to class III aminotransferases. *Dev. Biol.* **235**, 211.
- Ray, S., Park, S. S. & Ray, A. 1997 Pollen tube guidance by the female gametophyte. *Development* **124**, 2489–2498.
- Russell, S. D. 1996 Attraction and transport of male gametes for fertilization. *Sex. Pl. Reprod.* **9**, 337–342.
- Sage, T. L., Bertin, R. I. & Williams, E. G. 1994 Ovary and other late-acting self-incompatibility systems. In *Genetic control of self-incompatibility and reproductive development in flowering plants* (ed. E. G. Williams, A. E. Clarke & R. B. Knox), pp. 116–140. Dordrecht, The Netherlands: Kluwer.
- Sanzol, J. & Herrero, M. 2001 The effective pollination period in fruit trees. *Sci. Hort.* **90**, 1–17.
- Seavey, S. R. & Bawa, K. S. 1986 Late-acting self-incompatibility in angiosperms. *Bot. Rev.* **52**, 195–218.

- Sedgley, M. & Griffin, A. R. 1989 *Sexual reproduction of tree crops*. London: Academic.
- Sedgley, M. & Scholefield, P. B. 1980 Stigma secretion in watermelon before and after pollination. *Bot. Gaz.* **141**, 428–434.
- Shimizu, K. K. & Okada, K. 2000 Attractive and repulsive interactions between female and male gametophytes in *Arabidopsis* pollen tube guidance. *Development* **127**, 4511–4518.
- Tilton, V. R. 1980 The nucellar epidermis and micropyle of *Ornithogalum caudatum* (Liliaceae) with a review of these structures in other taxa. *Can. J. Bot.* **58**, 1872–1884.
- Tilton, V. R. & Horner Jr, H. T. 1980 Stigma, style and obturator of *Ornithogalum caudatum* (Liliaceae) and their function in the reproductive process. *Am. J. Bot.* **67**, 1113–1131.
- Wilhelmi, L. K. & Preuss, D. 1996 Self-sterility in *Arabidopsis* due to defective pollen tube guidance. *Science* **274**, 1535–1537.
- Williams, E. G., Knox, R. B. & Rouse, T. 1982 Pollination subsystems distinguished by pollen tube arrest after interspecific crosses in *Rhododendron*. *J. Cell Sci.* **53**, 255–273.
- Willson, M. F. & Burley, N. 1983 *Mate choice in plants. Tactics, mechanisms and consequences*. Princeton University Press.

Discussion

S. C. H. Barrett (*Department of Botany, University of Toronto, Toronto, Canada*). Do you have any evidence that the presence of self pollen on the stigma or in the style influences the various ‘gates’ or selective barriers in the ovary that you have described? All of the processes that you have described were from flowers that were exclusively cross-pollinated. I am wondering whether self pollen may influence these processes.

M. Herrero. No, we have not added self pollen to stigmas as well as outcross pollen. All our studies have involved cross pollen (outcrossing) or unpollinated flowers.

A. J. Richards (*School of Biology, University of Newcastle upon Tyne, Newcastle upon Tyne, UK*). In *Primula*, intraspecific incompatibility occurs in the style or above, but interspecific incompatibility between style base and micropyle. Could this be an example where an intraspecific ‘gate’ has been commandeered as a speciation mechanism?

M. Herrero. It seems very probable.