Self-Sterility in *Arabidopsis* Due to Defective Pollen Tube Guidance

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In flowering plants, a series of cell-cell interactions govern the delivery of sperm to the ovules through precise guidance of pollen tubes. Two *Arabidopsis* genes, *POP2* and *POP3*, were found that mediate pollen tube guidance and are critical for self-fertility in diploid reproductive cells. The *pop2* and *pop3* mutations exhibited genetic redundancy: Self-sterility occurred only when male and female tissues were defective in both genes. This phenotype resembles that found in many self-incompatible species.

The guidance of pollen tubes to individual ovules is critical to successful plant reproduction. In *Arabidopsis*, these tubes grow through female pistil tissue and are guided toward the micropyle with near perfect efficiency (*1*) (Fig. 1A), avoiding ovules that have interacted with other pollen tubes. In the crucifer family, this process is accompanied by tight adhesion of pollen tubes to the surface of ovule cells (*2*) and probably requires an intricate exchange of attractive and repellent signals, as in the functionally analogous process of axon guidance in animals (*3*). Signaling molecules important in early steps of pollen tube migration have been identified (*4*, *5*), and signals necessary for the final stages of pollen tube guidance are not transmitted by immature pistils (6) or structurally aberrant ovules (*7*). After screening more than 80,000 *Arabidopsis* plants (*8*), we identified a sterile mutant that was defective in two genes that severely affect targeting of pollen tubes to structurally normal ovules.

Pollen tubes did not grow toward the micropyle in self-pollinated pistils from the sterile mutant; instead they grew in random directions throughout the ovary locule (Fig. 1, B and C) regardless of proximity to other pollen tubes or to the micropyle. The mutant pollen tubes did not adhere to pistil cells, consistent with a model in which intercellular adhesion contributes to pollen tube guidance (9).

Pollen tube guidance to the ovules was the only apparent defect in the mutant plants, which exhibited a decrease in seed yield by a factor of 300 but otherwise grew as vigorously as the wild type (Fig. 1, D and E). Mutant pollen production, pollen germination (in vitro and in vivo), the growth rate of mutant pollen tubes (in vitro and in wild-type pistils), and transportation of sperm cells to the tip of the pollen tube were comparable in mutant and wild-type plants (Table 1).

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To determine whether sterility resulted from a male or a female defect, we performed reciprocal crosses, applying wild-type pollen to mutant pistils and mutant pollen to wild-type pistils. Full seed sets were obtained in either case (*10*), indicating that the mutant is self-sterile and its phenotype reflects a combination of defects in male and female tissues. Crosses between individual sterile mutants also failed to set seed, indicating that the self-sterile phenotype is determined solely by the genotype of the plant and not by self-pollination per se. Self-sterility as a result of an early defect in pollen germination and tube growth has been observed in maize and petunia (*11*), and self-incompatibility, a highly evolved mechanism that limits inbreeding often by affecting pollen tube development, has been characterized in many species of flowering plants (*12*).

To characterize the genetic basis for the self-sterile phenotype, we crossed the mutant to a wild-type mapping strain. F_1 plants from this cross were fully fertile, indicating that sterility is recessive. Analysis of 110 sterile F_2 individuals showed linkage to two genes, *POP2* (on chromosome 3) and *POP3* (on chromosome 5), required for pollen-pistil interactions (Fig. 2) (*13*). All the sterile individuals were homozygous for the *pop2* mutation, yet only one-third (41/110) were homozygous for *pop3*; the remainder were heterozygous (*13*). These data indicate two genotypes (*pop2/pop2*, *pop3/pop3* and *pop2/pop2*, *pop3/POP3*$^+$) that result in sterility, and, although *pop2* is recessive, the *pop3* allele is dominant in a *pop2/pop2* background. The low frequency with which mutants of this phenotype arise is consistent with alteration of multiple genes or generation of unusual alleles.

Many *Arabidopsis* mutations, such as those in the tryptophan biosynthetic pathway (*14*), exhibit aberrant transmission through either male or female gametes. Because mutations altering reproduction itself could have similar or more severe consequences, we examined whether combinations of the *pop2* and *pop3* alleles adversely affect gamete fertility or zygote viability by analyzing (i) the transmission of the sterile phenotype, (ii) the genotype of fertile progeny from a *pop2/POP2*$^+$, *pop3/POP3*$^+$ plant, and (iii) the results of a cross between *pop2* and *pop3* single mutants.

The *pop* alleles are transmitted normally; inheritance of sterility follows the ratio expected for two independently assorting genes, given the dominant nature of the *pop3* mutation and its interaction with *pop2*. Of 1110 progeny from a plant with a *pop2/POP2*$^+$, *pop3/POP3*$^+$ genotype, 225 were sterile, consistent with a fertile-to-sterile ratio of 13:3 ($0.5 > P > 0.1$; χ^2 test). Subse-

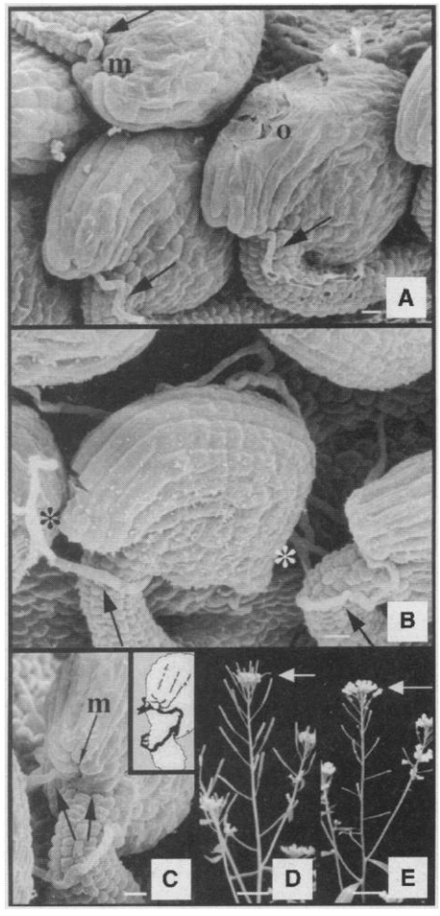

Fig. 1. Scanning electron micrographs and line drawing depicting ovules and pollen tubes within pollinated pistils, as prepared in (*1*). In the wild type (**A**), one pollen tube (arrows) grows directly toward each micropyle (m), adhering tightly to the cells that support the ovule (o) (n = 200 observations). In the sterile mutant (**B** and **C**), pollen tubes migrate randomly ($n > 300$) and do not enter the micropyle or adhere tightly to ovule cells; multiple tubes are observed on one ovule (asterisks). Panels (B) and (C) show different views of the same ovule; the pollen tube closely approached the micropyle (large arrows) but did not enter. The large seed pods (arrows) present in wild type (**D**) are not formed in sterile plants (**E**). Bars represent 10 μm in (A) through (C) and 1.3 cm in (D) and (E).

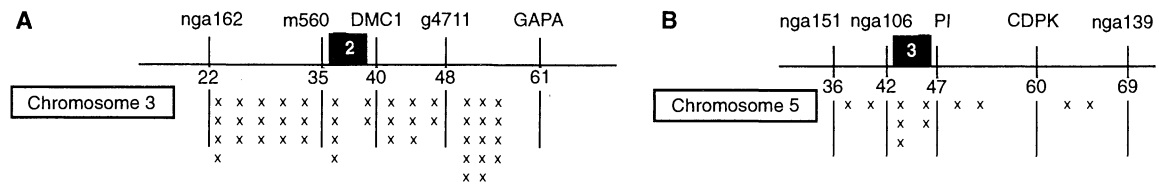

Fig. 2. Genetic mapping of *POP2* (**A**) and *POP3* (**B**). Genetic markers and distance in centimorgans are indicated. The symbol × indicates detected recombination events; ■ represents predicted position of *POP* genes.

quent crosses with these sterile plants confirmed there are two genotypes (Fig. 3). Crossing a sterile *pop2/pop2, pop3/pop3* plant to a wild-type plant yields F_1 plants that always produce sterile progeny. In contrast, crossing a sterile *pop2/pop2, pop3/POP3*$^+$ strain to wild type yields two classes of F_1 plants: one (*pop2/POP2*$^+$, *pop3/POP3*$^+$) that gives rise to sterile F_2 plants and another (*pop2/POP2*$^+$, *POP3*$^+$*/POP3*$^+$) that produces only fertile descendants.

To test whether all genotypes other than *pop2/pop2, pop3/pop3* and *pop2/pop2, pop3/POP3*$^+$ are viable and fertile, 68 fertile plants from the mapping population were assigned genotypes by scoring markers that flank *POP2* and *POP3* (*15*). The genotype of 64 of these plants was confirmed by examining the phenotypes of their progeny (*15*). All possible fertile genotypes were found among the F_2 plants, indicating that no combination of the *pop2* and *pop3* mutations is lethal.

To confirm that *pop2* and *pop3* can be transmitted through both male and female gametes, and that both mutations are required for sterility, we crossed fertile strains with a *pop2/pop2, POP3*$^+$*/POP3*$^+$ genotype to strains with a *POP2*$^+$*/POP2*$^+$, *pop3/pop3* genotype. Each single mutant was crossed as a pollen donor or acceptor; in either case, all of the F_1 progeny tested (6/6) produced sterile descendants in the predicted ratios and with the expected genotypes.

These genetic tests indicate that pollen tube guidance is impaired in sterile *pop2/pop2, pop3/POP3*$^+$ plants, although one-half of the male and female gametes are predicted to have a *POP3*$^+$ allele. Likewise, all gametes from fertile *pop2/POP2*$^+$, *pop3/POP3*$^+$ plants are functional, even though three-quarters carry mutant alleles. Thus, in both cases, *pop2, POP3*$^+$ gametes are produced, yet the function of these gametes depends only on the parental genotype. This is typical of sporophytic inheritance, in which function of the haploid gametes is governed by the diploid tissues from which they were derived. Interactions between the haploid pollen grains by means of a diffusible component could account for these observations, so that haploid *POP2*$^+$, *POP3*$^+$ pollen from a mixed population might rescue *pop2, pop3* pollen. To test this possibility, we pollinated sterile pistils with a mixture of pollen from sterile and wild-type plants. All offspring from this cross had a wild-type male parent, demonstrating that the pollen tube guidance defect is cell autonomous. The sporophytic property of the *pop2* and *pop3* mutations is unexpected because aspects of late pollen tube growth are thought to be controlled by the haploid genome (*16*). These mutations thus provide an opportunity to investigate the contribu-

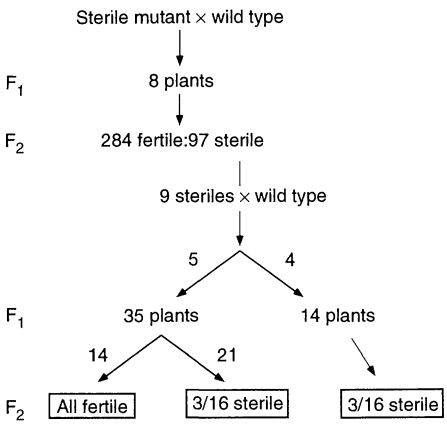

Fig. 3. Inheritance of self-sterility. The sterile isolate (*8*) was backcrossed to wild type, and all F_1 plants (8/8) produced ~3/16 sterile progeny, suggesting that the sterile parent was *pop2/pop2, pop3/pop3*. The progeny from a second backcross of nine sterile individuals indicated that five had a *pop2/pop2, pop3/POP3*$^+$ genotype and four were likely *pop2/pop2, pop3/pop3*, as their progeny showed segregation similar to the first backcross.

Table 1. Pollen phenotype. Total pollen washed from four open flowers was measured for 3 wild-type and 12 sterile strains. These samples were also used to determine percent germination in vitro (*8*), counting tubes >40 μm long. To assess the progression of sperm cell nuclei, mutant pollen tubes >0.3 mm long were stained with 4′,6′-diamidino-2-phenylindole; in every case (n = 24), sperm migrated normally to the tip of the tube. Germination in vivo was determined by staining stigmas of self-pollinated flowers with aniline blue (*8*). Pollen tubes >100 μm long were counted: The average from 12 mutant (range, 77 to 100%) and 3 wild-type (89 to 94%) strains is shown. An in vitro germination assay was used to determine the length of pollen tubes from five wild-type and five sterile plants (standard deviation represents a comparison between individuals). Growth rates in vivo were measured by placing >100 pollen grains on six pistils from the *ms1* mutant (which lacks viable pollen) for 45, 90, 135, 180, 225, 270, 315, and 360 min. Aniline blue staining was used to determine the progression of the population of tubes, which, in a given pistil, exhibited about the same growth rate. Distances were estimated by using a standard of 2.3 mm per pistil and 0.109 ± 0.026 mm per ovule.

Assay	Self-sterile mutant (n)	Wild type (n)
Pollen production (grains per flower)	146 ± 63 (12)	158 ± 57 (3)
Average pollen germination in vitro (%)	34.2 ± 7 (3510)	47.2 ± 14 (1620)
Average pollen germination in vivo (%)	94.8 ± 14 (530)	92.4 ± 2.1 (171)
Average pollen tube length in vitro (mm)	0.204 ± 0.026 (316)	0.196 ± 0.007 (320)
Average pollen tube growth rate in vivo (μm/min)	5.4 ± 3.0 (24)	5.3 ± 2.7 (24)

Table 2. Crosses between wild-type and mutant plants. Diploid genotypes are indicated; both self-sterile genotypes were tested. In reciprocal crosses (3 to 10 plants each), pollen was applied to emasculated pistils. The fraction of crosses with ≥80% of wild-type seed set is indicated. Manual self-pollination did not rescue self-sterility (17 plants tested, 0 seeds from 52 pods), nor did crosses between different sterile individuals (n = 7). Fertile designations indicate ~50 seeds per pod after self-pollination.

Pistil	Pollen			
	POP2$^+$, *POP3*$^+$	Self-sterile	*pop2, POP3*$^+$	*POP2*$^+$, *pop3*
POP2$^+$, *POP3*$^+$	Fertile	76/91	4/5	5/5
Self-sterile	116/133	0/52	16/16	21/22
pop2, POP3$^+$	5/5	13/19	Fertile	6/6
POP2$^+$, *pop3*	7/7	10/10	7/7	Fertile

tion of genes expressed in diploid cells to pollen tube guidance.

The *pop2, pop3* pollen tube guidance defect results from alteration of both pollen and pistil components. In pairwise reciprocal crosses with homozygous single mutants (*pop2/pop2* or *pop3/pop3*), the double mutant (*pop2/pop2, pop3/±*), or wild type (Table 2), all combinations produced seeds except a cross between pollen from a *pop2, pop3* plant and *pop2, pop3* pistils. These observations indicate that *POP2* and *POP3* function in both the pollen and pistil, consistent with a model in which these genes encode molecules present on the surface of pollen tubes and pistil cells, much like the agglutinins required for adhesion of mating yeast cells (*17*).

In many plant species, self-fertilization is inhibited at a late stage, sometimes through self-incompatibility mechanisms (*12*). Self-sterility in these systems, as in the *Arabidopsis pop2, pop3* mutant, may involve defects in pollen tube guidance and redundant signaling pathways active in male and female tissues.

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15. Markers flanking *POP2* (nga162, g4711) and *POP3* (nga139, nga151) defining an ~30-cM interval were used to determine the genotype of 68 fertile strains (*13*). From 64 of these, ~25 F_3 seeds were planted and ~95% germinated, indicating that all combinations of *pop2* and *pop3* are viable. The ratio of sterile to fertile F_3 plants was determined; 40 plants predicted to lack either *pop2* or *pop3* (or both) produced only fertile F_3 progeny. Of 24 fertile plants predicted to contain both mutant alleles (*pop2/POP2*$^+$, *pop3/POP3*$^+$ or *pop2/POP2*$^+$, *pop3/pop3*), 21 produced sterile progeny. The three exceptions (3/64 or ~5%) most likely were due to double crossovers, which are expected to occur at a frequency of $(0.3)^2$ or ~9%.
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Diversification of C-Function Activity in Maize Flower Development

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The *Arabidopsis* gene *AGAMOUS* is required for male and female reproductive organ development and for floral determinacy. Reverse genetics allowed the isolation of a transposon-induced mutation in *ZAG1*, the maize homolog of *AGAMOUS*. *ZAG1* mutants exhibited a loss of determinacy, but the identity of reproductive organs was largely unaffected. This suggested a redundancy in maize sex organ specification that led to the identification and cloning of a second *AGAMOUS* homolog, *ZMM2*, that has a pattern of expression distinct from that of *ZAG1*. C-function organ identity in maize (as defined by the A, B, C model of floral organ development) may therefore be orchestrated by two closely related genes, *ZAG1* and *ZMM2*, with overlapping but nonidentical activities.

The discovery of conserved genetic mechanisms of flower morphogenesis in dicots such as *Arabidopsis thaliana* and *Antirrhinum majus* (*1–3*) suggests that an ancestral floral developmental plan has been preserved during angiosperm evolution (*4*). Monocots diverged from dicots about 180 million years ago and have evolved flowers that are distinct from those of dicots. Among the monocots, flowers in the grasses are the most highly derived. We previously reported (*5*) the isolation of ZAG*1*, the maize homolog of AGAMOUS (AG) from *Arabidopsis* and PLENA (*PLE*) from *Antirrhinum*, both of which are required for reproductive organ development (*6–8*). Although the onset of ZAG*1* expression in the cells that give rise to stamens and carpels is analogous to that observed for AG and *PLE*, ZAG*1* expression (unlike AG and *PLE* expression) diminishes during stamen development (*5*). Here, we identified a transposon-induced mutant allele of ZAG*1* and used it to examine the functional homology between these monocot and dicot genes.

We used reverse genetic technology to screen for transposon insertions in the ZAG*1* gene (*9*). A ZAG*1* mutant allele, designated *zag1-mum1*, was isolated that carried a Robertson's *Mutator* (*Mu*) transposable element inserted adjacent to the region encoding for the K domain (Fig. 1, A and B), a motif thought to be involved in protein-protein interactions (*10*). No ZAG*1* transcript was detected in RNA samples from *zag1-mum1* homozygotes upon hybridization with a probe 3′ of the *Mu* insertion site, and only reduced amounts of transcript were detectable with a ZAG*1* probe 5′ of the insertion site (Fig. 1C). These results suggest that in the *zag1-mum1* allele, transcription proceeds into but not through the *Mu* element, and that the chimeric message generated is unstable. Any possible translation product of the chimeric transcript (Fig. 1B) would lack 77 amino acids of the normal protein, including the COOH-terminus of the K box. Mutations

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