Evaluating variation in meiosis and the impact of light intensity on growth and development in *Pellaea truncata* Goodd. (Pteridaceae)

By: Jaina Preisen

Advisor: Dr. Amanda Grusz

University of Minnesota-Duluth Honors Program

Abstract

Ferns are an ancient lineage of vascular plants and the closest relatives of seed plants, which include conifers and flowering plants. Additionally, many ferns provide ecosystem services, for example, by filtering heavy metals and toxins from the surrounding environment (Dhir, B., 2018). In this study, I examine the growth and development of a desert-adapted fern from the southwestern United States and Mexico, *Pellaea truncata.* Specifically, I studied variation in reproductive propagules (spores) to better understand how genome size differs among the offspring that are produced by a single leaf. Spores were removed from individual sporangia growing on pinnules spanning a single mature leaf. Spores from this specimen were also sown on nutrient-enriched agar and placed under multiple light-intensity treatments to determine if light intensity impacts the germination rate.

Introduction

Pellaea truncata Goodd. (Pteridaceae) is a fern species commonly found in the southwestern United States and northern Mexico. *Pellaea truncata* is a sexual diploid ($2n = 2x$) with an average spore size of 38.3 µm (Windham et al., 2022). Spores are the cellular products of meiosis and their size can be used as a proxy for nuclear genome size if calibrated with a chromosome count.

Understanding variation genome traits and light requirements for germination in fern species is important for characterizing growth and development (Krieg & Chambers, 2022). Previous studies have examined optimal light conditions, optimal Photosynthetic Photon Flux Density (PPFD) level, and the response of light on biosynthesis pathways. Hevly (1963) focused on the optimal amount of photoperiod of exposure to allow for germination, with 16-plus hours of continuous light exposure allowing for germination. Optimal light intensity for normal

prothallus growth was also observed with 750 ft-c being an optimal intensity. While the study was focused on the closely related genus *Notholaena* (Pteridaceae), this work is applicable to *Pellaea*. Other recent studies have measured optimal PPFD levels and their effect on the estimated area and perimeter of the growing gametophyte (Cai et al., 2023). These authors also examined the production of flavenoid and other biosynthesis pathways in relation to the levels of PPFD. By examining light levels and how they affect the plant at the molecular level, we can better understand associated pressures in wild gametophytes.

This study aims to answer two questions: (1) Does genome size vary among spores on one leaf of *P. truncata*? and (2) Does light intensity impact rates of germination and growth in gametophytes of *P. truncata*?

Methods

To assess genome size across a single leaf, spore length was measured across multiple sporangia on a single frond of *P. truncata* (*Grusz 522A*) using a dissecting microscope. Individual sporangia were carefully removed and placed on a clean microscope slide. An insect pin wiped with 70% ethanol was used to rupture the wall of the sporangium in a drop of glycerol. Spores were aggregated together on the slide and examined using a Nikon compound microscope equipped with a digital camera. Images were taken and spores were measured individually using Nikon NIS Elements software.

To assess whether light intensity impacts rates of germination and growth, spore rain from multiple specimens was collected and pooled (*Grusz 522A*, *Grusz 507A & B*, *Parker Upper Creek 2022*). Sampled spores were sterilized in 1% Clorox solution after I conducted an optimization experiment for 0.1%, 1%, and 2% Clorox solutions. Spores were vacuum dried,

placed in 25 mL distilled water, and gently mixed before plating 1 mL onto 100 mm sterile petri dishes filled with Hevly's medium (Hevly, 1963) and then wrapped with parafilm. Plates were organized haphazardly within a light chamber on styrofoam boxes at 4 heights: Box 1: 16.03 cm, Box 2: 32.26 cm, Box 3: 68.33 cm, Box 4: 88.65 cm. Plates were monitored biweekly for germination. Each trial had four replicates per light-intensity treatment.

Results

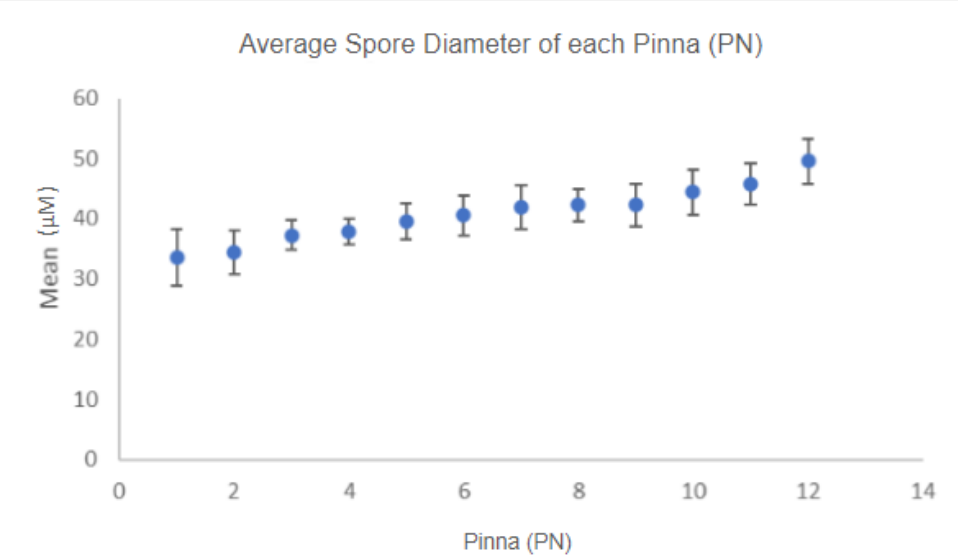

Figure 1. Average spore diameter (µm) across 12 pinnae on the *P. truncata* specimen *Grusz 522A*.

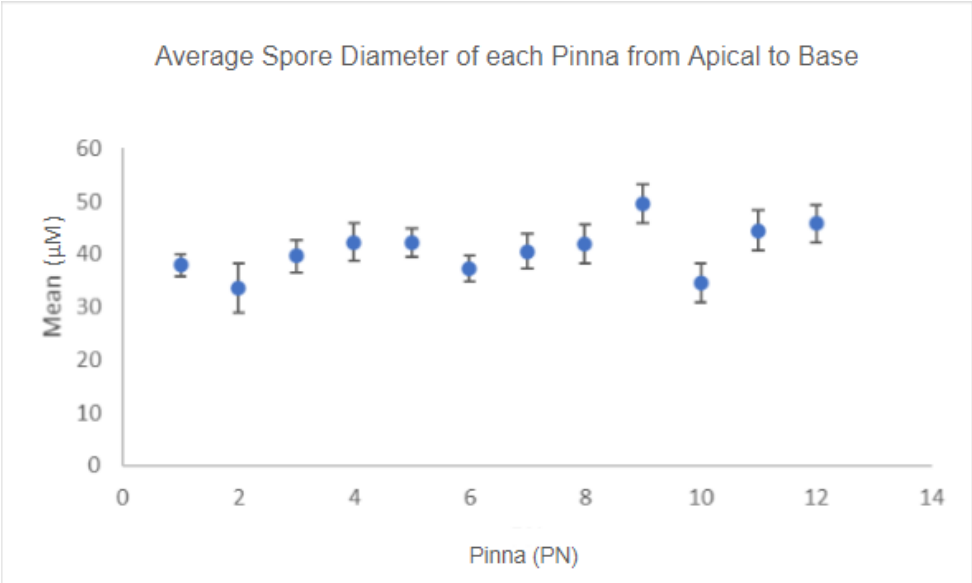

Figure 2. Average spore diameter (µm) for sporangia collected across 12 pinnae on a single leaf from specimen *Grusz 522A* arranged from apex (left) to base (right).

Figure 3. Specimen *Grusz 522A* with indicated labels (2-11, 13-14) at each pinnae where

sporangia were measured.

Figure 1 shows an increase in spore diameter from 34.4–49.54 µm. Figure 2 expands upon Figure 1, arranging based on the position on the frond, showing variation in spore size, a proxy for genome size, across the specimen. Error bars represent the standard deviation of spore diameter collected from individual sporangia. Pinnules 3, 4, and 8 have the least variation in spore size from the individual sporangia taken. Figure 3 visualizes specimen *Grusz 522A* which was used to obtain results of Figures 1 and 2.

Table 1. Shows the height intervals of each set of plates in the growth chamber.

	Height (cm)	**Large Plates**	**Small Plates**
Box 1	16.03	5, 3, 4, 6	9, 4, 5
Box 2	32.26	7, 2, 1, 8	8, 11, 7
Box 3	68.33	16, 15, 14, 13	1, 12, 10
Box 4	88.65	9, 12, 10, 11	6, 2, 3

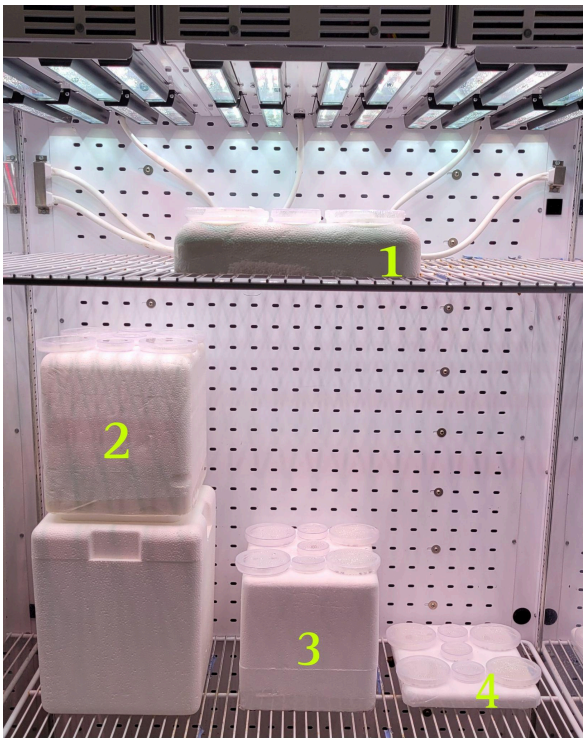

Figure 4. Experimental setup of the growth chamber with plates situated at four heights: Box 1: 16.03 cm, Box 2: 32.36 cm, Box 3: 68.33 cm, Box 4: 88.65 cm (see Table 1).

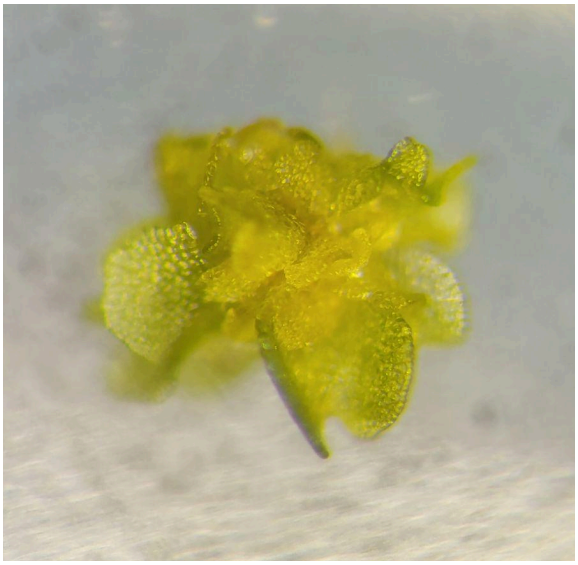

Figure 5. Initial growth of *Pellaea truncata* gametophyte grown from spores in my second experiment; light intensity treatment at height interval 68 cm from light source at 6 weeks.

Figure 5 shows the initial growth of gametophyte from box 3 (68 cm). Table 1 shows height variations of plates from boxes 1-4. Box three was set as the control height from the fixed light source with the specimen growing farthest back on the styrofoam box. Figure 4 expands on Table 1, showing the experimental setup described.

Discussion

Spore diameter varied across the leaf (Figs. 1 & 2). There is no discernible pattern between the age of the pinna and the genome size of the spores it produces. Average spore diameter across the *Grusz 522A* frond is consistent with a sexual diploid.

The second experiment focusing on light intensity was run three times with no results from trials 1 and 2. Trial 1 failed because the concentration of bleach was too high during the spore sterilization step. To optimize bleach sterilization, I ran a trial with spores plated on 0.1%, 1%, and 2% Clorox solutions, including 3 replicates per treatment, to determine the optimum for spore germination without contamination by fungi and bacteria. All three concentrations successfully yielded growth, but I chose the 1% treatment because it had the best germination. Trial 2 was with 1% bleach to sterilize spores which were then plated on Hevly's medium. However, trial 2 experienced extensive contamination with no spore germination. Contamination of the plates could have occurred because of old media, insufficient sterilization of the spores, or overexposure to air when checking for germination. Cracks in the parafilm could have been a potential cause of contamination as well. To mitigate contamination in Trial 3, I worked to improve the sterility of the environment and I used gloves when sowing spores. The spores were also plated on freshly made media with limited air exposure using new parafilm. The third trial of the experiment resulted in the growth of a single gametophyte on plate 10 at box 3. Figure 3, shows the germinated gametophyte at two weeks from initial germination. The third trial is ongoing with no other germination observed for any light treatments, but contamination has occurred on some of the plates.

Conclusions

By examining the spore diameter across a singular frond, I conducted the first in-depth study of variation in genome size among spores produced by a single leaf. Based on my preliminary results, *Grusz 522A* is a sexual diploid leaf. In terms of the experiment on light intensity, there are no conclusive results on the variation of growth at different levels of light intensity tested because only one plate exhibited growth at the time of this summary; additional

time is needed to determine if light intensity impacts germination or growth rates.

Trial 3 of my light-intensity experiment will continue to be monitored for other growth and development in the growth chamber. The single gametophyte observed (Fig. 3) will be allowed to grow and will be monitored, with the goal of conducting physiological analyses after more growth has occurred. Further analyses and future trials of this experiment should focus on improving germination rates.

Ferns and Society

As ferns are a diverse group of vascular plants, and research into the growth of these plants under environmental stress is important for understanding the long-term impacts of local and global climate. Because ferns are sessile and unable to move in the face of environmental stress, their ability to adapt and thrive is of profound importance. Understanding the mechanisms of ferns in their local environment can further shed light on their adaptation strategies in a changing climate.

References

Cai, Z., Wang, X., Xie, Z., Wen, Z., Yu, X., Xu, S., Su, X., & Luo, J. (2023). Light response of gametophyte in *Adiantum flabellulatum*: transcriptome analysis and identification of key genes and pathways. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1222414

Dhir, B. (2018). Role of Ferns in Environmental Cleanup. *Current Advances in Fern Research*, 517–531. https://doi.org/10.1007/978-3-319-75103-0_25

Hevly, R. H. (1963). Adaptations of cheilanthoid ferns to desert environments. *Journal of the Arizona Academy of Science*, *2*(4), 164. https://doi.org/10.2307/40026172

Krieg, C. P., & Chambers, S. M. (2022). The ecology and physiology of fern gametophytes: A methodological synthesis. *Applications in Plant Sciences*, *10*(2). https://doi.org/10.1002/aps3.11464

Windham, M. D., Huiet, L., Metzgar, J. S., Ranker, T. A., Yatskievych, G., Haufler, C. H., & Pryer, K. M. (2022). Once more unto the breach, dear friends: Resolving the origins and relationships of the *Pellaea wrightiana* hybrid complex. *American Journal of Botany*, *109*(5), 821–850. https://doi.org/10.1002/ajb2.1850