Risky sex? Pollen and pollination in the grasses (Poaceae)

PROJECT DESCRIPTION

INTELLECTUAL MERIT

**Introduction and Objectives** (including Hypotheses)

Introduction

Imagine the remarkable and seemingly random journey a pollen grain must travel to accomplish pollination in anemophilous plants. Grasses, the most diverse wind-pollinated family of seed plants, have evolved a wide array of spikelet morphologies that apparently uniformly rely on anemophily. We propose to explore the critical junction of flower form and function by constructing virtual models of grass spikelets (the flower with associated bracts) for computational fluid dynamic simulations to understand the aerodynamics of anemophily and investigate to what extent spikelet morphology has been shaped by it. Do variations in spikelet morphology (particularly compression, presence or absence of awns, and stigma position at anthesis) affect pollination? Do these variations correlate to ecological niche? Grass pollen is often assumed to be relatively uniform, but forest grasses produce more highly ornamented pollen than those of open habitats. Microchannels in the outer pollen wall are also assumed to be diagnostic for the grass family, but this trait and others have not been investigated in a phylogenetic context. This research will increase our understanding of the macroevolutionary history of grass spikelet morphology and pollen.

Grasses, with ca. 12,000 species, are the 5th most diverse plant family and the most diverse wind-pollinated one; Cyperaceae (with ca. 5,000 species depending on the source) is in the top 10.

Wind pollination is ecologically common, but only a relatively small % of seed plants, in terms of species, have evolved this mode of pollination. Gymnosperms are generally assumed to be wind-pollinated, but only the conifers and Gingko are exclusively wind-pollinated, whereas cycads and Gnetales are insect-pollinated (Peñalver et al. 2012). Approximately 1/5 of angiosperm families have evolved wind pollination; it is estimated that 10% of angiosperms (species?) are wind-pollinated (Friedman & Barrett 2009). Wind pollination is common in higher latitudes and elevations, especially in temperate areas, but is considered rare in the tropics, especially in lowland rainforests.

Inflorescence/flower characteristics of wind-pollinated angiosperms. List of predictions: feathery stigmas, high pollen:ovule ratio, 10-50um pollen diameter, pollen with slow terminal velocities 2 – 6 cm/s (Adams et al. 1981), smooth pollen surface, few and circular pollen apertures, long stamen filaments, absent or reduced nectaries fragrance perianth, **usually unisexual**, **pendulous catkin like inflorescence,** inflorescence held away from vegetation, low to moderate optimum wind speed habitat, low humidity habitat, infrequent precipitation habitat, open surrounding vegetation habitat, moderate to high plant density. (see bickel and freeman 1993 for plant geometry)

In addition to wind, pollen release, transport and deposition also depend on other abiotic factors such as humidity, rainfall and temperature.

Anemophily in grasses. Grass spikelet structure, bracteate, reduced flowers, feathery stigmas, versatile anthers. Grass pollen development (peripheral pollen). Grass pollen (uniporate, annulus, operculum, supposedly smooth walls, microchannels as a potential synapomorphy). Note similarities of Cyperaceae (bracteate spikelets, reduced flowers, independent evolution of peripheral pollen) but that these evolved independently. [Does peripheral pollen occur in any other wind-pollinated angiosperms, or in any angiosperms?]

However, insect visitation to grass inflorescences (and not just forest grasses) and those of a number of wind-pollinated plants have been documented. Ambophily vs. insect-assisted pollination.

Objectives

-to examine the evolution of grass spikelets in the context of adaptation to wind pollination

-to examine the evolution of traits associated with wind pollination in the family in a phylogenetic context

-to examine the surface and ultrastructure of grass pollen in a phylogenetic framework

Hypotheses

*Spikelet/spikelet trait-related*

H0 Spikelet has the same pollen capture potential in all orientations

H1There are positions where pollen capture potential is greater than other positions

H0 Spikelet has the same pollen capture potential in all wind speeds

H1 A range of wind speeds increases the chance the pollen capture

H0 There is no correlation between habitat and pollen capture potential

H1 Spikelets differ in their aerodynamics (to a degree that influences pollination) that is specific to habitat, while also considering phylogenetic relatedness.

[Do the above capture these questions: stigma exsertion is always the same (perpendicular to the larger faces of the spikelet); spikelet shape has no relation to pollen release/capture (i.e., dispersal requirements are entirely driving spikelet evolution); awns have no effect on pollen release/capture]

*Pollen-related*

H0 Intra-exinous channels are not a synapomorphy of the Poaceae

H1 Intra-exinous channels are a synapomorphy of the Poaceae

H0 Intra-exinous channel presence and/or structure is not taxonomically significant at the subfamily level in the Poaceae

H1 The presence and/or structure of intra-exinous channels is taxonomically significant at the subfamily level in the Poaceae

H2 Intra-exinous channels are a synapomorphy of the BOP + PACMAD clade

H0 The presence of intra-exinous channels does not correlate with habitat (forest-associated vs. grassland)

H1 The occurrence of intra-exinous channels differs between forest-associated taxa and grassland taxa

[might need to modify depending on what the literature search shows]

**Significance**

[If we include this section, we have to be careful not to be redundant with the Broader Impacts. But this would give us a chance to highlight the novelty of the virtual wind tunnel.]

**Proposed Work**

Pollination and Spikelet Evolution

Sampling

Spikelet modeling

Virtual wind tunnel experiments [anything we might do with peripheral pollen should go here]

Pollen

Sampling (go for 36)

LM

SEM

TEM

**Preliminary Results**

Pollination and Spikelet Evolution

Method for spikelet modeling has been developed (cite APPS paper)

Preliminary tests of virtual wind tunnel (with Festuca, maybe also others)

Pollen

Discuss what is known regarding the distribution of the intra-exinous channels

Include anything relevant about experience with processing pollen

**Timetable**

Year 1: Pollen sampling and microscopy; collection of spikelets and anthers. Year 2: Sectioning of spikelets and preparation of models; virtual wind tunnel experiments. Year 3:

BROADER IMPACTS

Educational: Grass spikelet models that are digitally dissectible.

Human Resources: Training of two Ph.D. students, one of whom is female and a first generation college and graduate school student; training of at least two undergraduates during the project.

Methods: Development of 3D methods and analyses

Other fields: potential impact on paleoecology/paleoclimatology

[work in BPMI?; Carver students?; SACNAS?]

**Results from Prior NSF Support**

Clark. DEB-1120750, $291,057, 2011-2016, COLLABORATIVE RESEARCH: **Ecological Diversification and Molecular Evolution of Grasses (Poaceae)***, with M. Duvall (DEB-1120761) and S. Kelchner (DEB-1120856)*.

DEB-1342787 to PI M. Ungerer (originally to J. Craine) at Kansas State University, $1,419,186 (**ISU/Clark subcontract** $207,121), 2013-2019, Dimensions/NASA: COLLABORATIVE RESEARCH: **The Biogeography and Evolution of Drought Tolerance in Grasses**, *with M. Duvall (DEB-1342782) and C. Still (DEB-1342703)*.

The questions, activities, and results of these two projects were complementary and overlapping. To simplify reporting, we integrated the results in the text below, with DEB-1120750 products labeled with an asterisk (\*) and DEB-1342787 labeled by a cross (†).To date, we have published 11 papers (9\* and 2\*†) and 2\*† are currently in review or revision. We have produced three permanent slide collections : 1) ca. 4,500 slides, representing sections in all three planes, of ca. 30 non-grass monocots and ca. 300 grass species; 2) leaf clearings of ca. 220 grass species, and 3) root cross sections of ca. 80 grass species. These are currently housed in the Clark lab at ISU (access available upon request), but we will select representative slides and images to be archived at the Cornell University Plant Anatomy Collection/Website.

*Intellectual Merit*

Major activities undertaken by the Clark lab were: 1) collection of critical taxa; 2) facilitation of plastome sequencing; 3) a family-wide leaf shape evolution analysis; 4) a survey of 3D leaf anatomy in the grasses; 5) a survey of grass root anatomy; 6) collection of root and leaf anatomical data as part of experimental drought tolerance and gene expression studies; and 7) analyses of leaf and root anatomy to test the evolutionary and ecological significance of climate and light. These activities produced several key findings that improved our understanding of grass evolution, as well as the genetic and functional bases of water and light relations.

Our plastome phylogenetic estimations confirmed the previous topology of three early-diverging grass lineages sister to two major clades (BOP + PACMAD), suggested Panicoideae as sister to ACMAD (instead of Aristidoideae), provided improved resolution of relationships within subfamilies, and revealed a new genus of bamboo (\*Attigala et al. 2014; \*Burke et al. 2014; \*Cotton et al. 2015; \*Saarela et al. 2015; \*Wysocki et al. 2015; \*†Attigala et al. 2016a,c; \*Burke et al. 2016b; \*Duvall et al. 2016). Our results date the origin of the grass family into the Early Cretaceous (\*†Burke et al. 2016a; \*†Gallaher et al., in revision), 20-30 My earlier than previous estimates.

Our studies of grass leaf shape evolution showed that strongly linear leaves occur in climates receiving <1,000 mm MAP, whereas in habitats with greater precipitation, all three shape categories (linear, intermediate, ovate) may be found. Ancestral grasses most likely had moderately broad leaves, and grew in mesic, partially shaded habitats. Strong leaf linearity evolved multiple times and preceded the evolution of C4 photosynthesis (\*†Gallaher et al., in revision). Anatomically, grass adaxial chlorenchyma cells are usually lobed in at least one plane, often the proximo-distal or lateral planes; the presence of lobes is plesiomorphic and general, with some reversions to the non-lobed condition. Five major morphotypes were identified; a species may have more than one, but we modeled the dominant adaxial cell shape adjacent to the sheaths of 1st order bundles. Using all data, adaxial chlorenchyma cell shape is significantly correlated with both photosynthetic pathway (PS) and light regime. Several descriptors (e.g., cell length:width, number of lobes in cross section) have significant phylogenetic signal, and some also show other significant associations (e.g., L:W with light regime and PS) (\*†Gallaher et al., in prep.).

We documented significant variation in transverse mature stelar root anatomy in grasses (\*†Cox et al., in prep.). Novel findings included internal (pith) phloem in two species and an apparent multiseriate endodermis (up to 7 layers) in ca. half of the species we sampled. Surprisingly, root anatomical variation showed no phylogenetic or photosynthetic pathway signal, and only a few, weak correlations with climatic variables.

*Broader Impacts*

Two postdoctoral fellows, 4 graduate students (3 Ph.D., 1 M.S.), 7 undergraduate students and 1 high school student (8 of whom were female) received training in aspects of Next Generation sequencing/assembly, plastome phylogenetics/bioinformatics, preparation and imaging of paraffin sections and leaf clearings, quantitative anatomy, 3D modeling/printing, and statistical analysis in R. We reported results in 8 (6\*, 2\*†) invited seminars, 10 (5\*, 5\*†) contributed talks and 3\* posters at national and international meetings/institutions. We are preparing animations and protocols for 3D printing are to be used in educational applications. Other products included an interactive software package for developing botanical keys (\*Attigala et al. 2016b).

**Details/Budget**

3-year project, with a start date as soon as possible in 2019 (but might have to be mid-semester in the spring or not until the start of summer semester)

12 months of RA support per year, plus benefits and tuition

Hourly for undergrads, plus benefits

Supplies for anatomical work for both components of the project

LM imaging, measurements, etc.

SEM training and beam time

TEM samples

Travel to meetings

Greenhouse supplies/costs

Publication costs

[likely no request for computer or software, but what about server time?]

ME:

Sampling paragraph –

Narrative about sampling scheme (phylogenetic basis)

Number of tribes, etc. from Soreng, filling in gaps, resampling early diverging since C&R 67 since tech has improved

Methodology –

Microscopy methods for all 3

Character lists – pollen diameter/size/annulus/ornamentation/microchannels