生物学各論Ⅰ

第7回　花粉管のガイダンス（2）

- シロイヌナズナにおける分子遺伝学
- ガイダンス分子（誘引物質）の同定に迫る研究の流れを知る

雌性配偶体(=胚嚢)による花粉管ガイダンスを分子遺伝学的に調べた最初の研究

1: Plant Cell. 1995 Jan;7(1):57-64.

Genetic Evidence for a Long-Range Activity That Directs Pollen Tube Guidance in Arabidopsis.

Hulskamp M, Schneitz K, Pruitt RE.

Department of Molecular and Cellular Biology, The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138.

The fertilization process of plants is governed by different kinds of cell-cell interactions. In higher plants, these interactions are required both for recognition of the pollen grain by the female reproductive system and to direct the growth of the pollen tube inside the ovary. Despite many years of study, the signaling mechanisms that guide the pollen tube toward its target, the ovule, are largely unknown. Two distinct types of principles, mechanical and chemotropic, have been suggested to account for the directed growth of the pollen tube. The first of these two types of models implies that the guidance of the pollen tube depends on the architecture and chemical properties of the female reproductive tissues, whereas the latter suggests that the ovule provides a signal for the target-directed growth of the pollen tube. To examine such a role for the ovules, we analyzed the growth path of pollen tubes in mutants defective in ovule development in Arabidopsis. The results presented here provide unique in vivo evidence for an ovule-derived, long-range activity controlling pollen tube guidance. A morphological comparison of the ovule mutants used in this study indicates that within the ovule, the haploid embryo sac plays an important role in this long-range signaling process.

PMID: 12242351 [PubMed - as supplied by publisher]

PMCID: PMC160764

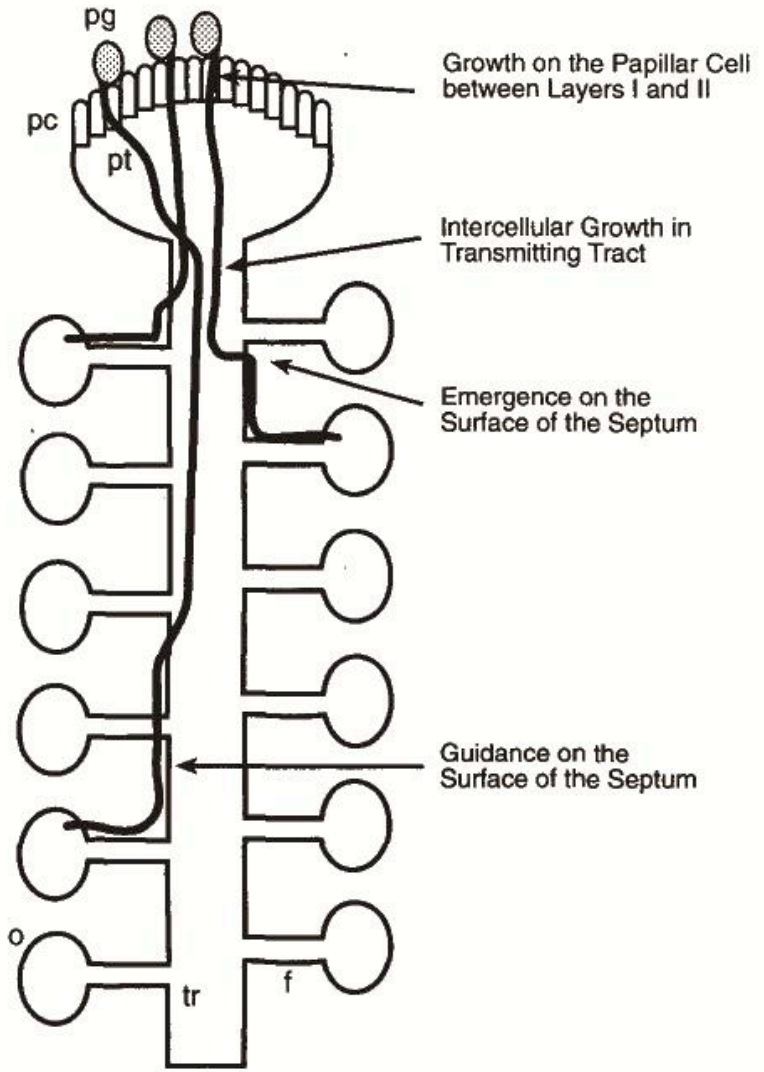
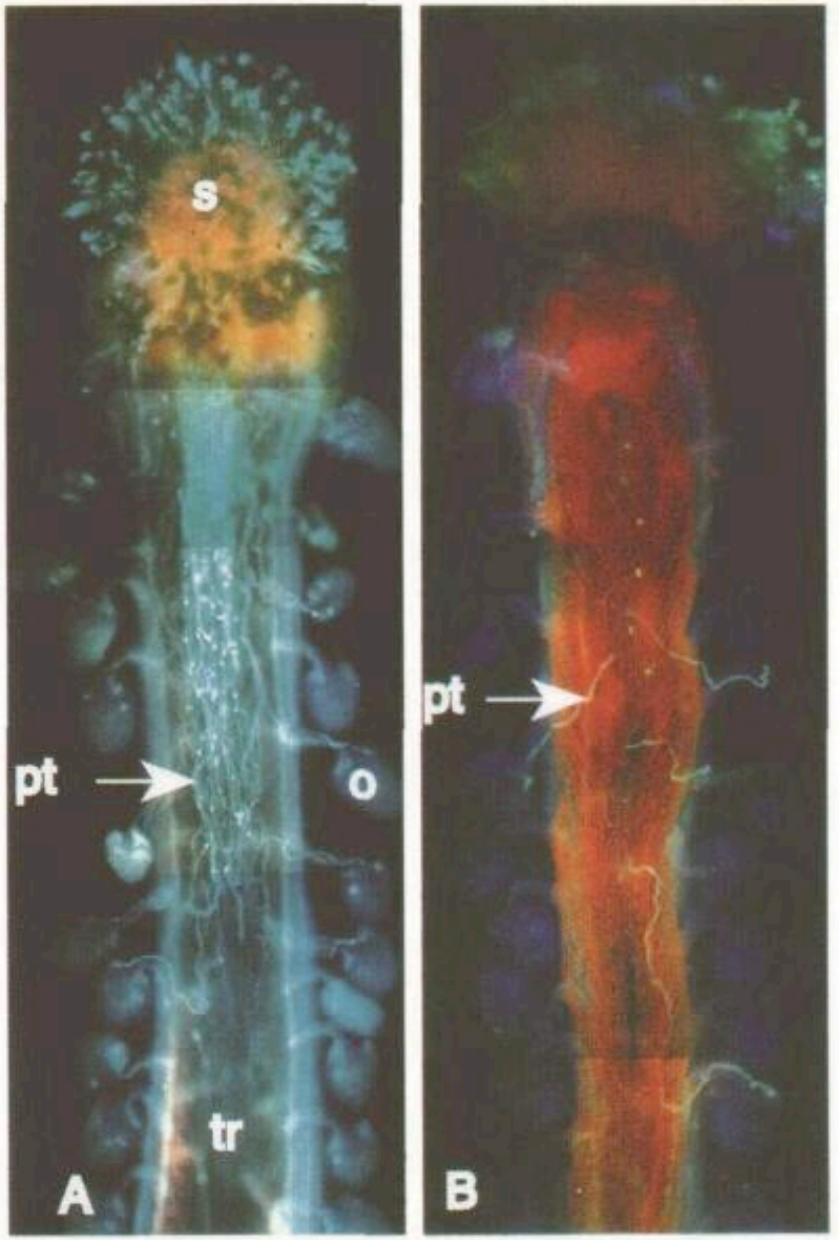


Figure 1. Schematic Representation of Pollen Tube Growth in the Pistil.

This schematic drawing depicts the four different growth phases of the pollen tube. pc, papillar cell; pg, pollen grain; pt, pollen tube; o, ovule; tr, transmitting tract; f, funiculus.



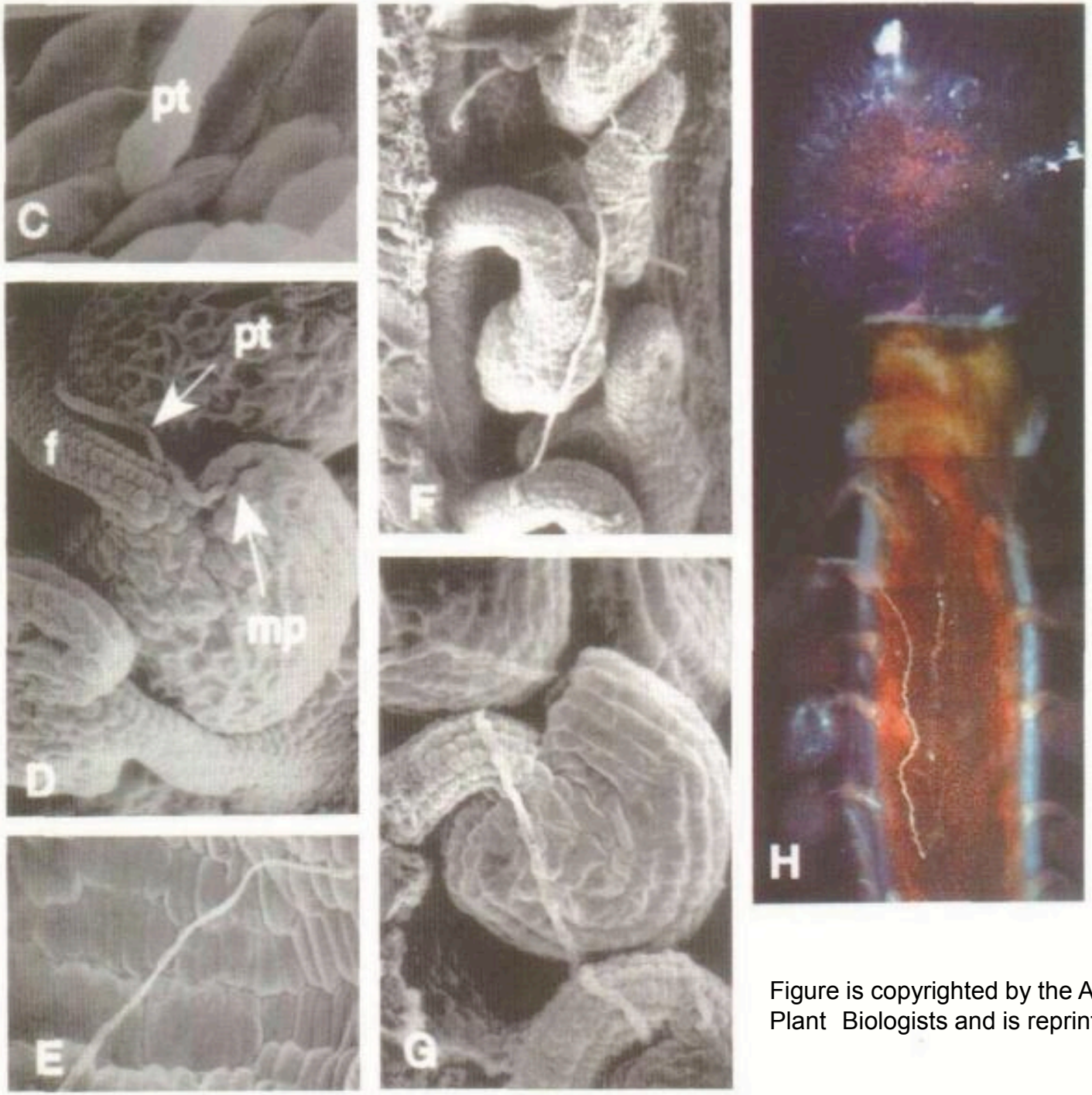


Table 2. Correlation of Developmental Arrest of Ovule Development with Guidance Competence in the Mutant 54D12

Ovule Phenotype[a]	No. of Ovules[b]	Associated with Pollen Tube[c]	Not Associated with Pollen Tube[c]
No embryo sac	181 (57%)	0 (0%)	181 (100%)
Partial embryo sac	88 (27%)	25 (28%)	63 (72%)
Wild type	51 (16%)	47 (92%)	4 (8%)

[a] 54D12 mutants show a very variable phenotype with respect to embryo sac development. There was no, partial, or full embryo sac development.

[b] The numbers in parentheses give the relative frequency of the different classes with respect to the total number of ovules scored (320).

[c] The number of ovules in each class that were or were not associated with a pollen tube, respectively. The numbers in parentheses denote the relative frequencies with respect to the total number of ovules per class.

Q1. この表から何が言えるか？

雌性配偶体が本当に花粉管をガイドするのか？

1: Development. 1997 Jun;124(12):2489-98.

Pollen tube guidance by the female gametophyte.

Ray SM, Park SS, Ray A.

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

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.

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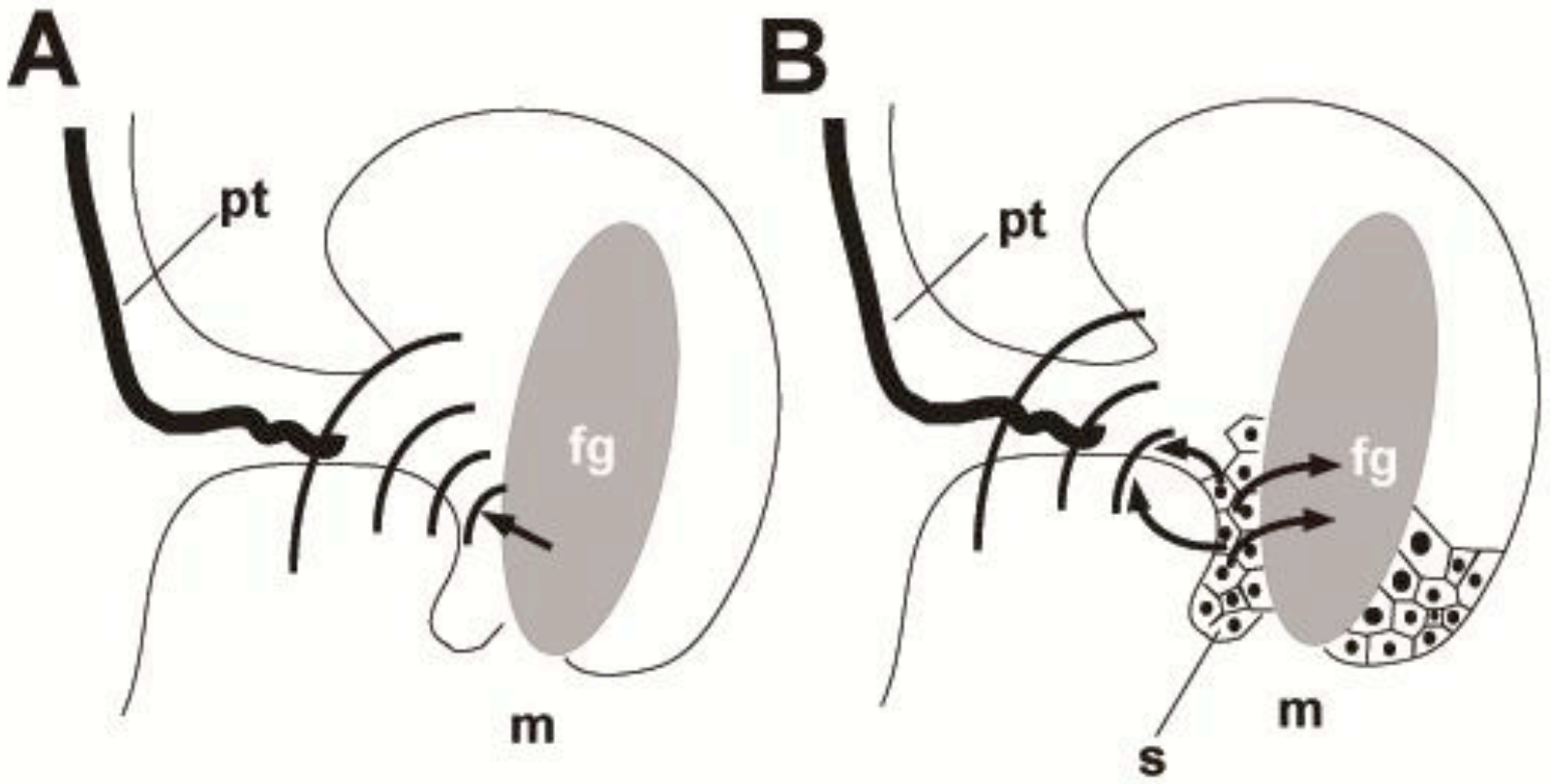
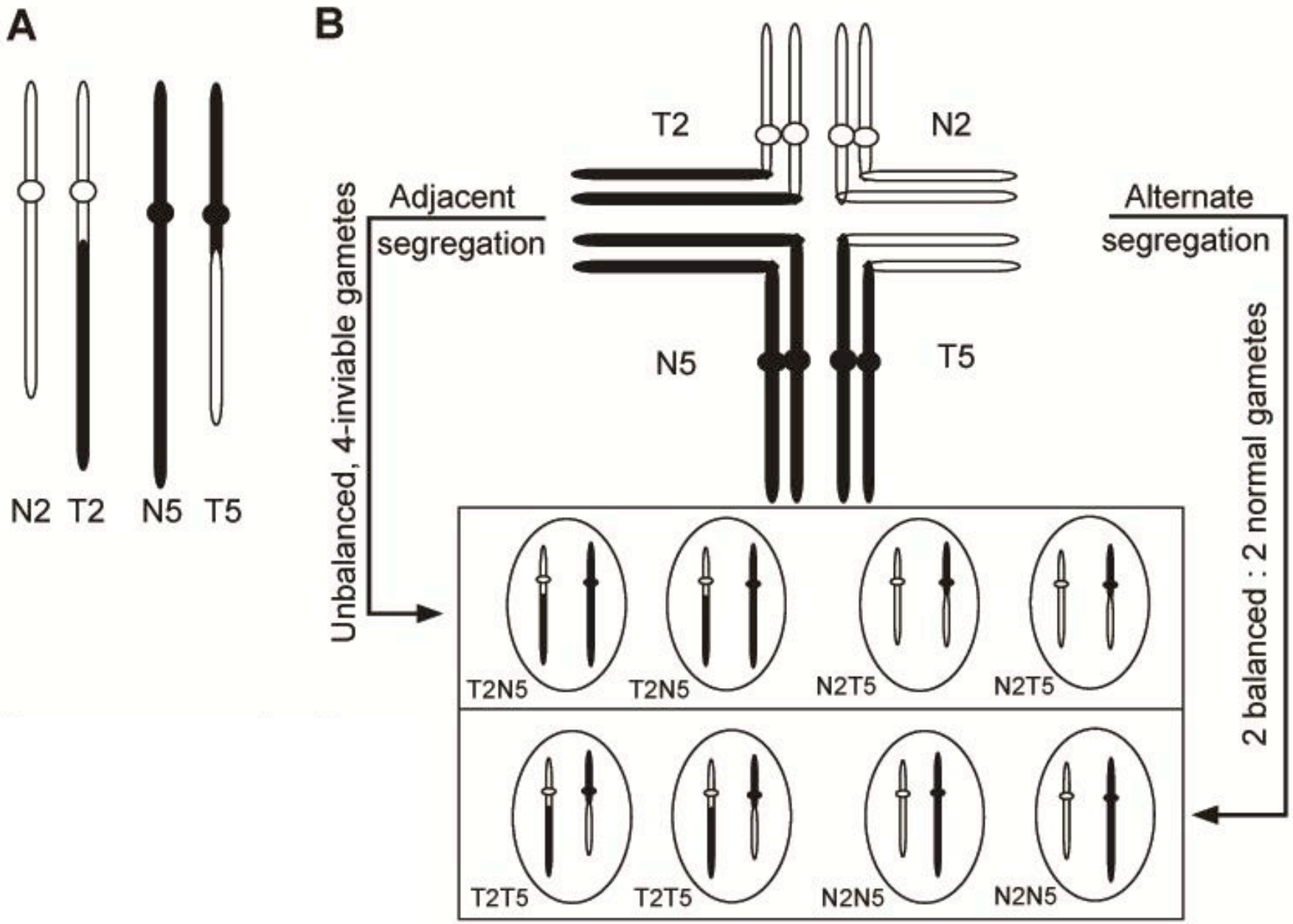
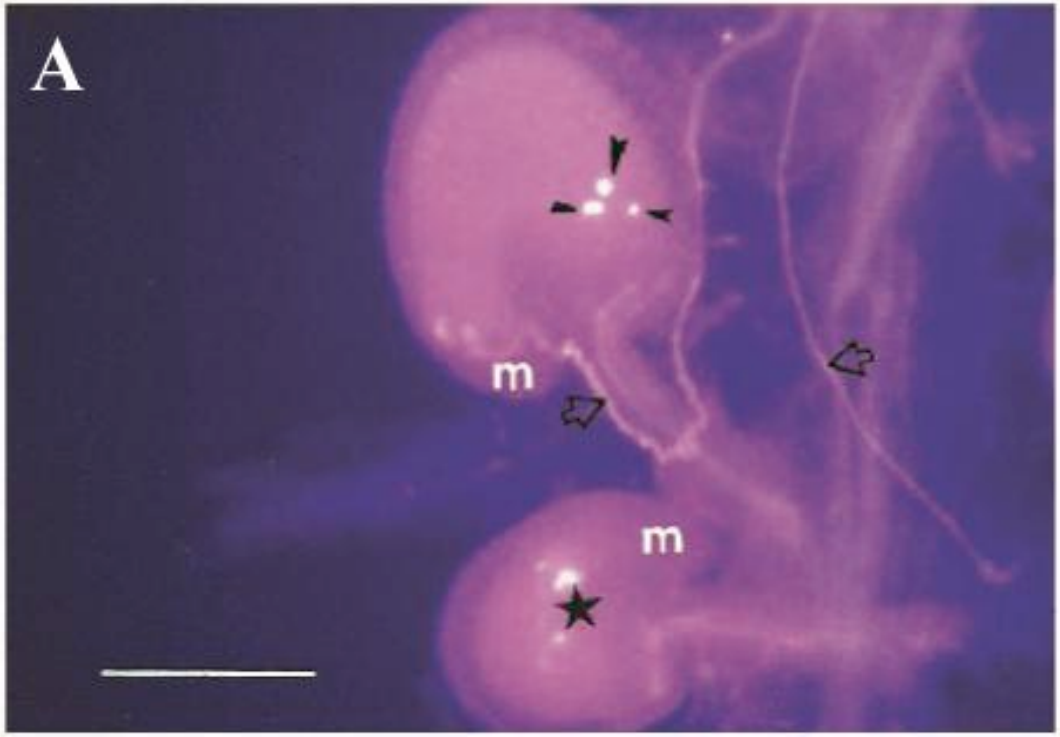


Fig. 1. Models of pollen tube guidance by the ovule. (A) The female gametophyte (fg) guides the emergent pollen tube (pt) along the ovular stalk to the micropyle (m) by emitting (arrow) a hypothetical guidance signal (arcs). (B) A hypothetical group of sporophytic cells (s), shown in an arbitrary location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



http://dev.biologists.org/content/124/12/2489.full.pdf

Q2. この表から何が言えるか？



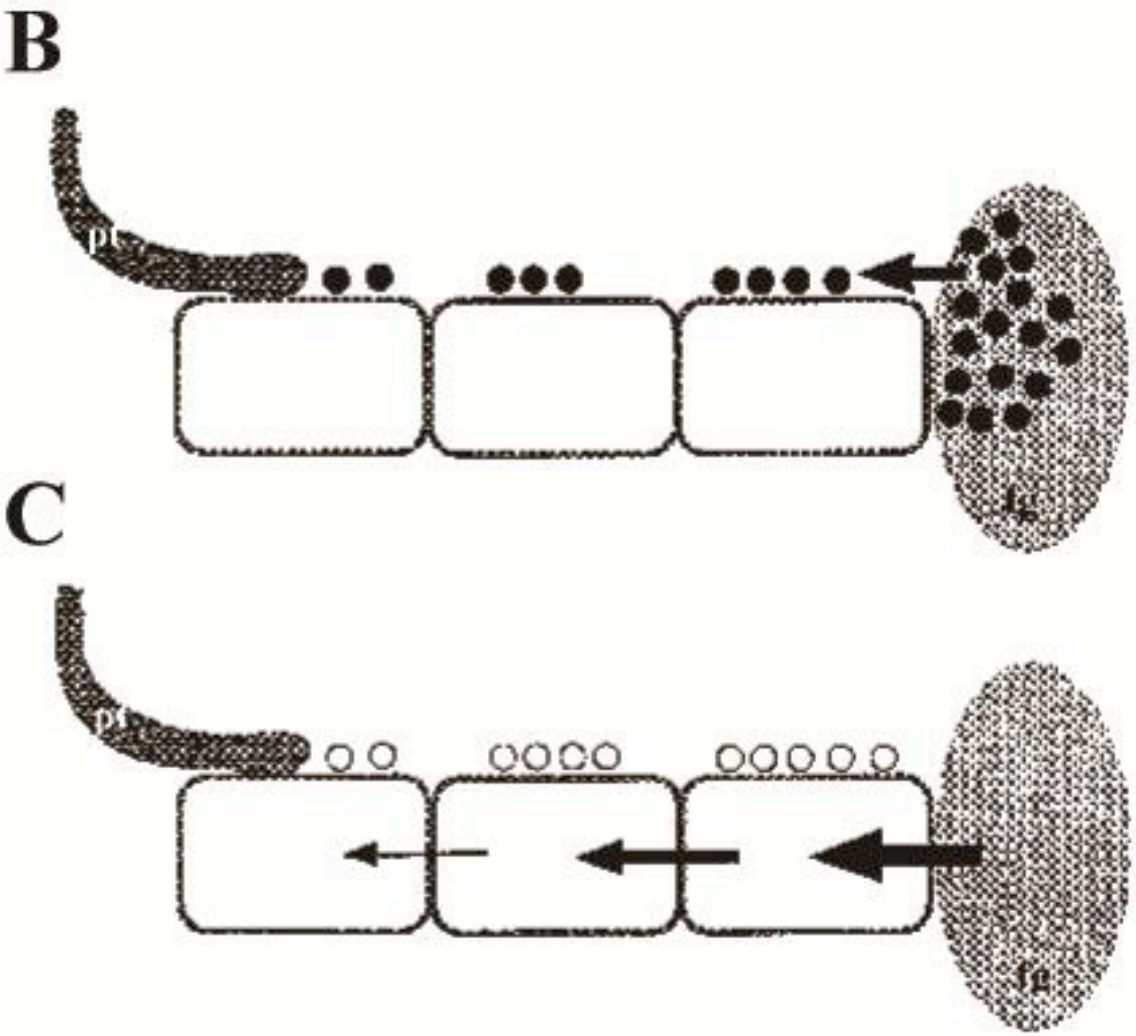
http://dev.biologists.org/content/124/12/2489.full.pdf

Table 4. Effect of translocation on pollen tube guidance

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

http://dev.biologists.org/content/124/12/2489.full.pdf

雌性配偶体による花粉管ガイダンスのモデル



http://dev.biologists.org/content/124/12/2489.full.pdf

雌性配偶体が拡散性シグナルにより花粉管をガイドする(トレニア)

1: Plant Cell. 1998 Dec;10(12):2019-32.

Guidance in vitro of the pollen tube to the naked embryo sac of torenia fournieri

Higashiyama T, Kuroiwa H, Kawano S, Kuroiwa T.

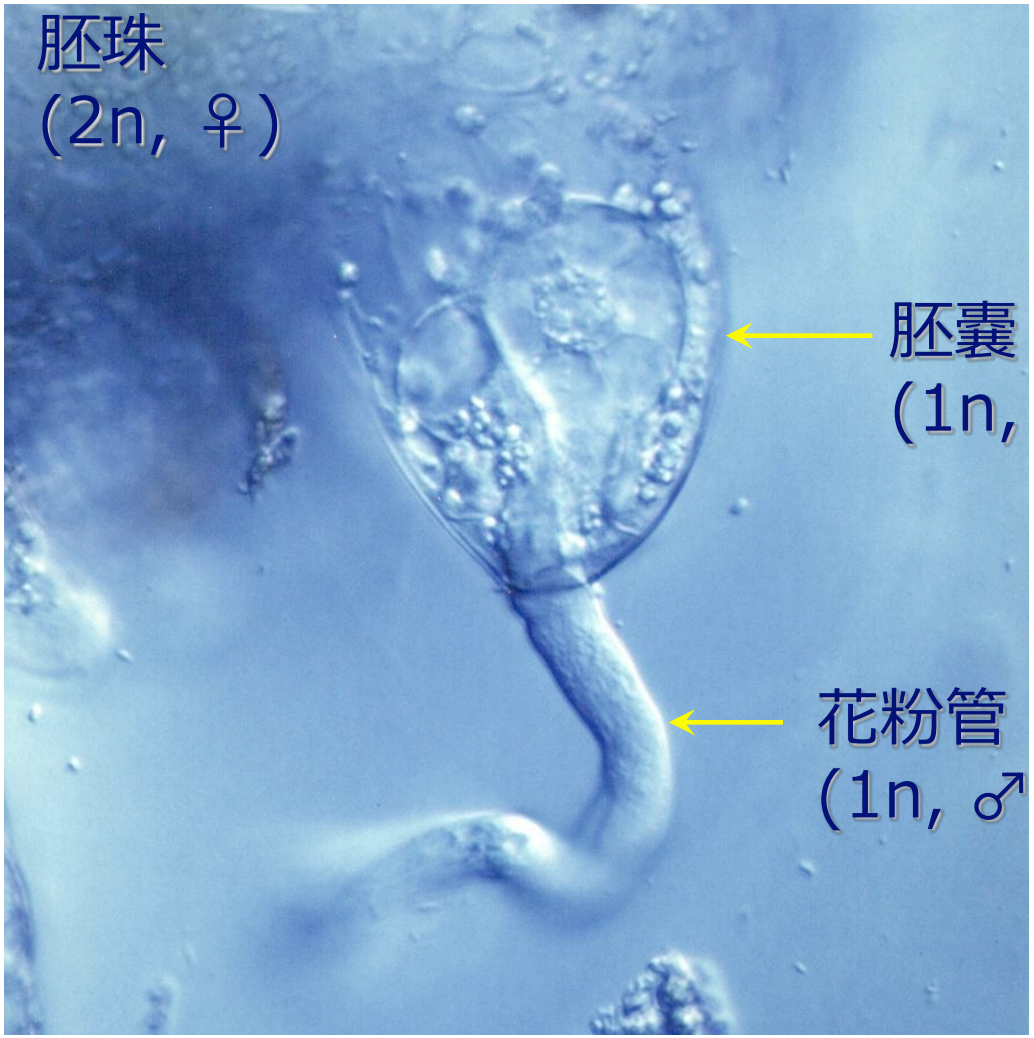
Department of Biological Sciences, Graduate School of Science, University of Tokyo, Hongo, Tokyo 113-0033, Japan.

The precise guidance of the pollen tube to the embryo sac is critical to the successful sexual reproduction of flowering plants. We demonstrate here the guidance of the pollen tube to the embryo sac in vitro by using the naked embryo sac of Torenia fournieri, which protrudes from the micropyle of the ovule. We developed a medium for culture of both the ovule and the pollen tube of T. fournieri and cocultivated them in a thin layer of solid medium. Although pollen tubes that had germinated in vitro passed naked embryo sacs, some pollen tubes that grew semi-in vitro through a cut style arrived precisely at the site of entry into the embryo sac, namely, the filiform apparatus of the synergids. When pollen tubes were unable to enter the embryo sac, they continuously grew toward the same filiform apparatus, forming narrow coils. Pollen tubes selectively arrived at complete, unfertilized embryo sacs but did not arrive at those of heat-treated ovules or those with disrupted synergids. These results convincingly demonstrate that pollen tubes are specifically attracted to the region of the filiform apparatus of living synergids in vitro.

PMID: 9836742 [PubMed - as supplied by publisher]

PMCID: PMC143976

培地中で胚嚢の先端に正確に誘引される花粉管



雌性配偶体によるガイダンスは少なくとも二段階ある

1: Development. 2000 Oct;127(20):4511-8.

Attractive and repulsive interactions between female and male gametophytes in Arabidopsis pollen tube guidance.

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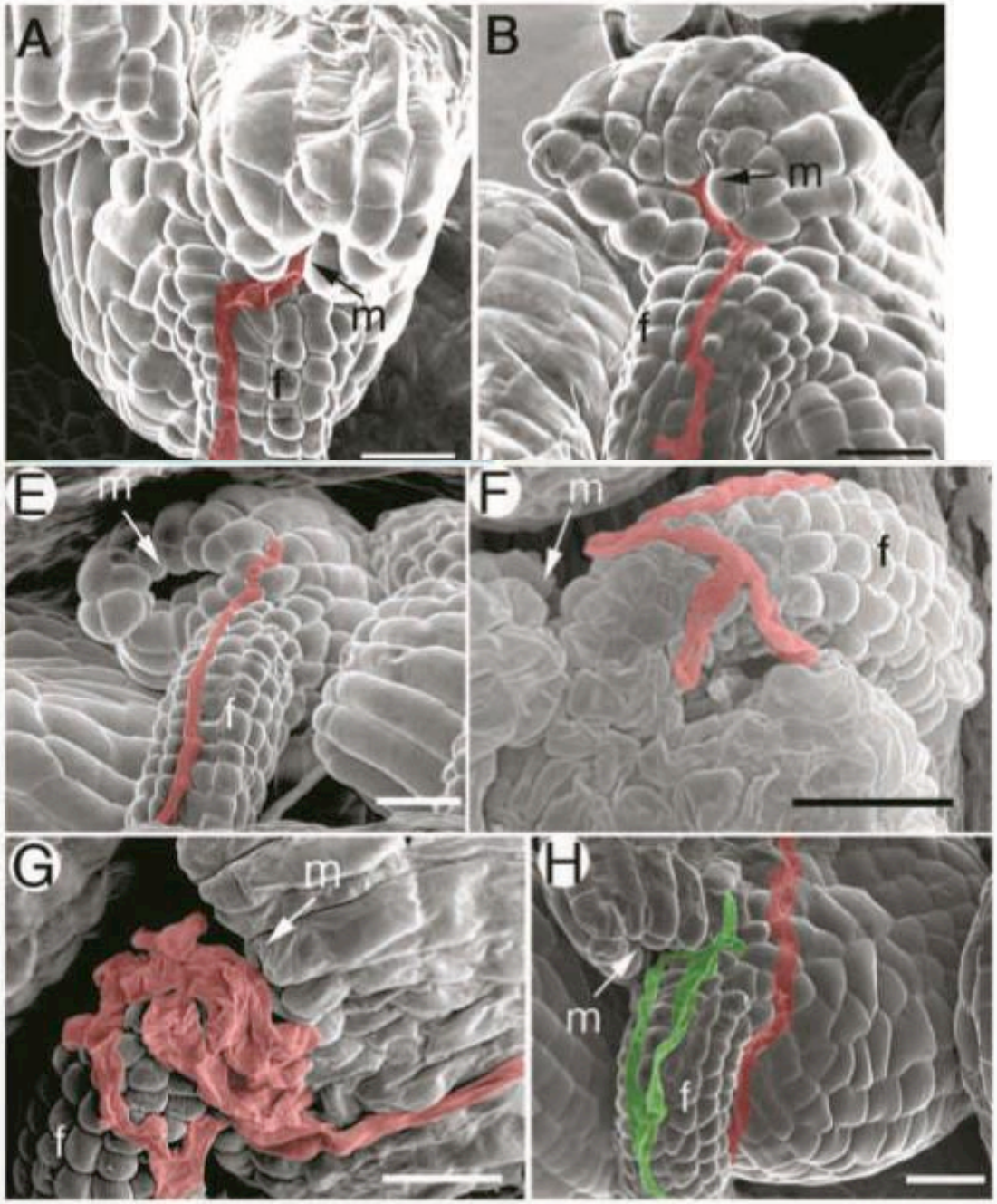
Sexual reproduction in plants, unlike that of animals, requires the action of multicellular haploid gametophytes. The male gametophyte (pollen tube) is guided to a female gametophyte through diploid sporophytic cells in the pistil. While interactions between the pollen tube and diploid cells have been described, little is known about the intercellular recognition systems between the pollen tube and the female gametophyte. In particular, the mechanisms that enable only one pollen tube to interact with each female gametophyte, thereby preventing polysperm, are not understood. We isolated female gametophyte mutants named magatama (maa) from Arabidopsis thaliana by screening for siliques containing half the normal number of mature seeds. In maa1 and maa3 mutants, in which the development of the female gametophyte was delayed, pollen tube guidance was affected. Pollen tubes were directed to mutant female gametophytes, but they lost their way just before entering the micropyle and elongated in random directions. Moreover, the mutant female gametophytes attracted two pollen tubes at a high frequency. To explain the interaction between gametophytes, we propose a monogamy model in which a female gametophyte emits two attractants and prevents polyspermy. This prevention process by the female gametophyte could increase a plant's inclusive fitness by facilitating the fertilization of sibling female gametophytes. In addition, repulsion between pollen tubes might help prevent polyspermy. The reproductive isolations observed in interspecific crosses in Brassicaceae are also consistent with the monogamy model.

PMID: 11003848 [PubMed - indexed for MEDLINE]



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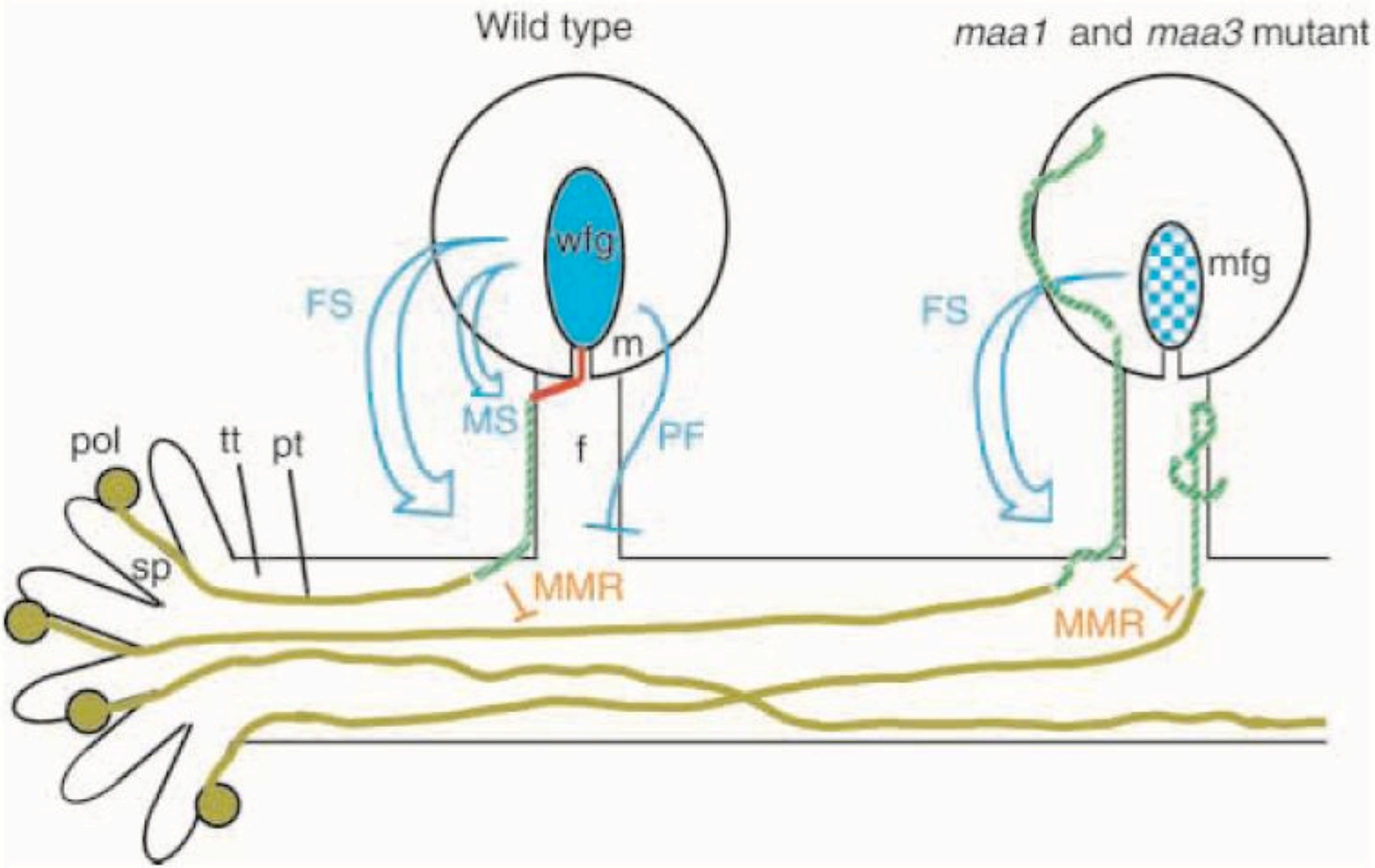


http://dev.biologists.org/content/127/20/4511.full.pdf

助細胞が花粉管をガイドする(トレニア)

1: Science. 2001 Aug 24;293(5534):1480-3.

Comment in:
Science. 2001 Aug 24;293(5534):1441-2.

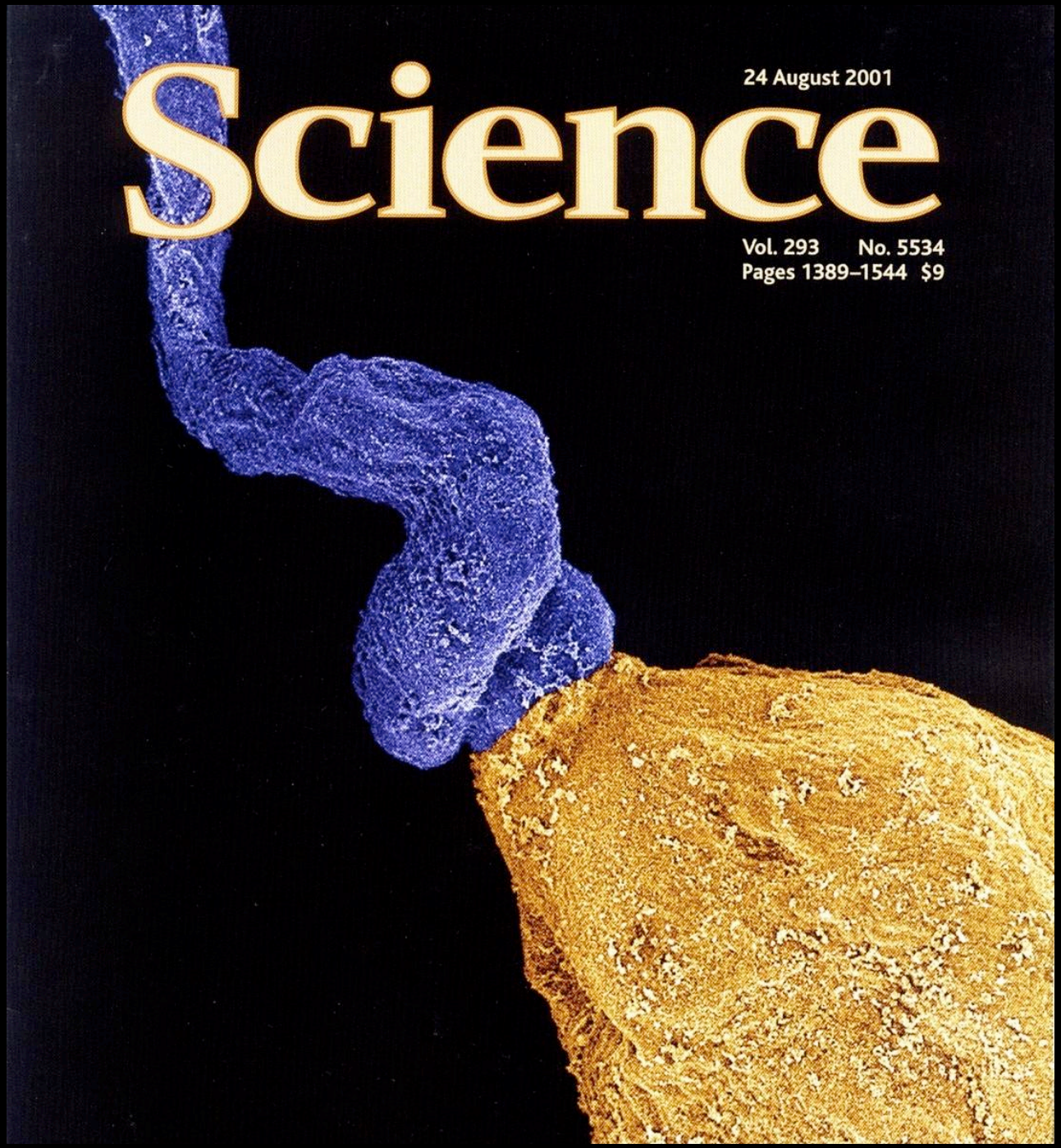
Pollen tube attraction by the synergid cell.

Higashiyama T, Yabe S, Sasaki N, Nishimura Y, Miyagishima S, Kuroiwa H, Kuroiwa T.

Department of Biological Sciences, Graduate School of Science, University of Tokyo, Hongo, Tokyo 113-0033, Japan. higashi@biol.s.u-tokyo.ac.jp

In flowering plants, guidance of the pollen tube to the embryo sac (the haploid female gametophyte) is critical for successful fertilization. The target embryo sac may attract the pollen tube as the final step of guidance in the pistil. We show by laser cell ablation that two synergid cells adjacent to the egg cell attract the pollen tube. A single synergid cell was sufficient to generate an attraction signal, and two cells enhanced it. After fertilization, the embryo sac no longer attracts the pollen tube, despite the persistence of one synergid cell. This cessation of attraction might be involved in blocking polyspermy.

PMID: 11520985 [PubMed - indexed for MEDLINE]



シロイヌナズナにおいても助細胞が花粉管ガイダンスに関わる

1: Plant Cell. 2005 Nov;17(11):2981-92. Epub 2005 Oct 7.

MYB98 is required for pollen tube guidance and synergid cell differentiation in Arabidopsis.

Kasahara RD, Portereiko MF, Sandaklie-Nikolova L, Rabiger DS, Drews GN.

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The synergid cells of the female gametophyte play a role in many steps of the angiosperm fertilization process, including guidance of pollen tube growth to the female gametophyte. However, the mechanisms by which the synergid cells become specified and develop their unique features during female gametophyte development are not understood. We identified MYB98 in a screen for Arabidopsis thaliana genes expressed in the female gametophyte. MYB98 is a member of the R2R3-MYB gene family, the members of which likely encode transcription factors. In the context of the ovule, MYB98 is expressed exclusively in the synergid cells, and mutations in this gene affect the female gametophyte specifically. myb98 female gametophytes are affected in two unique features of the synergid cell, pollen tube guidance and the filiform apparatus, but are otherwise normal. MYB98 also is expressed in trichomes and endosperm. Homozygous myb98 mutants exhibit no sporophytic defects, including trichome and endosperm defects. Together, these data suggest that MYB98 controls the development of specific features within the synergid cell during female gametophyte development.

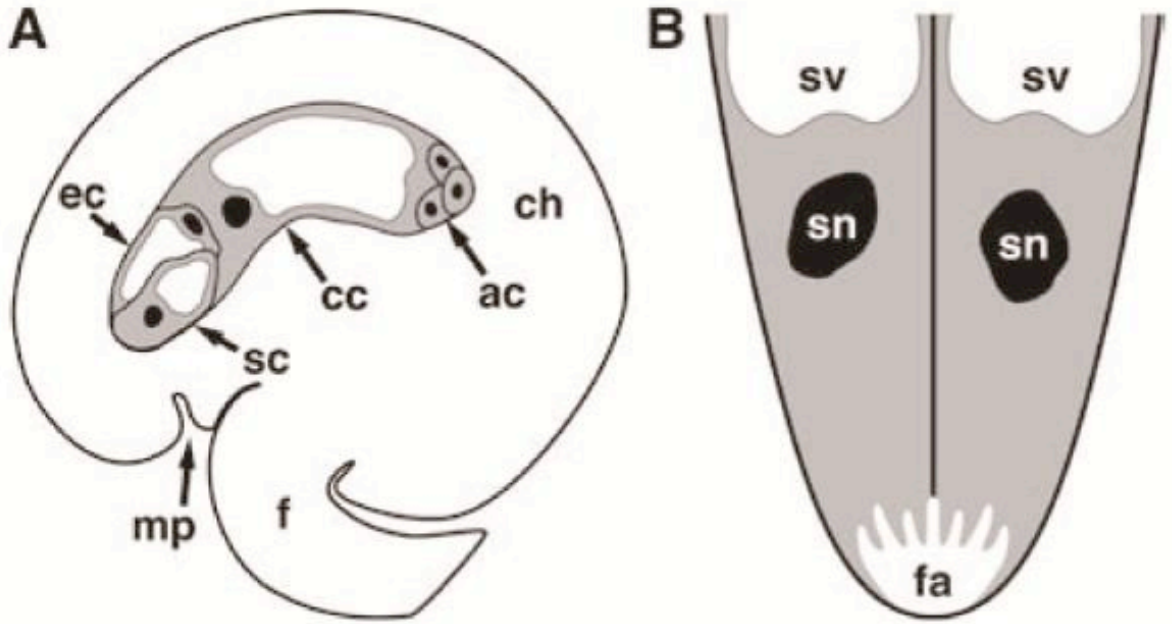


Figure 1. Depictions of the Female Gametophyte and Synergid Cells.

(A) Depiction of the *Arabidopsis* female gametophyte. Modified from Drews et al. (1998).

(B) Depiction of the synergid cells. This view is perpendicular to that in **(A)**.

The cytoplasm is gray, vacuoles are white, and nuclei are black. ac, three antipodal cells; cc, central cell; ch, chalazal region of the ovule; ec, egg cell; f, funiculus; fa, filiform apparatus; mp, micropyle; sc, synergid cell; sn, synergid cell nucleus; sv, synergid cell vacuole.

逆遺伝学により同定されたMYB98遺伝子

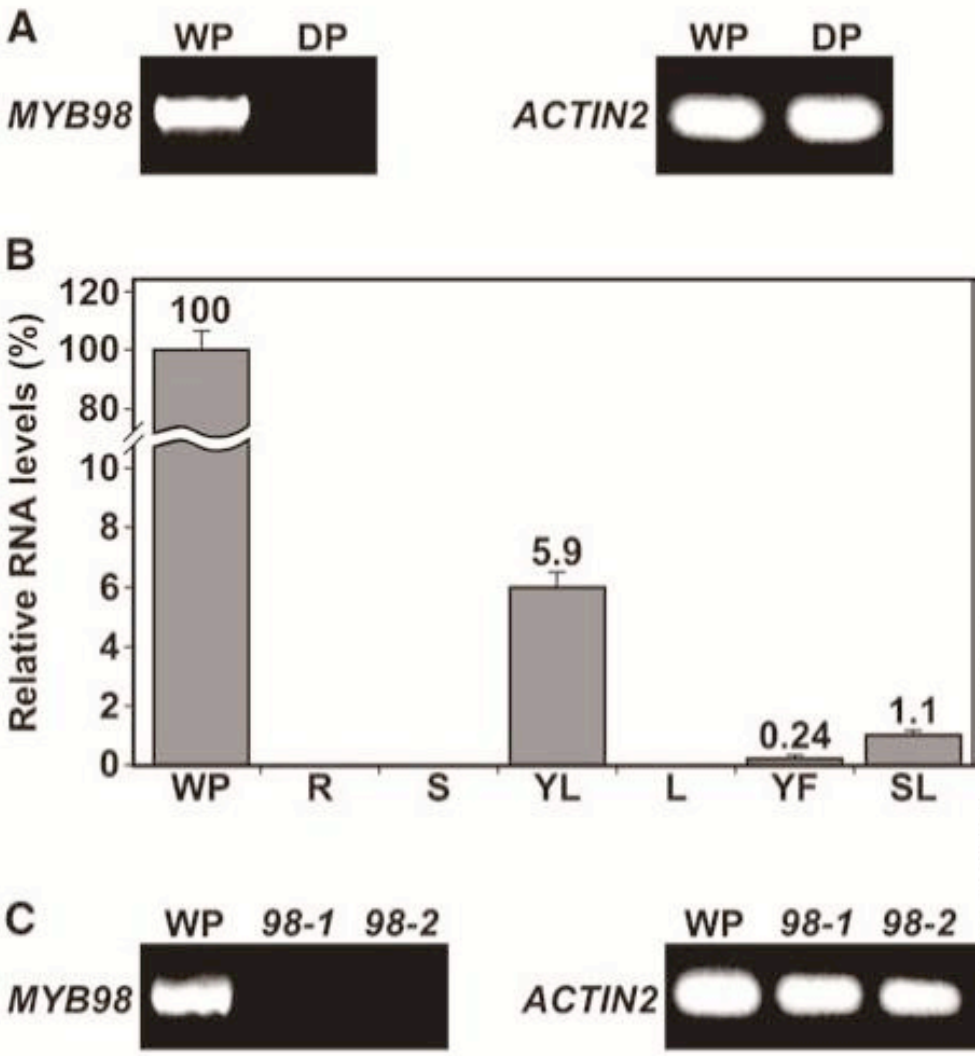


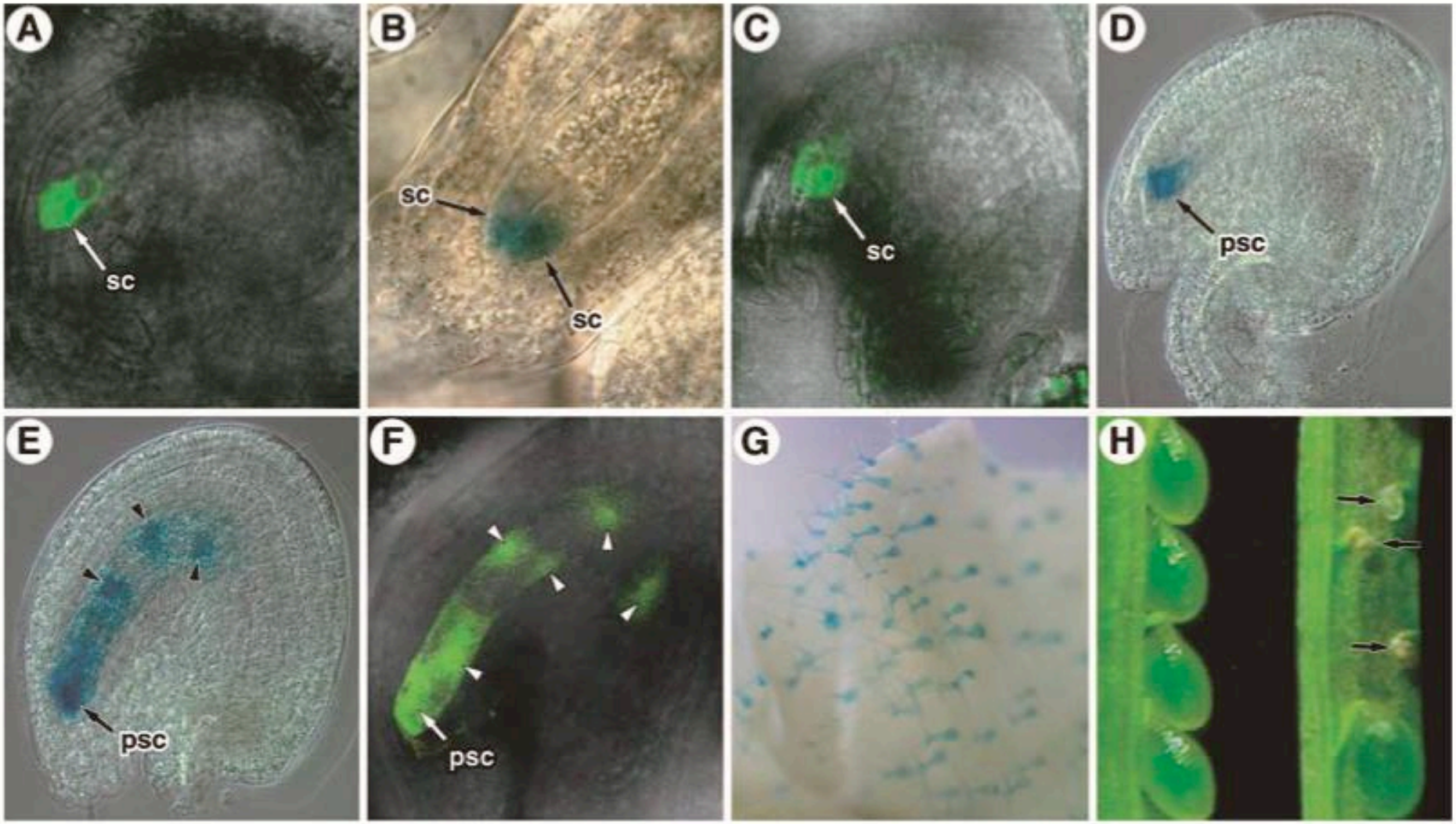
Figure 2. RT-PCR Analysis of *MYB98* Expression.

(A) Expression of *MYB98* in wild-type pistils (WP) and *dif1-2/dif1-2* pistils (DP). Expression of the *ACTIN2* gene was used as a control.

(B) Real-time RT-PCR analysis of *MYB98* expression in pistils (WP), roots (R), floral stems (S), young leaves at <5 mm in length (YL), older leaves at 10 to 20 mm in length (L), flowers at stages 1 to 11 (YF), and siliques at 1 to 2 d after pollination (SL). Each bar represents an average of four to five independent reactions. Error bars indicate SD. The number above each bar represents RNA level relative to pistil (WP).

(C) Expression of *MYB98* in wild-type pistils (WP), *myb98-1/myb98-1* pistils (*98-1*), and *myb98-2/myb98-2* pistils (*98-2*). Expression of the *ACTIN2* gene was used as a control.

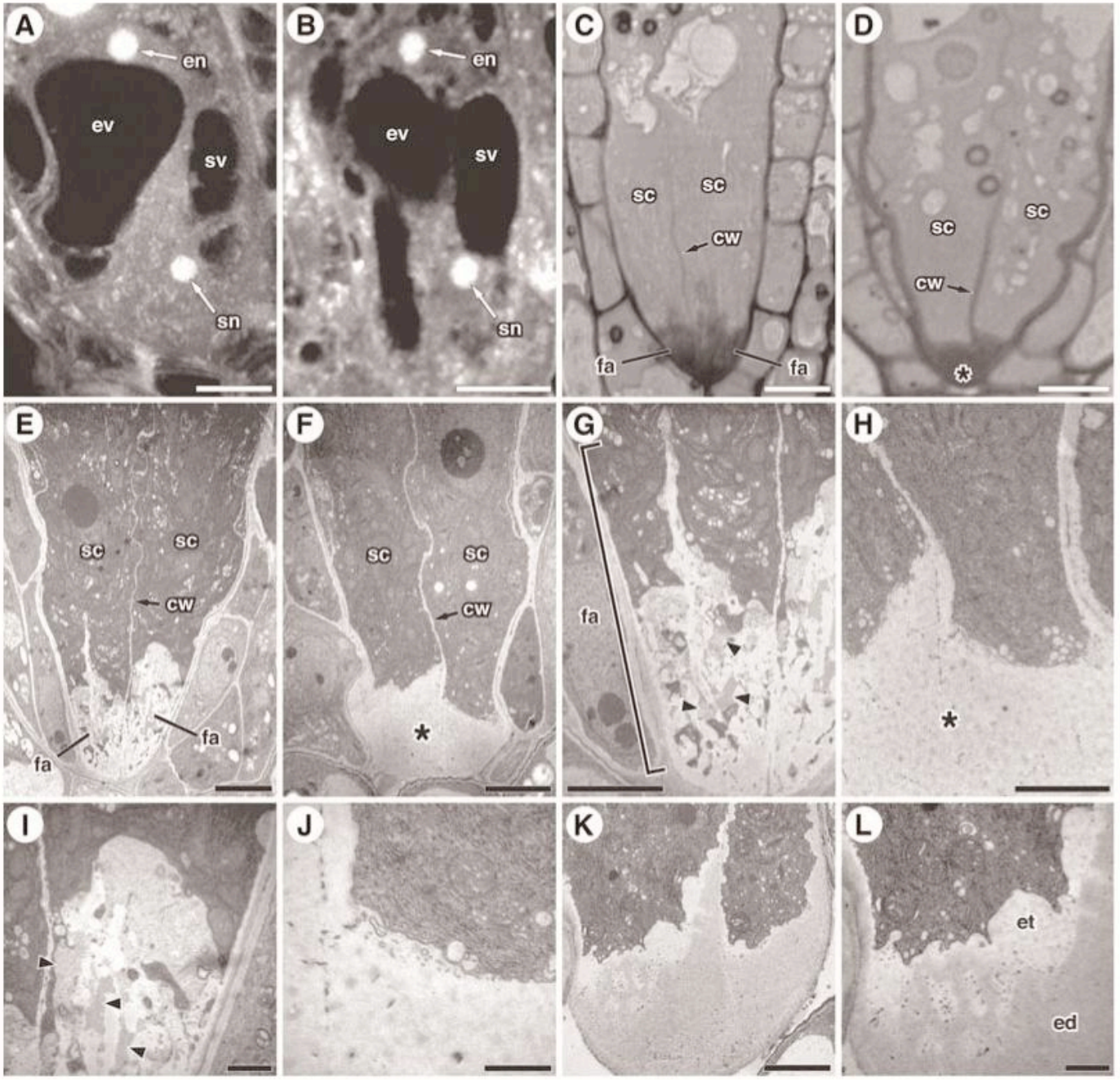
レポーター遺伝子を用いた発現解析と、T-DNAノックアウトラインを用いた機能解析



Q3. この表から何が言えるか？

Table 1. Segregation of the *myb98-1* and *myb98-2* Mutations

Parental Genotypes		Progeny Genotypes		
Male	Female	*MYB98/MYB98*	*myb98/MYB98*	*myb98/myb98*
myb98-1/MYB98	*myb98-1/MYB98*	109	113	5
MYB98/MYB98	*myb98-1/MYB98*	304	3	–
myb98-1/MYB98	*MYB98/MYB98*	160	172	–
myb98-2/MYB98	*myb98-2/MYB98*	367	388	13
MYB98/MYB98	*myb98-2/MYB98*	368	5	–
myb98-2/MYB98	*MYB98/MYB98*	187	217	–



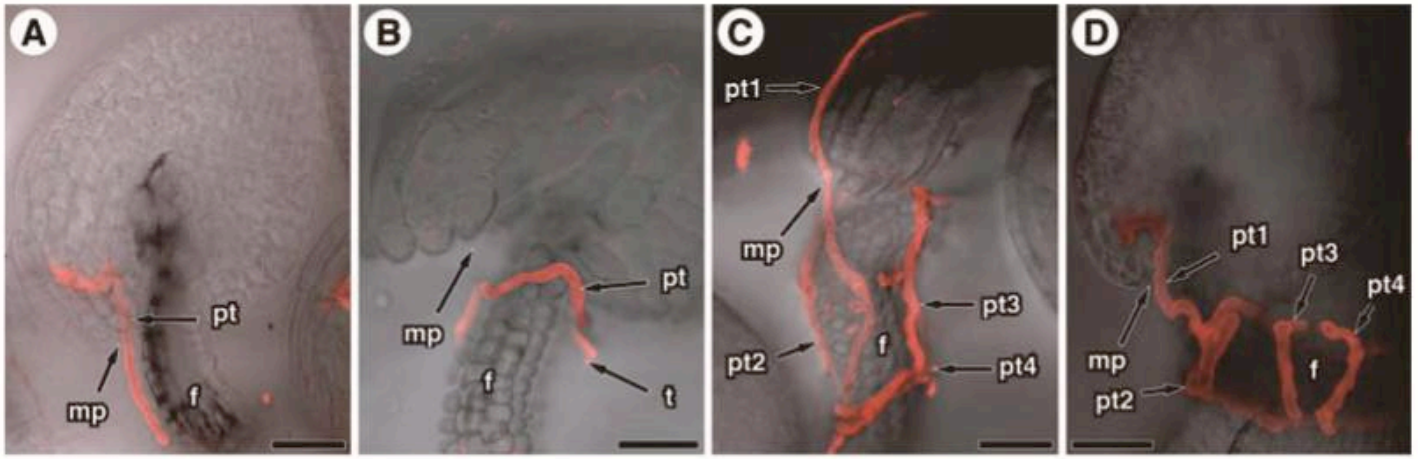


Figure 6. Pollen Tube Guidance Is Affected in *myb98* Female Gametophytes.

(A) Wild-type pollen tube on a wild-type ovule at 12 h after pollination.
(B) Wild-type pollen tube on a *myb98-1/myb98-1* ovule at 12 h after pollination.
(C) Wild-type pollen tubes on a *myb98-1/myb98-1* ovule at 24 h after pollination. Four pollen tubes (pt1 to pt4) are present on the ovule.
(D) Wild-type pollen tubes on a *myb98-1/myb98-1* ovule at 24 h after pollination. Four pollen tubes (pt1 to pt4) are present on the ovule. One of the pollen tubes (pt1) is in the micropyle.
All images are composites composed of CLSM micrographs of the pollen tubes merged with bright-field images of ovules. All images are projections of multiple 1-μm sections. f, funiculus; mp, micropyle; pt, pollen tube; t, tip of pollen tube. Bars = 25 μm.

助細胞が、___から___にかけての花粉管ガイダンスに関わる（最終ステップ；~100μm）

Acknowledgements

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- Pictures/Tables from Development are reproduced /adapted with permission.(reference [5,6])

- Some Materials are from National Library of Medicine (NLM) Web pages.

参考文献(references)

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- [2] Tetsuya Higashiyama, Haruko Kuroiwa, Shigeyuki Kawano, and Tsuneyoshi Kuroiwa. (1998). Guidance in Vitro of the Pollen Tube to the Naked Embryo Sac of Torenia fournieri. *Plant Cell* 10: 2019-2032.
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- [4] Full Cover from *SCIENCE* volume. 293, Issue 5534 (24 Aug 2001) .
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- [6] K.K. Shimizu and K. Okada. (2000). Attractive and repulsive interactions between female and male gametophytes in Arabidopsis pollen tube guidance *Development .* 127: 4511-4518