Development 124, 2489-2498 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
DEV0106

Pollen tube guidance by the female gametophyte

Sumita Ray, Sung-Sik Park and Animesh Ray*

Department of Biology, University of Rochester, Rochester, NY 14627, USA

*Author for correspondence (e-mail: ray@ar.biology.rochester.edu)

SUMMARY

In flowering plants, pollen grains germinate on the pistil and send pollen tubes down the transmitting tract toward ovules. Previous genetic studies suggested that the ovule is responsible for long-range pollen tube guidance during the last phase of a pollen tube's journey to the female gametes. It was not possible, however, to unambiguously identify the signaling cells within an ovule: the haploid female gametophyte or the diploid sporophytic cells. In an effort to distinguish genetically between these two possibilities, we have used a reciprocal chromosomal translocation to generate flowers wherein approximately half the ovules do not contain a functional female gametophyte but all ovules contain genotypically normal sporophytic cells. In these flowers, pollen tubes are guided to the normal but not to the abnormal female gametophytes. These results strongly suggest that the female gametophyte is responsible for pollen tube guidance, but leave open the possibility that the gametophyte may accomplish this indirectly through its influence on some sporophytic cells.

Key words: *Arabidopsis*, reproduction, ovule, pollen, cell guidance, cell signaling

INTRODUCTION

Long range guidance of cells to their targets is a common theme in metazoan development. Examples include the homing of pollen tubes (Heslop-Harrison, 1987; Lord and Sanders, 1992; Pruitt and Hülskamp, 1994; Hülskamp et al., 1995; Wilhelmi and Preuss, 1996) and sperms (Amanze and Iyengar, 1990; Russell, 1993) to the female gamete, and the guidance of axonal growth cones to their target cells (Kolodkin, 1996). Cellular guidance may involve at least two distinct mechanisms. The guided cell may respond to a gradient of positive or negative signal issuing from the target or its surrounding cells (Heslop-Harrison and Heslop-Harrison, 1986; Baier and Bonhoeffer, 1994; Hülskamp et al., 1995; Dodd and Schuchardt, 1995). Alternatively, the guided cell may show a polarized growth along a pre-established track (Sanders and Lord, 1989; Amanze and Iyengar, 1990; Lord and Sanders, 1992).

In flowering plants, a pollen grain alighting on the stigma of a compatible flower germinates to produce a pollen tube that enters the intercellular space of the ovary (Elleman et al., 1992; Nasrallah and Nasrallah, 1993; Hiscock et al., 1996). The pollen tube grows through a specialized ovarian tissue, the transmitting tract, to emerge at an apparently random point on the inner surface of the carpel (Mascarenhas, 1993; Kandasamy et al., 1994; Pruitt and Hülskamp, 1994). From that point, the pollen tube grows along an ovule stalk towards the micropyle where it liberates two sperm cells on their way to double fertilization inside the embryo sac (Pruitt and Hülskamp, 1994; Hülskamp et al., 1995).

Pollen tube guidance has at least two distinct phases that may be mechanistically different. The initial guidance of the tube through the transmitting tract probably occurs along a pre-established path laid down by sporophytic tissues (Sanders and Lord, 1989; Lord and Sanders, 1992). Pollen tube re-emergence on the inner cell surface of the ovary, and the tube's subsequent guidance to an ovule, requires genes that are expressed both in the maternal sporophyte and the male gametophyte (Wilhelmi and Preuss, 1996). Adhesion of the pollen tube to the sporophytic cell surface plays an important role in this process (Wilhelmi and Preuss, 1996; reviewed by Smyth, 1997). The final phase of guidance appears to occur by a response of the pollen tube to an yet unidentified signal(s) produced by the ovule (Hülskamp et al., 1995).

An ovule consists of diploid sporophytic cells that physically surround the haploid female gametophyte (Robinson-Beers et al., 1992; Reiser and Fischer, 1993) (Fig. 1). The source of the signal(s) responsible for pollen tube guidance to the egg apparatus could in principle originate either in the sporophytic or in the gametophytic cells of the ovule. An attempt to distinguish between these two possibilities was made by Hülskamp et al. (1995). They isolated an incompletely penetrant, recessive, female sterile mutant line, 54D12. In the homozygous 54D12 line, some ovules in each flower contain a normal female gametophyte but others contain an incomplete gametophyte or none at all. The pollen tubes were guided mostly to ovules that housed a normal female gametophyte, rarely to ovules with an incomplete gametophyte and never to those lacking a gametophyte. These results prompted the suggestion that the female gametophytic cells are probably important for pollen tube guidance to the ovule.

The above results forced us to entertain two alternative models (Fig. 1) that could not be distinguished in the previous study. One model proposes that the gametophyte produces a signal that directly, or indirectly through its effects on sporophytic cells, causes pollen tube guidance to the ovule (Fig. 1A).

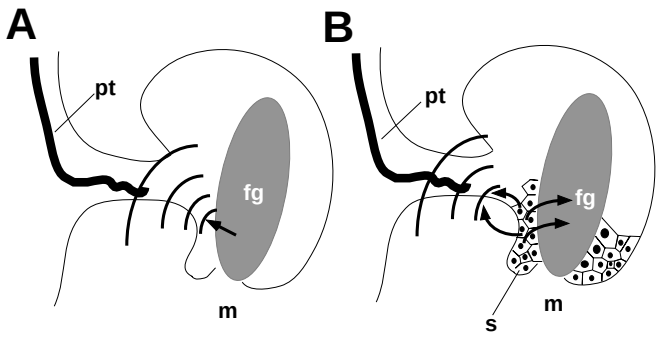

Fig. 1. Models of pollen tube guidance by the ovule. (A) The female gametophyte (fg) guides the emergent pollen tube (pt) along the ovular stalk to the micropyle (m) by emitting (arrow) a hypothetical guidance signal (arcs). (B) A hypothetical group of sporophytic cells (s), shown in an arbitrary location near the micropyle, controls the formation and function of the female gametophyte as well as emits the guidance signal.

The second model, equally consistent with the results of Hülskamp et al. (1995), proposes that some sporophytic cells of the ovule primordia control female gametophyte differentiation by cell non-autonomous signaling. These sporophytic cells, or their mitotic descendants, also cause pollen tube guidance (Fig. 1B). The observations of Hülskamp et al. (1995) may suggest that some ovules in the incompletely penetrant mutant 54D12 fail to generate a normal female gametophyte because some homozygous mutant sporophytic cells in the ovule primordia fail to induce or maintain the female gametophyte. It is possible that the inability of these sporophytic cells to induce/maintain a normal female gametophyte simultaneously makes them incompetent for pollen tube guidance. Thus, a possible role of the sporophyte in controlling female gametophyte development was not genetically separable in that mutant line from the sporophyte's possible role in pollen tube guidance.

In this communication, we describe an explicit genetic test that distinguishes between the above two models. Using a chromosomal translocation, we have generated ovules that contain genotypically wild-type sporophytic cells but have either a functional or a nonfunctional female gametophyte. We show that pollen tubes are guided only to an ovule having a functional female gametophyte. Therefore, at least the final phase of pollen tube guidance to the ovule is controlled by the female gametophyte. These results raise further questions concerning the precise origin and the nature of the guidance signal. We leave open the possibility that the guidance signal may be produced either by the gametophyte itself or by some sporophytic cells upon induction by the female gametophyte.

MATERIALS AND METHODS

Plant growth and genetic techniques

Conditions for seed germination, plant propagation, techniques for genetic crosses, and mapping have been described before (Lang et al., 1994; Ray et al., 1996). The wild-type Columbia strain WC1, and the mapping strains were described by Lang et al. (1994). Molecular markers were described by Hauge et al. (1993), Konieczny and Ausubel (1993), and Bell and Ecker (1994). T-DNA mutagenized lines were made by Dr Kenneth Feldmann (University of Arizona) and were obtained from the Arabidopsis Genetic Stock Center (Ohio State University, Columbus). The strain harboring the *quartet1* mutation (in the Landsberg *erecta* background) was generously provided by Dr Daphne Preuss (University of Chicago). The latest map positions were taken from either the Integrated Genetic and Physical map, or the latest Recombinant Inbred map (Jarvis et al., 1994) in the *Arabidopsis thaliana* database (accessible through the Web page, http://genome-www.stanford.edu/Arabidopsis/aboutgenmaps.html). The sterility index is the mean number of aborted ovules per silique, which was calculated from the fraction of aborted ovules in the third, fourth and the fifth flowers. These flowers have the least frequency of background ovule abortion. A semi-sterile plant is defined as one with a sterility index of over 0.4, and a fully fertile plant has a sterility index below 0.12.

Microscopic techniques

Techniques for cleared whole mounts of ovules were described by Lang et al. (1994). For the determination of ploidy of ovule cells in sections, isolated pistils were fixed in 0.3% glutaraldehyde in sodium phosphate buffer (pH 7.3), dehydrated through graded ethanol series, infiltrated with and embedded in JB4-Plus (Polysciences, Inc.) resin according to the manufacturer's instructions. 4-5 μm microtome sections were placed on poly L-lysine coated glass slides, stained with 1 μg/ml of DAPI in phosphate-buffered saline, rinsed, and mounted in Gelvatol (Monsanto Co.) containing 2.5% DABCO (1,4-diazobicyclo-[2.2.2]-octane) (Aldrich Chemicals). Fluorescence in individual nuclei was digitized straight from the microscope with a Nuvicon MTI-65 video camera after manually adjusting gain and correcting for dark and light backgrounds. Fluorescence intensity was measured using the densitometry menu of the IMAGE 1 image processor program. Pollen viability was assayed as described by Regan and Moffatt (1990). For tetrad analysis, whole flowers were fixed in ethanol-acetic acid for 30 minutes, briefly rinsed in water, and dissected anthers were mounted in a drop of water on a slide. The cover slip was gently tapped to release the pollen tetrads and which were counted under the Nomarski optics. Pollen tube turning was assayed by the technique of Hülskamp et al. (1995) following pollination of isolated flowers on agar plates (Kandasamy et al., 1994).

RESULTS

Genetic strategy

To test whether pollen tube guidance is effected by the female gametophytic cells, we wished to generate flowers in which all sporophytic cells are wild type but some of the female gametophytes are aborted. If the female gametophyte abortion is due to the expression of a gametophytically autonomous lethal condition, then we should be able to genetically separate the putative effect of a sporophyte from that of a gametophyte on the pollen tube guidance.

A diploid plant heterozygous for a reciprocal translocation between two chromosomal arms should exhibit no somatic abnormality unless at least one chromosomal break-point lands within a critical gene. During meiosis, however, the translocated and the normal chromosomes should pair in a typical cross-shaped structure in the pachytene stage of prophase I (Fig. 2). In organisms with metacentric chromosomes, as in *Arabidopsis*, such cross-paired chromosomes should segregate in two different ways at equal frequency. These alternative segregations are expected to produce either four normal haploid products, each of which contains a complement of the full chromosomal set, or four abnormal haploid cells, each of

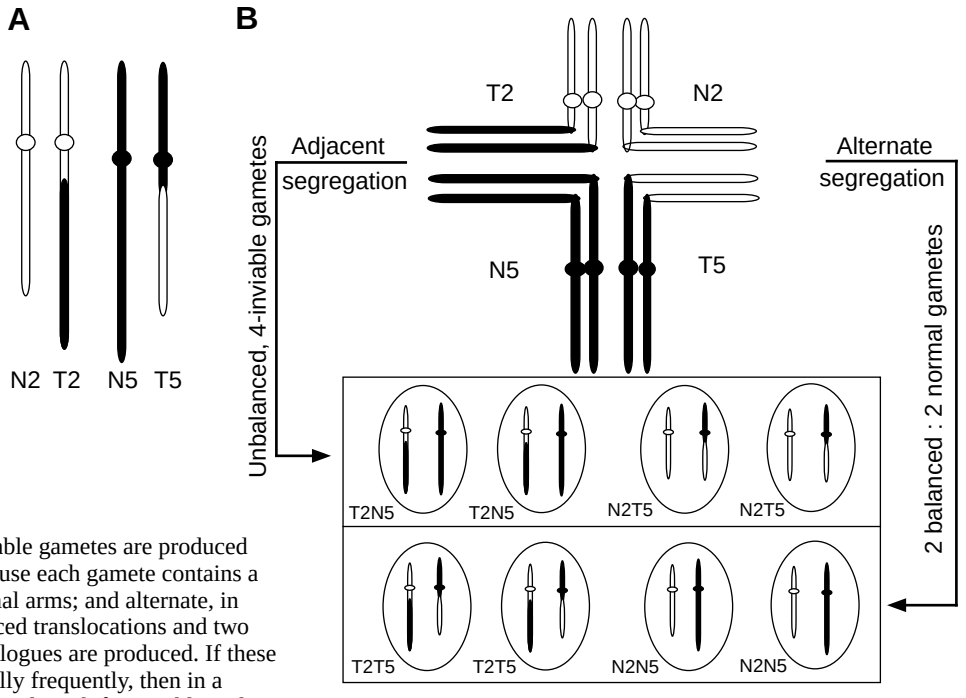

Fig. 2. Meiotic segregation of translocated chromosomes. (A) Homologous chromosomes in a translocation heterozygote. Only two pairs of chromosomes (those involving the translocation) are shown. The chromosomes are named by their centromeres: N2 is normal chromosome 2; N5 is normal chromosome 5; T2 is a translocation chromosome in which the lower arm of chromosome 2 is linked to the lower arm of chromosome 5; T5 contains the lower arm of chromosome 2 linked to chromosome 5. (B) The cruciform structure of paired homologues in the pachytene stage of meiotic prophase I. The two non-homologous centromeres co-segregate in meiosis prophase I in two different ways: adjacent, in which four inviable gametes are produced following the completion of meiosis II because each gamete contains a deficiency and a duplication for chromosomal arms; and alternate, in which two viable gametes containing balanced translocations and two viable gametes containing the normal homologues are produced. If these two segregations are assumed to occur equally frequently, then in a homozygous *quartet* mutant background, tetrads with four viable and four inviable pollens are expected to be recovered equally frequently. When a translocation heterozygote is self-crossed, gametes arising from adjacent segregation do not contribute to the progeny. Therefore, selfing produces in the F_2 generation a ratio of 2 translocation heterozygotes : 1 normal homozygote : 1 translocation homozygote. All three classes are viable. The first class, like the parent, is semi-sterile, but the latter two classes are fully fertile. The latter two classes can be distinguished by crossing to wild-type testers, when a translocation homozygote will produce semi-sterile F_1 offsprings while the normal homozygote will produce fully fertile F_1 progeny.

which contains a duplication and a deficiency of two chromosomal arms (Fig. 2). In plants, such deficiencies and duplications are almost invariably lethal at the gametophytic phase (Anderson, 1936; Stebbins, 1971). Each ovule contains one female gametophyte that is derived from one of the four meiotic products of the megasporocyte; the other three haploid products degenerate. Subsequent mitotic divisions of the surviving haploid megaspore results in the formation of the mature female gametophyte. Therefore, a plant heterozygous for a reciprocal translocation is expected to produce approximately 50% ovules that should each contain a phenotypically normal female gametophyte that is either genotypically normal or contains a balanced translocation. In the remaining ovules the female gametophyte should either arrest or abort. Likewise, 50% of pollen meioses should lead to the abortion of all four meiotic products. Every sporophytic cell of this plant, however, should be normal.

In the following sections we first describe the isolation, genetic confirmation, and the effects on the female and the male gametophytes of a reciprocal translocation involving the lower arms of chromosomes 2 and 5. Then we describe use of the translocation line in distinguishing between the two models of pollen tube guidance presented in Fig. 1.

Isolation of the semi-sterile line TL-1

We isolated the chromosomal mutant line TL-1 from approximately 2,500 T-DNA mutagenized lines (Feldmann, 1991) during a systematic search for reduced-fecundity mutants displaying distorted segregation ratios for the inheritance of the sterility phenotype. The progeny of a selected mutant line that was back-crossed as a pollen donor to wild-type flowers for three generations, segregated 51±3% semi-sterile plants (*n*, number of progeny tested = 311) (see Materials and Methods for the definition of semi-sterility). The mean frequency of ovule abortion in the semi-sterile segregants was 51.8±1.7% (*n*=16) and in fully fertile segregants was 6.4±1.6% (*n*=12). There was a background level of 10.6±1.2% (*n*=17) ovule abortion in the parental wild-type strain. One of the reasons for such a pattern of segregation could be a reciprocal chromosomal translocation.

A reciprocal translocation should produce 50% lethality in the female gametophytes as well as in pollen. We did detect morphologically abnormal pollen grains among those produced by TL-1 anthers. Fluorochromatic test (Regan and Moffatt, 1990) on isolated pollen indicated that the frequency of pollen that failed to retain fluorescein diacetate (a measure of pollen inviability) was 32.0±7.1% (*n*=23; 4,802 pollen grains counted) in segregants of self-crossed TL-1 plants that also had frequent ovule abortion (presumed translocation heterozygotes). In those TL-1 segregants with normal levels of ovule development (presumed translocation homozygotes), the frequency of pollens unable to retain fluorescein diacetate was 18.9±5.5% (*n*=9; 1,904 pollen grains counted). Assuming a background pollen abortion rate of 19%, the expected level of pollen abortion for a strain with a reciprocal translocation is 59.5%. This is because 19% of the normal pollen, which should be 50% of the total, should also abort spontaneously, thus giving an overall abortion frequency of $(0.5+0.5\times0.19)\times100$, i.e., 59.5%. Thus the observed frequency of abnormal pollens in TL-1 plants was significantly less than that expected for a

Table 1. Transmission of the semi-sterility trait through male and female gametes

Cross type*	Cross number	Phenotype and no. of F1 plants	Mean sterility index (± s.e.)	Mean number of ovules per flower (± s.e.)	*n*
A	1	+ 5	0.09 (± 0.04)	49.4 (± 2.0)	8
		– 9	0.53 (± 0.02)	45.5 (± 0.9)	18
A	2	+ 9	0.05 (± 0.02)	47.8 (± 0.7)	21
		– 7	0.57 (± 0.08)	45.7 (± 0.8)	23
A	3	+ 12	0.02 (± 0.01)	46.9 (± 0.8)	28
		– 13	0.51 (± 0.02)	45.2 (± 0.7)	38
B	4	+ 4	0.08 (± 0.02)	53.8 (± 1.0)	8
		– 2	0.47 (± 0.03)	48.9 (± 2.2)	7
B	5	+ 5	0.03 (± 0.02)	47.9 (± 1.0)	9
		– 8	0.50 (± 0.05)	45.6 (± 1.2)	17

*Cross type A: Pollen of semi-sterile TL-1 × wild-type (WC1) stigma; cross type B: semi-sterile TL-1 stigma × wild-type (WC1) pollen. Individual F1 progeny was confirmed for heterozygosity of molecular markers polymorphic between the two parents. + denotes full fertility; – denotes semi-sterility. Sterility index is the frequency of aborted ovules per flower. *n*, total number of flowers (1-3 per plant) examined.

translocation. We suspected that the fluorochromatic test we employed may not be sufficiently accurate for our purposes. Therefore, we confirmed pollen abortion rate by tetrad analysis (see later).

Genetic analysis of TL-1

Lines established from individual TL-1 plants with reduced fecundity always produced progeny of which approximately half were semi-sterile and the other half were normal (fully fertile). Reciprocal crosses involving selected semi-sterile plants and a wild-type tester strain showed that approximately half of each kind of gamete (male or female) produced by a semi-sterile plant transmits the causative agent for reduced fertility to the progeny (Table 1). These observations are most compatible with a reciprocal chromosomal translocation. Since half of the F_1 progeny was semi-sterile, multiple mutations cannot explain the segregation data.

The self-crossed progeny of a line heterozygous for a reciprocal chromosomal translocation should be of three genotypic classes (Fig. 2). Half of the progeny plants are expected to be translocation heterozygotes, which, like their parents, should exhibit reduced fecundity. The remaining 50% of the progeny are expected to be fully fertile. Note, however, that two different genotypes are expected to be equally frequent among these phenotypically normal progeny plants: translocation homozygotes and normal homozygotes. When translocation homozygotes are out-crossed to a wild-type tester strain, all F_1 progeny plants should be translocation heterozygotes themselves, which should display reduced fecundity. Therefore, these crosses should produce F_1 plants that are either all fertile or all semi-sterile. To test this prediction for a reciprocal translocation, we crossed 17 phenotypically normal (i.e., fully fertile) plants segregating in a self-crossed progeny of TL-1 to a Landsberg *erecta* strain with an additional mutation in the *quartet1* (*qrt1*) gene (Preuss et al., 1994). At least four individual F_1 progeny plants from each cross were tested for high frequency ovule abortion (Table 2). The F_1 progeny from seven out of seventeen individual crosses produced all semi-sterile flowers, indicating that these progeny plants are most likely to be translocation heterozygotes. By inference, their fully fertile parents must have been homozygous for the translocated chromosomes. Since these parental plants were morphologically normal and fully fertile, the translocation break points must not have landed on any essential gene. Conversely, seven crosses produced only fully fertile progeny indicating that their pollen donor parents must have been homozygous for the normal complement of five chromosomes. Crosses 9, 10 and 14 (Table 2) produced both fully fertile and semi-sterile plants in their progeny, though one or the other class predominated. The semi-sterile plants in these progeny appeared unhealthy and stressed, apparently due to a fungal infection, which presumably contributed to their semi-sterility.

Mapping of the translocation break points

The above results strongly support, but do not prove, the presence of a reciprocal translocation in the mutant line TL-1. For example, these results are also compatible with a ‘meiotic drive’ model (Raju and Perkins, 1991) for the propagation of a particular chromosome in preference over its homologue. A reciprocal translocation, however, should generate novel

Table 2. The line TL-1 is heterozygous for a chromosomal translocation

Out-cross number	Number of F1 out-cross progeny with		
	Full fertility	Semi-sterility	Total
1	10	0	10
2	0	4	4
3	0	9	9
4	0	10	10
5	5	0	5
6	0	11	11
7	10	0	10
8	0	11	11
9	9	2	11
10	1	8	9
11	7	0	7
12	7	0	7
13	0	5	5
14	1	5	6
15	0	4	4
16	5	0	5
17	5	0	5

Fully fertile plants derived from a self-cross of TL-1 were out-crossed to a Landsberg-*erecta qrt1* tester, and F_1 out-cross progeny plants were analyzed for ovule abortion.

linkage relationships. The linkage relationships of eight markers (nga162, nga168, nga280, m246, *er*, *bp*, *ttg* and *yi*), distributed over all five linkage groups (Hauge et al., 1993; Konieczny and Ausubel, 1993; Bell and Ecker, 1994), were examined in crosses involving TL-1. Semi-sterile F_1 heterozygotes between a multiply marked tester (DP-24; see Lang et al., 1994) were self-crossed. Semi-sterile and fully fertile F_2 progeny from selected heterozygotes were examined for the relative segregation frequencies of each of the above markers. The following observations indicated the presence of a reciprocal translocation between the lower arms of chromosome 2 and 5.

There was an unexpected, though weak, linkage between the marker *erecta* (*er*; normally on chromosome 2 at 41.5 cM) and the two chromosome 5 markers, *transparent testa glabra* (*ttg*; normally on chromosome 5 at 35.5 cM) and *yellow inflorescence* (*yi*; normally on chromosome 5 at 96.0 cM). Among 103 F_2 progeny of TL-1 × DP-24 (*er*; *bp*; *ttg*, *yi*) significant departures from the 9 : 3 : 3 : 1 segregation ratio were observed: 70 Er^+ Yi^+ : 16 Er^- Yi^+ : 6 Er^+ Yi^- : 11 Er^- Yi^- ($\chi^2 = 15.5$, $P < 0.01$) and 66 Er^+ Ttg^+ : 14 Er^- Ttg^+ : 11 Er^+ Ttg^- : 12 Er^- Ttg^- ($\chi^2 = 11.1$, $P < 0.02$). This is possible if the translocation break points had involved chromosomes 2 and 5. The chromosome 4 marker *brevipedicellus* (*bp*) did not exhibit any linkage with the other three markers (data not shown). If the reciprocal translocation break points have no linkage to a recessive phenotypic marker (m), an F_2 segregation ratio of fully fertile m^+ : semi-sterile m^+ : fully fertile m^- : semi-sterile m^- of 3 : 3 : 1 : 1 should be obtained. The observed phenotypic ratios were significantly different (for *yi*, 33 : 50 : 13 : 7, $\chi^2 = 6.85$, $0.05 < P < 0.1$; for *ttg*, 30 : 49 : 17 : 7, $\chi^2 = 8.72$, $P < 0.05$; and for *er*, 50 : 27 : 25 : 1, $\chi^2 = 29.22$, $P << 0.01$). These data are consistent with the translocation break points having weak linkages to the chromosome 5 markers *yi* (linkage, 30.5±4.5 cM) and *ttg* (linkage, 26.9±4.4 cM), and a strong linkage with the chromosome 2 marker *er* (linkage, 3.8±1.9 cM). As expected, *bp* showed no linkage to the translocation break points (the segregation ratio was, 38 : 44 : 8 : 13, $\chi^2 = 2.61$, $P > 0.1$).

Given the location of the translocation break points on chromosome 2 near *er* and on the lower arm of chromosome 5 with weak linkages to *ttg* and *yi*, we tested the linkage between *er* and *quartet1* (*qrt1*; normal location between *DFR* and *LFY3* on the lower arm of chromosome 5; see Preuss et al., 1994) in a semi-sterile *ER*; *QRT1* TL-1 × *er qrt1* cross. As predicted, a strong linkage between *er* and *qrt1* was detected in the F_2. The observed phenotypic ratio was significantly different from 9 : 3 : 3 : 1 (62 Er^+ Qrt^+ : 4 Er^- Qrt^+ : 1 Er^+ Qrt^- : 19 Er^- Qrt^-; $\chi^2 = 48.4$, $P << 0.01$). Each of the five plants showing independent assortment of *er* and *qrt1* must have resulted from a crossover exchange between a translocation break point and a centromere at the cross-paired configuration. This computes a novel linkage between *er* and *qrt1* as 11.4±3.4 cM. As expected for a recombinant, the single Er^+ Qrt^- plant was also semi-sterile because it must be a translocation heterozygote.

Effect of translocation on ovule development

The basis for partial infertility was traced to a defect in a subset

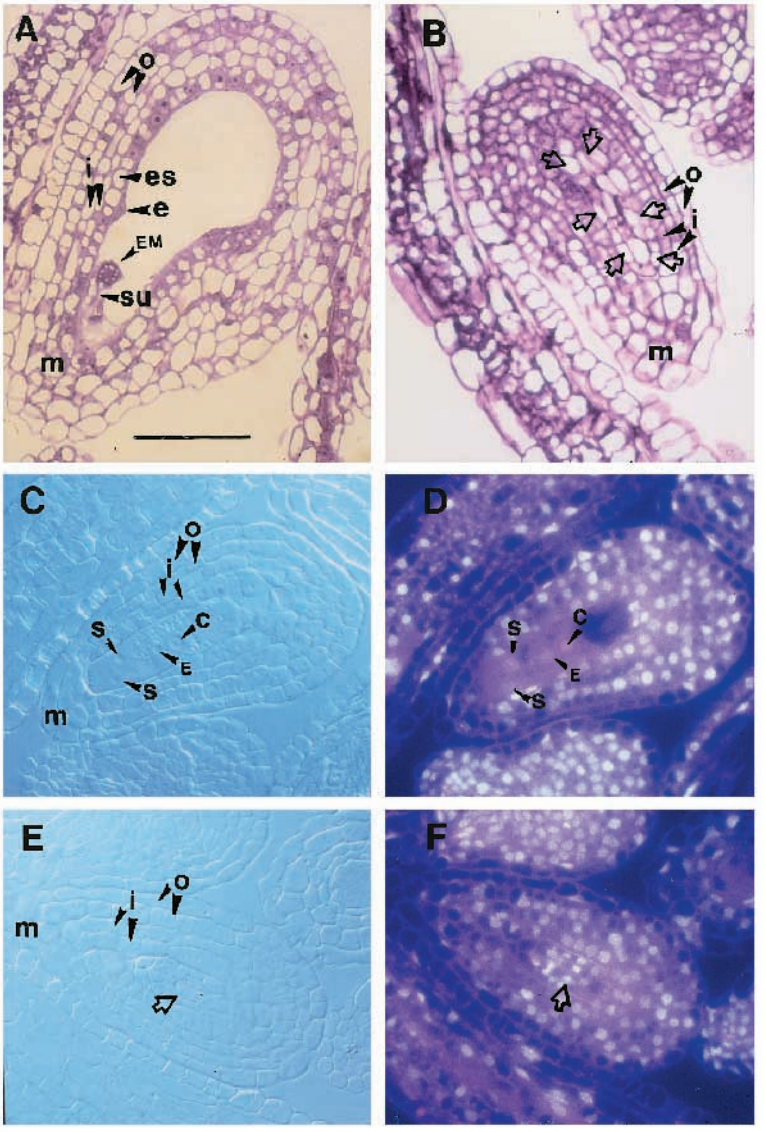

Fig. 3. Phenotypic analysis of TL-1 ovules. Sections through a normal (A) and an abnormal (B) ovule in a semi-sterile postfertilization flower. Histocryl sections 2.5 μm thick were stained with toluidine blue and photographed according to Lang et al. (1994). The normal post-fertilization stage ovule has an enlarged embryo sac with an embryo at the early globular stage and endosperm cells. By contrast, cells of unknown origin (open arrows) fill the void left by an absent embryo sac in the abnormal ovule in B. (C,D) An example of a JB4-Plus resin section through a normal ovule (prefertilization) stained with DAPI and photographed using Nomarski (C) and ultraviolet fluorescence optics (D). Four gametophytic nuclei, including two synergids, an egg and one of the two unfused central cell nuclei, are dimly fluorescent in this section. The sporophytic cells have highly fluorescent nuclei. (E,F) Photographs of a section through an abnormal ovule. The nuclei of cells of unknown origin (open arrow) in the central region of an abnormal ovule (F) are as strongly fluorescent as the surrounding sporophytic nuclei. c, central cell; E, egg; EM, embryo; e, endosperm; es, endothelium; i, inner integument; o, outer integument; s, synergid; su, suspensor. Scale bar, 75 μm for all panels.

of ovules. Ovule primordia of semi-sterile plants appeared identical to those of wild-type plants until after meiosis of the megaspore mother cell. At later stages, however, there were two distinct classes of ovules in semi-sterile flowers. Following pollination in fully mature flowers, some mature ovules from semi-sterile plants appeared normal and contained embryos at various stages of development (Fig. 3A). The remaining ovules had normal integuments but did not contain an embryo sac (Fig. 3B). Instead, the center of the nucellus was occupied by a cluster of cells with walls, which appeared to be enclosed within a common sac-like structure. It was difficult to determine the exact frequency of normal and abnormal ovules in plastic sections because the sections did not always pass through the same plane in all ovules, and serial sectioning of a large number of ovules in a single pistil was not possible. However, each section exhibited approximately equal numbers of ovules of both types. Approximately ten pistils of TL-1 flowers were compared with the wild-type pistils.

To determine whether these central cells in abnormal semi-sterile ovules were diploid or haploid, sections of mature ovules were stained with 4, 6-diamino-2-phenylindole (DAPI) and examined for fluorescence (Fig. 3C-F). While the haploid megaspore nuclei in normal ovules emit a low level of DAPI fluorescence (Fig. 3D), the nuclei of centrally located cells in abnormal ovules fluoresce strongly (Fig. 3D), suggesting that these cells are diploid. Measurement of DAPI fluorescence intensity by direct digitization and counting showed that the nuclei of cells in the central region of semi-sterile defective ovules had the same level of fluorescence as the surrounding sporophytic nuclei whereas the nuclei of female gametophytic cells had less than half the fluorescence levels: in normal ovules (five ovules in three independently stained sections), the fluorescence intensity per sporophytic nucleus was 147±19 units (number of nuclei counted, n=22) and that per gametophytic nucleus was 98±4 units (n=8), yielding a ratio of sporophyte to gametophyte of 2.9 after correcting for a background fluorescence of 72±4 units (n=15). In abnormal ovules (four ovules in two independently stained sections), the nuclear fluorescence intensity in the unidentified inner cells was 162±16 units (n=11), and that for the sporophytic cells was 158±19 units (n=10), yielding a ratio of sporophyte to central cells of 1.0. We thus conclude that the centrally located cells in defective ovules are not haploid.

To further characterize the exact developmental stage at which the ovule defect is first manifested, we counted the proportion of female gametophytes having one, two or more nuclei following meiosis of the megaspore mother cell until the time when anthesis occurs. Among ovules in wild-type plants, the proportion with a mononucleate megaspore decreased from approximately 100% in the earliest stages to zero at the last observed stage (Fig. 4A). This was accompanied by a corresponding increase in the frequency of ovules with a multinucleated megaspore. In semi-sterile mutants, however, the frequency of ovules with a mononucleate megaspore reached a minimum of approximately 40% and that of ovules with a multinucleate megaspore a maximum of approximately 50% (Fig. 4B). Approximately 10% of the megaspores remained binucleate until the end of the observed period. We conclude that the mutation causes an arrest of megaspore mitosis. The arrested cell was not obvious at a later stage of maturity, and probably degenerated.

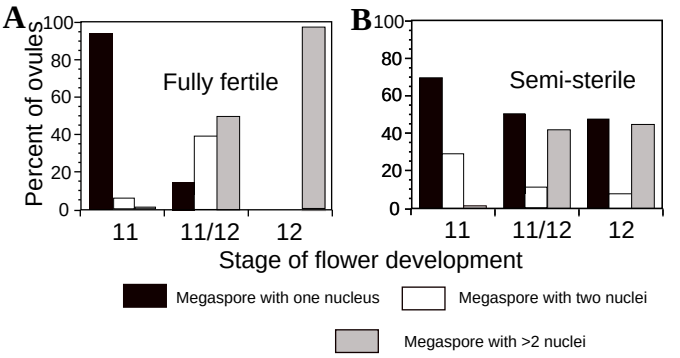

Fig. 4. Developmental arrest of the female gametophytes in semi-sterile TL-1 flowers. Relative frequency of ovules with one, two and more than two gametophytic nuclei in three successive flowers on the same inflorescence are plotted as a function of floral developmental stages. Floral developmental stage 11 corresponds to the time when the megaspore mother cell completes meiosis concomitant with stigmatic papillae arising on the carpel tip, and stage 12 corresponds to the appearance of the multinucleated female gametophyte simultaneously with petals becoming level with long stamens (Bowman et al., 1991). Flowers were fixed, cleared and whole mounts of ovules were examined for counting the number of gametophytic nuclei under Nomarski optics. The data are averages of three different experiments with each plant line. For each experiment, all ovules of three successive flowers were counted. The data in A are from a total of 256 ovules, and those in B from 193 ovules.

There was no discernible abnormality associated with semi-sterile plants in either floral structure or external ovule morphology. The aborted ovules were distributed randomly along the gynoecium, as were the seeds, indicating normal pollen tube growth through the style and transmitting tissues. The semi-sterile plants had normal whole-plant morphology.

Effect of translocation on pollen development

A reciprocal chromosomal translocation should produce equally drastic lethal effects on both the male and the female gametophytes. As mentioned above, however, fluorochromatic tests on mature pollen grains had failed to reveal a major effect on pollen viability. A trivial explanation could be that we systematically missed a proportion of the aborted pollens. Therefore we wished to examine the effect of the translocation by a more reliable genetic technique. In fungal genetics, a reciprocal translocation in which the translocation break points are tightly linked to the two centromeres is signaled in tetrad analysis by the occurrence of an equal frequency of four viable (4:0) and four inviable (0:4) spored tetrads (see Fig. 2B for explanation). The occurrence of other classes of tetrads, such as the three viable (3:1), two viable (2:2), and one viable (1:3) tetrads are either due to crossovers between the centromere and the translocation break points, or due to chromosome nondysjunction (Perkins and Raju, 1995). Crossover exchanges between a centromere and a translocation break point have been observed before in plants (Catcheside, 1947).

To examine the effect of translocation on pollen development in *Arabidopsis* by tetrad analysis (Preuss et al., 1994), we generated a semi-sterile F_2 segregant that was presumably heterozygous for the translocated chromosomes but homozygous for the *qrt1* mutation. Homozygosity for the *qrt1* mutation does

Table 3. Tetrad analysis of translocation in *qrt1* pollen

Number of plants	Ovule phenotype	Tetrad phenotype (viable:inviable)					Total tetrads
		4:0	3:1	2:2	1:3	0:4	
9	Fully fertile	111	50	23	2	1	187
9	Semi-sterile	28	28	58	23	33	170

not allow the four haploid pollen grains, products of a single meiosis, to separate (Preuss et al., 1994) (Fig. 5A). We scored the frequency of various tetrad types produced by fully fertile or semi-sterile progeny from self crosses of a semi-sterile *qrt1* plant (Table 3).

As expected for a reciprocal translocation, the frequency of four-abnormal tetrads (Fig. 5) is significantly higher in the semi-sterile plants compared to the fully fertile siblings (Table 3). The 4:0 and 0:4 tetrads occur approximately equally. Fluorochromatic tests on pollen tetrads indicated that the morphologically abnormal cells did not retain fluorescein diacetate, so were inviable, but several morphologically normal pollen grains also failed to retain the fluor. DAPI staining of the pollen nuclei (Preuss et al., 1994) showed that the abnormal pollens were never associated with DAPI fluorescence but that the normal pollens usually had strongly DAPI fluorescent nuclei. Thus, the abnormal pollen grains were most likely inviable. The high frequency of 2:2 pollen tetrads (Fig. 5) in the semi-sterile progeny indicates that meiotic crossovers do occur between the centromeres and the translocation break points. Abnormal pollen remains minute, with little associated cytoplasm. Minute, single pollen grains are easy to overlook in Qrt^+ strains, explaining why the fluorochromatic test on single pollen grains failed to reveal a significant effect of the translocation on pollen viability.

The 3:1, 2:2, 1:3 and 0:4 pollen tetrads from fully fertile plants were morphologically similar to the respective pollen tetrad classes made by semi-sterile plants, and these classes in the fully fertile plants probably represented the background level of pollen abortion. In addition to the tetrads containing one or more abnormal microspores, there were many pollen triads, dyads and monads in each flower (Fig. 5; see also, Preuss et al., 1994). These probably resulted from accidental dislodging of pollen tetrads or were due to incomplete penetrance of the *qrt1* mutant allele. These pollen classes were not included in the scoring of tetrad types. By contrast, the minute cells frequently associated with pollen tetrad classes from semi-sterile plants unambiguously marked them as products of selective gametophyte abortion.

Pollen tube guidance is controlled by the female gametophyte

Having determined that the semi-sterile plants in the TL-1 line are translocation heterozygotes, we next investigated whether pollen tubes are guided by ovules lacking a female gametophyte. Whole stage 12 flowers were placed upright on agar plates, the stigma surfaces were dusted with wild-type pollen, and they were allowed to incubate for 20 hours at 22°C (Kandasamy et al., 1994). The gynoecia were fixed, stained with decolorized aniline blue as described by Hülskamp et al. (1995), and observed for fluorescence of callose (a $\beta 1 \rightarrow 3$ glucan) that is usually associated with pollen tubes. Under these conditions, surface pollen tubes fluoresce strongly. When a pollen tube was seen associated with the micropylar tip of an ovule, which emits a strong fluorescent signal at the point of association, that particular ovule was scored as competent for pollen tube guidance (Fig. 6A).

In addition to that in the pollen tube, callose was found frequently associated with three cells near the chalazal pole and two cells near the micropylar pole of normal ovules (Fig. 6A). We believe that these three chalazal cells are the three haploid antipodal cells and the two micropylar cells are the haploid synergids. These five gametophytic cells are known to die soon after fertilization. Cell death, particularly programmed cell death, in plants is known to be associated with deposition of callose on the cell wall (Dietrich et al., 1994; Mittler et al., 1995). The abnormal ovules, by contrast, showed a strong cluster of aniline blue fluorescence in the center. We suggest that this cluster of fluorescence represents the dying megaspore or its descendants. These characteristic aniline blue fluorescence patterns, and the ovule size, made it possible to distinguish unambiguously between the two classes of ovules.

The relative frequencies of association of pollen tubes to the two classes of ovules were determined. The results are summarized in Table 4. Of the 184 morphologically normal ovules scored, 124 ovules were associated with a pollen tube at the micropylar end. By contrast, none of the 189 ovules without a normal female gametophyte had a pollen tube guided to the micropylar end. The morphology of 137 ovules could not be unambiguously scored in these flowers because these were either squashed too hard or were occluded by the surrounding opaque tissues; however, approximately half of these had some pollen tube association. These results convincingly demonstrate that pollen tube guidance by an ovule requires a functional female gametophyte, and excludes the model presented in Fig. 1B.

DISCUSSION

We have shown that a reciprocal translocation between the lower arms of chromosomes 2 and 5 causes lethality in half of the male and female gametophytes only when the plants are heterozygous for the translocated chromosomes. Since plants homozygous for the translocated chromosomes are normal in all respects, we infer that every sporophytic cell in a translocation heterozygote is potentially phenotypically normal.

Table 4. Effect of translocation on pollen tube guidance

Experiment number	Number of flowers	Number of ovules in semi-sterile flowers which contained				
		A normal embryo sac		An abnormal embryo sac		
		With associated pollen tube	Without associated pollen tube	With associated pollen tube	Without associated pollen tube	Unscorable
1	7	53	28	0	74	49
2	10	71	32	0	115	88

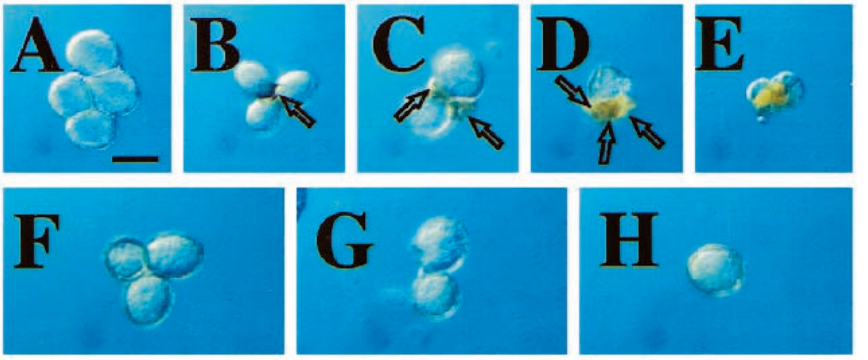

Fig. 5. Tetrad analysis of pollen abortion in a translocation heterozygote. A semi-sterile TL-1 plant was crossed to a *qrt1* mutant strain. A semi-sterile F_2 progeny that was homozygous for *qrt1* was self-crossed. F_3 descendants segregated 1:1 for semi-sterility. Pollen tetrads from individual F_3 plants were examined. A-E show examples of four normal (4:0) (A), three normal (3:1) (B), two normal (2:2) (C), one normal (1:3) (D), and four abnormal (0:4) (E) tetrad classes obtained in a semi-sterile line. An inviable microspore remains attached to the tetrad (arrows). By contrast, pollen triads (F), dyads (G), and monads (H) are clearly distinguishable from the tetrads by not being associated with any abnormal microspore. Scale bar, 30 μm.

Therefore, the partial gametophytic lethality of the heterozygote is due to chromosomal imbalance in a proportion of the gametophytes. We cannot exclude the formal possibility that the lack of a female gametophyte causes functional abnormality in some sporophytic cells of the ovule, which may indirectly affect the ability of these ovules to guide pollen tubes (more on this later).

Tetrad analysis has revealed that chromosomal imbalance causes male gametophytic death early in pollen morphogenesis. The exact course of events in the female gametophytic cells is less certain. It is clear that the female gametophyte does not develop normally within 50% of the ovules. It is possible that all four megaspores die in these ovules. The presence of a cluster of cells surrounded by a common wall in a subset of abnormal ovules may mean, however, that at least one megaspore may occasionally survive and subsequently divide in these abnormal ovules but that the specialized cell types of a normal female gametophyte do not appear. The measurement of DAPI-induced nuclear fluorescence intensity suggested that this group of cells within an embryo sac-like structure may be diploid, therefore, of sporophytic origin. We cannot eliminate the possibility that these cells may arise by polyploidization of a surviving haploid spore. These cells, nevertheless, are quite different from the normal female gametophytic cells in morphology. Whatever might be the exact cellular identity of these cells, the ovules containing these cells are morphologically clearly distinguishable from the normal ovules that constitute 50% of the total. These ovules do not form seeds.

Previous studies by Hülskamp et al. (1995) suggested that pollen tube guidance to the micropylar tip of the ovule may be controlled by the female gametophyte. Their experimental approach, however, could not distinguish between guidance by some special sporophytic cells of the ovule that also control female gametophyte development, from a direct role of the female gametophyte in pollen tube guidance. The results reported here allow us to conclusively reject the possibility that a group of sporophytic cells of the ovule are responsible for guiding the pollen tube as well as controlling the female gametophyte development. Our results also discourage models of guidance by default that involve repulsive guidance cues from non-target cells, which has been shown to play a significant role in neural path finding (Dodd and Schuchardt, 1995).

Hülskamp et al. (1995) showed that each ovule is capable of guiding a pollen tube autonomously, arguing for the involvement of a chemotactic mechanism. Since the pollen tube tracks along the cell surface, the putative chemotactic agent should be localized in a gradient along the cell surface. The two synergid cells, located at the micropylar end of the female gametophyte, through which the pollen tube enters the embryo sac, are plausible candidate cells for the originator of the guidance signal (Mascarenhas, 1993; Russell, 1993). If so, the signal must be sensed across approximately thirty to fifty cell diameters (~100 μm) away at the junction of the ovule with the placenta. In well-known cases of polarized cell growth by chemotaxis, such as in the 'shmooing' of haploid yeast cells in response to small peptide mating pheromones secreted by cells of the opposite mating type (Cross et al., 1988), the response is usually effective over much shorter distances: typically over two to five cell diameters (A. R., unpublished). Surface-localized diffusion of chemotactic signals that are effective over fifty cell diameters would probably require a small signal molecule of less than 1000 daltons (Crick, 1970). If the guidance is mediated by a larger molecule, then a mechanism for a polarized transport of the signal must be invoked. It is also conceivable that the pollen tube may be responding to an ionic or electrical potential gradient. Slow calcium wave has been implicated in long distance signaling during wound response in plants (Vian et al., 1996). Synergid cells are loaded with calcium (Chaubal and Reger, 1992), and calcium has been shown to elicit chemotactic response in pollen tubes (Mascarenhas and Machlis, 1964). Whatever the details of molecular basis of the guidance process is, there are two ways by which the female gametophyte may accomplish this. (1) The gametophyte may directly change the surface characteristics of the epidermal cells lining the ovule stalk and the outer integument by producing a surface-localized signal (Fig. 6B). (2) An alternative mechanism would be, a signal from the gametophyte indirectly causing changes in the surface properties of the sporophytic cells along which the pollen tube tracks (Fig. 6C). This latter model demands that the primary gametophytic signal is passed from the adjacent sporophytic cells to further neighboring sporophytic cells by the same or a different signaling mechanism.

A support for the second model (Fig. 6C) comes from the observation that the abnormal ovules of the *inner-no-outer* (*ino*) mutants are defective in the final stages of pollen tube guidance (Baker et al., 1997). In the *ino* mutants, the female gametophyte and the inner integument are morphologically normal but the outer integument cells are missing. Thus, a normal female

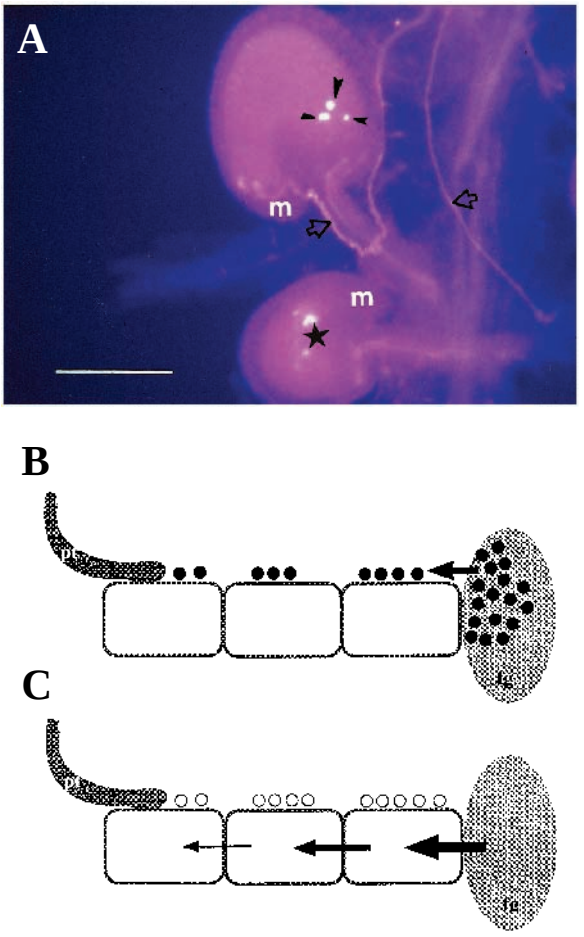

Fig. 6. Pollen tube guidance by the female gametophyte. (A) Aniline blue fluorescence micrograph of ovules of a semi-sterile TL-1 flower. The pollen tube (open arrows) is guided to the normal ovule (larger; top) but not to the abnormal (smaller; bottom) ovule. Arrowheads point to three fluorescent cells at the chalazal end that are presumably the three antipodal cells which undergo programmed death, along with the synergids, shortly after fertilization. The asterisk shows the cluster of highly fluorescent cells in the center of the abnormal ovule. These cells presumably die shortly after the time of fertilization although these ovules do not interact with any pollen tube. (B,C) Models of the guidance mechanism. fg is the female gametophyte. In B, one or more female gametophytic cells secrete (arrow) a morphogen (black circles) that forms a polar field on the surface of the sporophytic cells (boxes). The pollen tube (pt) responds by growing towards the female gametophyte. In C, the female gametophyte signals (arrow) the neighboring sporophytic cells that respond by altering their own surface (open circles) and by passing the signal on to their neighbors (thinner arrows). The pollen tube follows the altered surface features to the female gametophyte.

gametophyte is necessary but not sufficient for pollen tube guidance to the egg apparatus. The importance of cell-surface interaction in pollen tube guidance is shown by the recent work of Wilhelmi and Preuss (1996). Flowers homozygous for the recessive mutation *pop2* (*pollen pistil interaction2*) and at least heterozygous for the dominant mutation *pop3* fail to guide pollen tubes to the ovule only if the pollen is also mutant for *pop2* and *pop3*. The absence of guidance was associated with a lack of adhesion of the pollen tube to the inner ovarian wall. Thus, *POP2* and *POP3* genes are required in either parent for proper adhesion of the pollen tube to the cell surface. Failure to do so leads to a failure to guide. It is not yet known whether *POP2* and/or *POP3* expressions are under the female gametophyte's control, or whether these genes are necessary to predispose the pollen tube and/or the ovular sporophytic cells to respond to the guidance cues ultimately issuing from the female gametophyte. The distinction between these models may ultimately depend on the elucidation of the nature and the exact cellular source of the guidance signal within the female gametophyte. Selective genetic ablation of specific female gametophytic cells may be one way of identifying the cell responsible for generating the guidance signal.

We thank the Arabidopsis Biological Resource Center (Ohio State University) and Ken Feldmann (University of Arizona) for making T-DNA mutagenized seed pools publicly available, Daphne Preuss (University of Chicago) for providing the *qrt1* mutant strain, and Chuck Gasser (UC Davis) and David Smyth (Monash University) for discussions. We thank June Nasrallah (Cornell University), Muthu Kandasamy (currently at the University of Georgia), and especially Uzi Nur (University of Rochester) for advice. Comments from two anonymous reviewers significantly helped improve the manuscript. This laboratory was supported by grants from the US Department of Agriculture (Cooperative State Research Service Award 92-37304-7966) and by the National Science Foundation (MCB-9630402) to A. R.

REFERENCES

Anderson, E. G. (1936). Induced chromosomal alterations in maize, In *Biological Effects of Radiation* (ed. B. M. Duggar), pp. 1297-1310. McGraw-Hill, New York.

Amanze, D. and Iyengar, A. (1990). The micropyle: a sperm guidance system in teleost fertilization. *Development* **109**, 495-500.

Baier, H. and Bonhoeffer, F. (1994). Attractive axon guidance molecules. *Science* **265**, 1541-1542.

Baker, S. C., Robinson-Beers, K., Villanueva, J. M., Gaiser, J. C. and Gasser, C. S. (1997). Interactions among genes regulating ovule development in *Arabidopsis thaliana*. *Genetics* **145**, 1109-1124.

Bell, C. J. and Ecker, J. R. (1994). Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. *Genomics* **19,** 137-44.

Bowman, J. L., Drews, G. N. and Meyerowitz, E. M. (1991). Expression of the *Arabidopsis* floral homeotic gene *AGAMOUS* is restricted to specific cell types late in flower development. *Plant Cell* **3**, 749-758.

Catcheside, D. G. (1947). The *P*-locus position effect in *Oenothera*. *J. Genet.* **48**, 31-42.

Chaubal, R. and Reger, B. J. (1992). The dynamics of calcium distribution in the synergid cells of wheat after pollination. *Sex. Plant Reprod.* **5**, 206-213.

Crick, F. (1970). Diffusion in embryogenesis. *Nature* **225**, 420-422.

Cross, F., Hartwell, L. H., Jackson, C. and Konopka, J. B. (1988). Conjugation in *Saccharomyces cerevisiae*. *Annu. Rev. Cell. Biol.* **4**, 429-457.

Dietrich, R. A., Delaney, T. P., Uknes, S. J., Ward, E. R., Ryals, J. A. and Dangl, J. L. (1994). Arabidopsis mutants stimulating disease resistance response. *Cell* **77**, 565-578.

Dodd, J. and Schuchardt, A. (1995). Axon guidance: a compelling case for repelling growth cones. *Cell* **81**, 471-4.

Elleman, C. J., Franklin-Tong, V. and Dickinson, H. G. (1992). Pollination in species with dry stigmas: The nature of the early stigmatic response and the pathway taken by pollen tubes. *New Phytol*. **60**, 413-424.

Feldman, K. A. (1991). T-DNA insertion mutagenesis in *Arabidopsis*: mutational spectrum. *Plant J.* **1**, 71-82.

Hauge, B. M., Hanley, S. M., Cartinhour, S., Cherry, J. M., Goodman, H. M., Koornneef, M., Stam, P., Chang, C., Kempin, C., Medrano, L. and Meyerowitz, E. M. (1993). An integrated genetic/physical map of the *Arabidopsis thaliana* genome. *Plant J.* **3**, 745-754.

Heslop-Harrison, J. (1987). Pollen germination and pollen tube growth. *Int. Rev. Cytol.* **107**, 1-77.

Heslop-Harrison, J. and Heslop-Harrison, Y. (1986). Pollen-tube chemotropism: Fact or delusion? In *Biology of Reproduction and Cell Motility in Plants and Animals*, (ed. M. Cresti, and D. Romano), pp. 169-174, Siena, Italy: University of Siena Press.

Hiscock, S. J., Kües, U. and Dickinson, H. G. (1996). Molecular mechanisms of self-incompatibility in flowering plants and fungi – different means to the same end. *Trends Cell Biol.* **6**, 421-428.

Hülskamp, M., Schneitz, K. and Pruitt, R. E. (1995). Genetic evidence for a long range activity that directs pollen tube guidance in Arabidopsis. *Plant Cell* **7**, 57-64.

Jarvis, P., Lister, C., Szabo, V. and Dean, C. (1994). Integration of CAPS markers into the RFLP map generated using recombinant inbred lines of *Arabidopsis thaliana*. *Plant Mol. Biol.* **24**, 685-687.

Kandasamy, M. K., Nasrallah, J. B. and Nasrallah, M. K. (1994) Pollen-pistil interaction and developmental regulation of pollen tube growth in *Arabidopsis*. *Development* **120**, 3405-3418.

Kolodkin, A. L. (1996). Semaphorins: mediators of repulsive growth cone guidance. *Trends Cell Biol.* **6**, 15-22.

Konieczny, A. and Ausubel, F. M. (1993). A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. *Plant J.* **4**, 403-410.

Lang, J. D., Ray, S. and Ray, A. (1994). *sin1*, A mutation affecting female fertility in *Arabidopsis*, interacts with *mod1*, its recessive modifier. *Genetics* **137**, 1101-1110.

Lord, E. M. and Sanders, L. C. (1992). Roles for the extracellular matrix in plant development and pollination: A special case of cell movement in plants. *Dev. Biol.* **153**, 16-28.

Mascarenhas, J. B. (1993). Molecular mechanisms of pollen tube growth and differentiation. *Plant Cell* **5**, 1303-1314.

Mascarenhas, J. P. and Machlis, L. (1964). Chemotropic response of the pollen of *Antirrhinum majus*. *Plant Physiol.* **39**, 70-77.

Nasrallah, J. B. and Nasrallah, M. E. (1993). Pollen-stigma signaling in the sporophyte self-incompatibility response. *Plant Cell* **5**, 1325-1335.

Mittler, R., Shulaev, V., and Lam, E. (1995). Coordinated activation of programmed cell death and defense mechanisms in transgenic tobacco plants expressing a bacterial proton pump. *Plant Cell* **7**, 29-42.

Perkins, D. D. and Raju, N. B. (1995). Three-to-one segregation from reciprocal translocation quadrivalents in *Neurospora* and its bearing on the interpretation of spore-abortion patterns in unordered asci. *Genome* **3**, 661-672.

Preuss, D., Rhee, S. Y. and Davis, R. W. (1994). Tetrad analysis possible in *Arabidopsis with* mutation of the *QUARTET* (*QRT*) genes. *Science* **264**, 1458-1460.

Pruitt, R. E. and Hülskamp, M. (1994). From pollination to fertilization in *Arabidopsis.* In *Arabidopsis* (ed. E. M. Meyerowitz, and C. Somerville) Cold Spring Harbor, NY: Cold Spring Harbor Press.

Raju, N. B. and Perkins, D. D. (1991). Expression of meiotic drive elements Spore killer-2 and Spore killer-3 in asci of *Neurospora tetrasperma*. *Genetics* **129**, 25-37.

Ray, A., Lang, J. D., Golden, T. and Ray, S. (1996). *SHORT INTEGUMENT*, a gene important for ovule development in *Arabidopsis* also controls flowering time. *Development* **122**, 2631-2638.

Regan, S. M. and Moffatt, B. A. (1990). Cytochemical analysis of pollen development in wild type *Arabidopsis* and a male-sterile mutant. *Plant Cell* **2**, 877-889.

Reiser, L., and Fischer, R. L. (1993). The ovule and the embryo sac. *Plant Cell* **5**, 1291-1301.

Robinson-Beers, K., Pruitt, R. E. and Gasser, C. S. (1992). Ovule development in wild-type Arabidopsis and two female sterile mutants. *Plant Cell* **4**, 1237-1250.

Russell, S. D. (1993). The egg cell: Development and role in fertilization and early embryogenesis. *Plant Cell* **5**, 1349-1359.

Sanders, L. C. and Lord, E. M. (1989). Directed movement of latex particles in the gynoecia of three species of flowering plants. *Science* **243**, 1606-1608.

Sanders, L. C., and Lord, E. M. (1992). A dynamic role for the stylar matrix in pollen tube extension. *Int. Rev. Cytol.* **140**, 297-318.

Smyth, D. (1997). Attractive ovules. *Current Biol.* **7**, 64-66.

Stebbins, G. L. (1971). *Chromosomal Evolution in Higher Plants.* pp. 78-81. Addison-Wesley Publishing Company: Reading, Massachusetts.

Vian, A., Henry-Vian, C., Schatz, R., Ledoigt, G., Frachisse, J. M., Desbiez, M. O. and Julien, J. L. (1996). Is membrane potential involved in calmodulin gene expression after external stimulation in plants? *FEBS Lett.* **380**, 93-96.

Wilhelmi, L.K. and Preuss, D. (1996). Self-sterility in *Arabidopsis* due to defective pollen tube guidance. *Science* **274**, 1535-1537.

(Accepted 14 April 1997)