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One Protein Can Produce a Fundamental Change in the Flower: Fatty Acid Hydroxylase Knockdown Converts Wet Stigma to Dry Stigma

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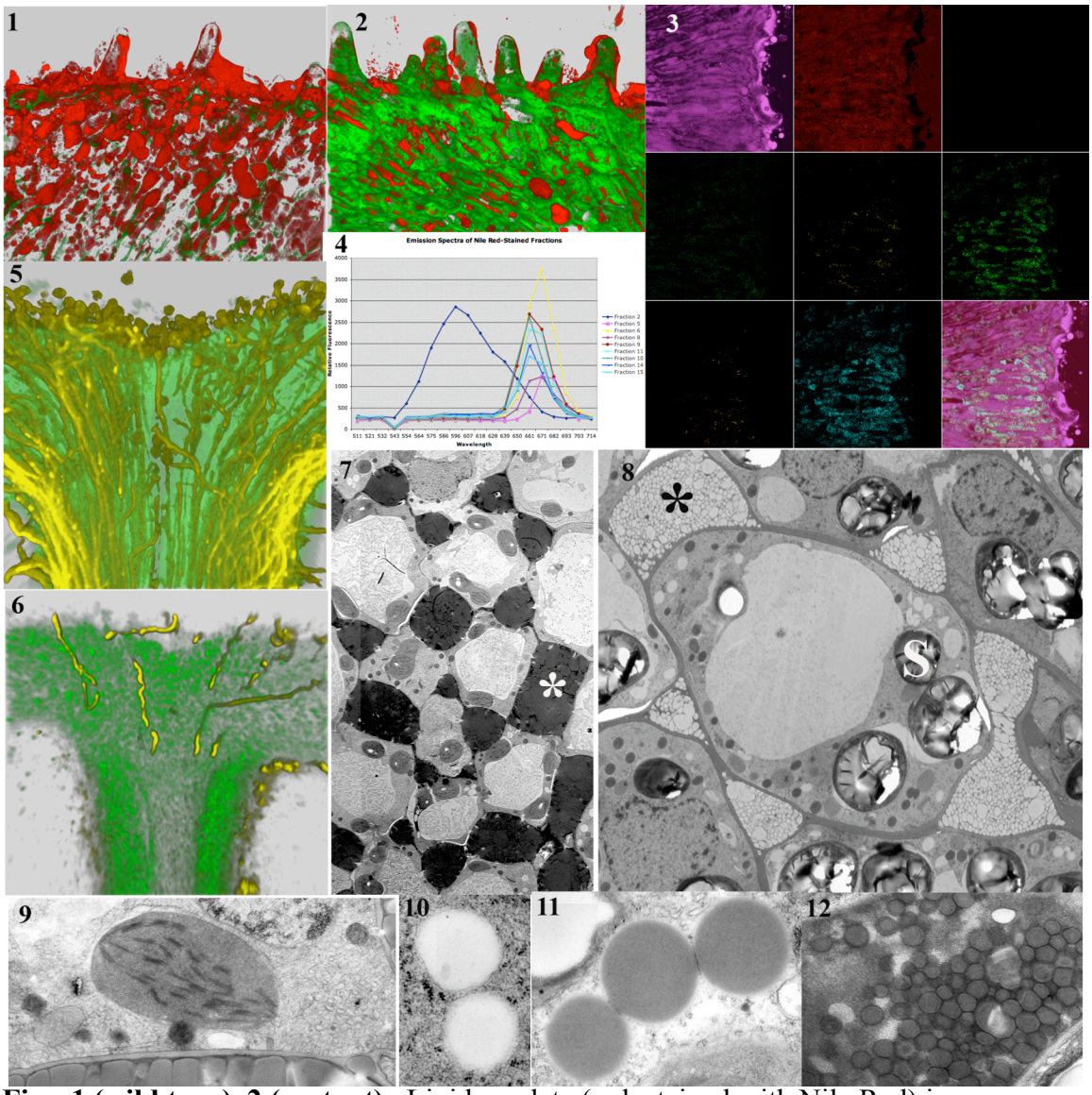
Evolutionary changes in plant organs do not have to involve a complex series of mutations. Here we alter one gene to effect conversion of the petunia flower stigma from a wet stigma into a dry stigma. We show the significant changes this brings, from the cell to the tissue level, using correlative light and transmission electron microscopy.

In the plant floral organ, the stigma functions as a site for binding pollen and facilitating its germination in route to forming the pollen tube. Pollen binding and germination is facilitated by a variety of macromolecules whose composition varies with different classes of plants [1]. In petunia, the stigma tissue is "wet" due to copious exudation of lipids, in the form of triacylglyeride polymers. The "wet" stigma surface is conducive to pollen adherence, germination, and pollen tube growth into the stylar tissue [2]. The material for electron microscopy in this study was prepared by high pressure freezing, followed by freeze substitution (FS) in 2% osmium in acetone.

Mutation by RNAi knockdown of fatty acid hydroxylase dramatically reduces secretion of exudates (Figs.1,2), which in the wild type are comprised of hydroxy fatty acid polymers. Spectral unmixing of lipid classes in the stigma (Figs. 3,4) shows a variety of polar lipids are present in stigma tissue, and that the exudate is non-polar triacylglyeride (shown in pink). The paucity of exudates in the mutant, forming the dry stigma phenotype, causes poor pollen adherence, germination and pollen tube growth (Fig. 6, vs Fig. 5, wild type). In both wild type and mutant plants, exudates accumulate in the extracellular space, first in the middle lamella, then as large accumulations at cell corners (Figs.7,8), and finally at the stigma surface. The exudates that *are* produced in the mutant differ from wild type, lacking electron density (Fig.8). Osmium staining cytochemistry (Figs. 10-12) shows that aqueous osmium (chemical fix at room temperature) preserves the osmiophilic exudates in the mutant (Fig.12). Lack of their preservation compared to the wild type in *freeze substituted* specimens (Figs. 7 vs 8) is likely due to extraction of the non-polymeric exudates in the mutant by acetone, while in the wild type extraction is prevented because the exudate is polymeric. In the mutant plant the high carbon flux normally secreted as exudate instead accumulates in cytosolic lipid bodies (Figs. 10,11) and, further upstream in carbon flow, there is a massive accumulation of starch in chloroplasts of secretory cells (Fig.8, compare to wild type chloroplasts in Fig.9). Cytosolic lipid bodies do not form in wild type plants, and in mutants are extracted by freeze substitution (Figs. 10,11). We are interested in understanding the mechanism for exudate secretion in wet stigmas. No secretory organelles have yet been identified, but smooth ER is implicated in the process and the cytosolic lipid bodies in the mutant apparently derive from the smooth ER.

[1] A.F. Edlund et al., Plant Cell 16 (2004) S84.
[2] M. Wolters-Arts et al., Nature 392 (1998) 818.
[3] P. Greenspan and S.D. Fowler, J Lipid Research (1985) 781.



Figs. 1 (wild type), 2 (mutant). Lipid exudate (red, stained with Nile Red) is significantly reduced in stigmas of plants mutated to form RNAi knock-down of fatty acid hydroxylase: lack of hydroxyl groups on the fatty acid components of the exudate prevents polymerization of the neutral lipids and this leads to a number of unusual effects on stigma cell biology. **Figs. 3,4.** Analysis of lipid polarity classes by spectral imaging. Reference spectra of Nile Red-stained exudate fractions (Fig.4) were used to unmix these spectral fingerprints from the spectral image (Fig.3, stigma surface facing right) The panel of micrographs shows unmixes of fractions with increasing polarity from upper left to lower right (final image is composite). **Figs. 5 (wild type) and 6 (mutant)**. Pollen (yellow, Aniline Blue stain) has poor adherence in mutant stigmas, and pollen tube growth is poor in the absence of exudates. **Figs. 7 (wild type), 8 (mutant).** Thin sections of freeze substituted (FS) stigma cells. Exudate in the mutant (asterisk) is reduced in amount (cf. Figs. 1,2) and it has been leached during FS (cf. Fig. 12, aqueous osmium fix of mutant exudate). Chloroplasts in the mutant accumulate starch (S, relative to wild type, Fig.9), correlating with the intracellular accumulation of carbon due to reduced secretion. Lipid also accumulates in the mutant as lipid bodies (Fig. 11), not found in controls, that also are extracted by FS (Fig.9).

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