Can you extract DNA from a single pollen grain?: A methodology useful for plant conservation research

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Background

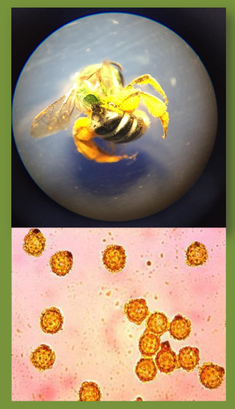
- There is less than 5% of the presettlement tallgrass prairie remaining (Samson and Knopf 1994).
- Most herbaceous tallgrass prairie species are self-incompatible and therefore need pollinators to successfully reproduce.
- Conservation biologists are interested in if pollinators are able to successfully carry pollen between fragmented populations.

Species & Field Method

- We used a self-incompatible, perennial commonly found in tallgrass prairies, *Echinacea angustifolia* (Leuszler *et al*. 1996).
- We collected pollinators as they visited *E. angustifolia* flowers during July 2014.
- Commonly used methods to determine the distance pollinators carry pollen are either time consuming or expensive or both. (For example using fluorescent dye or pollinator observation and genotyping of offspring.)
- **The goal of this study was to create and optimize a less time consuming and expensive method.**

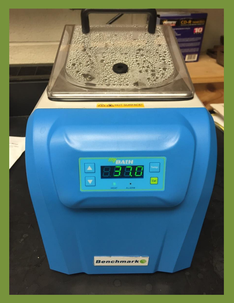
Specific Methodology

- The method was modified from Matsuki *et al*. (2007).



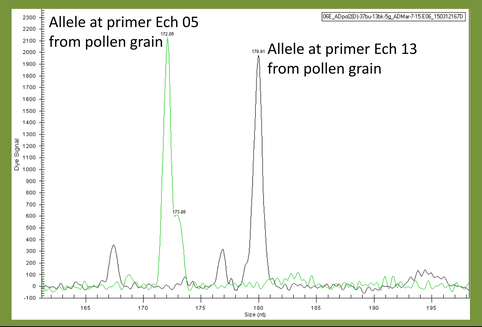
Step 1: Collect a single pollen grain and place in 5 µL of reaction buffer. The buffer consists of 1.5 mM $MgCl_2$, 10 mM Tris-Hcl (pH of 8.3), 50 mM KCl, 0.01% Proteinase-K, and 0.01% SDS.

Step 2: Place the tubes into a water bath at 37.0°C for one hour.



Step 3: Heat the tubes in a PCR machine at 95°C for 10 minutes. Then amplify the DNA using *Echinacea* specific microsatellite primers in a PCR.

Step 4: Genotype the pollen grain using fragment analysis on a capillary gel electrophoresis machine.



Research Question

Can DNA be extracted from directly from a single pollen grain carried on a pollinator?

Discussion

- Using this method, researchers can now quantify distance and number of pollen donors carried on a single pollinator.
- This method can be modified for use on other plant species.
- Current research includes optimizing multiplex method for multiple primers.
- Future research includes using DNA fingerprinting to determine the species diversity of pollen pollinators are carrying.

Citations:

Leuszler, HK, VJ Tepedino, & DG Alston (1996). Reproductive biology of purple coneflower in southwestern North Dakota. *Prairie Nat,* 28, 91–102.

Matsuki, Y, Y Isagi, & Y Suyama (2007). The determination of multiple microsatellite genotypes and DNA sequences from a single pollen grain. *Molecular Ecology Notes,* 7, 194–198. Pictures from the Echinacea Project

Samson, F & F Knopf (1994). Prairie conservation in North America. *BioScience,* 44, 418–442.

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