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Identification of Genes May Tell How Plants Recognize Pollen

Researchers have identified the genes that code for proteins that coat the pollen of the flowering plant *Arabidopsis thaliana*. The studies may help scientists understand how plants recognize pollen from their own species.

Understanding the basis of species recognition by plants could enable crop scientists to cross previously incompatible plant species or to prevent genetically engineered plants from crossing with other strains.

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— Daphne Preuss

The researchers, led by Howard Hughes Medical Institute investigator Daphne Preuss at the University of Chicago, reported on the identification of the *Arabidopsis* pollen coat protein gene families in an article published in the June 29, 2001, issue of the journal *Science*.

Arabidopsis thaliana is a member of the mustard family that also includes cabbage and radish. *Arabidopsis* is small, prolific, easily grown and has a rapid life cycle. In December 2000, an international consortium of scientists announced that it had sequenced the entire *Arabidopsis* genome, an achievement that plant scientists believe will lead to advances in understanding plant physiology.

Earlier experiments by Preuss and others had shown that switching or altering pollen coat proteins can change plants' species specificity or eliminate pollination altogether. "Scientists have understood for quite a while that the pollen coating is critical for launching the process of pollination in a large number of species," said Preuss. "But a comprehensive analysis of all of the pollen protein coat genes in one plant had never been done before, and that's where this work is unique."

The search for the pollen coat protein genes began with work by lead author Jacob A. Mayfield, who extracted and identified all of the *Arabidopsis* pollen coat proteins. Researchers at Stanford University Medical Center provided protein sequence information. "Although we had these protein sequences, it was not until the *Arabidopsis* genome was sequenced that our search revealed that these proteins are encoded in tandem arrays in the genome," said Preuss.

The scientists discovered that the coat protein genes existed in two distinct clusters of the *Arabidopsis* genome, an exciting finding, said Preuss, because it indicates that the plants have 'mating clusters' of genes that are kept together. One of the gene clusters coded for enzymes called lipases that cleave lipid molecules; and the other cluster coded for lipid-binding proteins called oleosins. According to Preuss, the lipid-related enzymes and proteins enable the dry pollen coat to interact with the stigma cells of plant flowers during pollination.

After finding that the genes were contained in two clusters, the scientists began experiments designed to reveal the role of the clusters. "Whenever genes are clustered, it raises important evolutionary questions about why they are in clusters and what maintains the cluster," Preuss said. To explore whether the clusters are maintained in related *Arabidopsis* "ecotypes" — distinctive plant strains from different geographic regions — co-author Aretha Fiebig sequenced the clustered genes from five ecotypes.

Fiebig discovered that the gene clusters were maintained across ecotypes. She also found that any variation within the genes did not disrupt the functionality of the genes. "These findings argued that there was evolutionary pressure to maintain the genes," said Preuss.

When Mayfield compared the pollen protein coat gene clusters from *Arabidopsis* to those of its relative, broccoli, he found a large genetic divergence. "We believe that this divergence means that these genes are important for speciation, and we would like to begin seeking similar genes in other plants," said Preuss.

In future studies, Preuss's team plans to mutate the *Arabidopsis* pollen coat protein genes and swap gene clusters from other plants to explore the proteins' basic function in species recognition and their role in speciation. Understanding these functions could lead to powerful new techniques for crossing plants, said Preuss.

"Understanding the basic recognition molecules would allow two main problems to be addressed that are opposite sides of the same coin," she said. "First, manipulating pollen recognition would enable crosses that are otherwise difficult to do — such as mating an apple tree that is cold-tolerant with an otherwise incompatible variety that has delicious fruit. The other side of the coin would be to engineer pollen to inhibit crosses, for example so that the pollen from genetically engineered crops would not be recognized by another variety."

According to Preuss, the discovery of the pollen protein coat genes is an example of the kind of research that plant biologists can do now that the *Arabidopsis* genome has been sequenced. " *Arabidopsis* has all the basic parts that plants need for doing what they do, so it's an excellent model. The sequencing of its genome has put us into a wonderful discovery phase, in which we can race ahead and test the function of large numbers of genes very quickly.

"The *Arabidopsis* genome sequence allows plant scientists to work from just a small bit of gene sequence to obtain the entire sequence of a gene," said Preuss. "Also, we already have many known mutations of this plant, so when we make new mutants, we can compare them with these previously characterized mutants. Finally, since much work has been done relating *Arabidopsis* to other species, now that we have the *Arabidopsis* genome nailed, it's very easy to make comparisons."