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Screening male-sterile mutants in Berkeley for anther development mutants

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We are interested in identifying mutants that have specific defects in early anther development that occur in the window of developmental time between initiation of the anther and initiation of meiosis. Studying these mutants will allow us to identify processes involved in anther cell fate acquisition, including acquisition of meiotic cell fate. In order to identify appropriate mutants, we are screening all known male sterile mutants that we can find, including those at the Maize Genetic Stock Center, the majority of which contain *RescueMu*, and other resources. **Please send us your male sterile mutants!!** This screen is part of a new NSF sponsored plant genome research project, Cell Fate Acquisition in Maize which we call "The Anther Project" (PI: Virginia Walbot, CoPI: Zac Cande). Here we describe our screen of existing male sterile mutants for mutants with defects in early anther development.

The Screen. In the summer of 2007, we planted 117 families segregating for male sterile mutants from the coop and from the mutants Inna Golubovskaya identified as male sterile from the MTM project (identified summers of 1999 and 2000), representing 6 known male sterile mutants and 57 uncharacterized male sterile mutants. In order to determine the nature of the male sterile phenotype, we collected immature anthers at the stage of early meiosis for every plant, and fixed in 3:1 ethanol:acetic acid. This took about 17 person hours per 100 plants in the field. About two weeks later, when the plants started to shed pollen, plants were scored for male sterility and appropriate crosses were made. During the scoring, it was possible to identify many families segregating for very late shedders. In the initial screens performed by others, such lines were probably identified as male steriles if sterility was checked only at the normal shedding time, or alternatively, the mild Berkeley climate allowed these male steriles to escape. After the field season, the immature anthers from identified male sterile plants where examined microscopically. For a screen of this size, it is too time consuming to embed and section anthers from each male sterile plant. Instead, acetocarmine squashes were performed on several stages of anther development (based on anther length) and the organization and viability of cells in the anther wall, tapetum and meiocytes could be determined. This turned out to be a very productive way to screen the male sterile anthers and identify mutants with early anther development defects.

Results. From 57 uncharacterized mutants screened so far, 7 segregate for classic male sterile pollen development defects, 3 have defects in meiosis, and 5 have defects in early anther development (the class we want). Thus, of the 57 uncharacterized mutants examined, 5 warrant further analysis in our study; almost 9%! The rest appeared normal in the acetocarmine squashes, probably indicating post meiotic defects. After we complete complementation tests, we will know how many new genes these 5 mutants represent.

Conclusion. Screening male sterile mutants will be an extremely productive way to identify early anther defects. Examining immature anthers by acetocarmine squashes is a productive way to identify even mutants with abnormal anther wall morphology. During the winter, we will continue to examine the 5 early anther defects by careful microscopic examination of embedded and sectioned immature anthers.

Where to find information. All information from this screen, including images and the criteria used to determine which class mutants fall into, will be deposited at MaizeGDB.