A Tale of Two Ferns
Examining the Relationship Between Polyploidy, Reproductive Mode, and Distribution in *Pteris vittata* and *Pteris quadriaurita*

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Introduction

Figure 1. Specimens of globally distributed fern species *Pteris vittata* (a) and *Pteris quadriaurita* (b).

Asexuality and polyploidy are important drivers of plant speciation that are especially rampant in ferns. The predominant form of asexual reproduction in this group is apomixis, a process resulting in the production of unreduced spores and gametophytes (the typically haploid phase) that, in turn, give rise to sporophytes (the typically diploid phase) without fertilization. While only 1% of angiosperms are thought to reproduce via apomixis, upwards of 10% of ferns may exhibit this form of asexuality. Polyploidy, or whole genome duplication, is also overrepresented in ferns, with perhaps 31% of fern species originating through polyploid speciation events. Such duplications can be viewed as a fast-track to speciation or ecological adaptation, with polyploidization allowing for the rapid evolution of novel phenotypes. However, despite evidence that both apomixis and polyploidy play outsized roles in shaping fern diversity, reproductive mode and ploidy level variation are under-researched in ferns, particularly in the context of globally distributed species.

The fern genus *Pteris* is emerging as a model system for studying the incidence and importance of both apomixis and polyploidy. One of the most diverse fern genera, *Pteris* comprises over 300 species that vary widely in morphology, ecology, and distribution. Cytological studies have demonstrated that apomixis and polyploidy are widespread, making *Pteris* an ideal lineage for studying both the frequency and distribution of these two evolutionary strategies within a biogeographical context.

Here, we focused on two widespread fern species, *P. vittata* and *P. quadriaurita* (Fig. 1a and 1b) that are known to have multiple ploidy levels and, in the case of *P. quadriaurita* multiple reproductive modes, across their ranges. Spore analysis was used to establish reproductive mode and estimate ploidy level variation for a wide geographic sampling of each species, providing important insight into the potential roles polyploidy and apomixis play in fern diversification.

Methods

Within a species, variation in mean spore diameter is suggestive of differences in ploidy level, with larger spores correlating to increased genomic content. In most leptosporangiate ferns (i.e., ferns whose sporangia develop from a single cell), sexually reproducing specimens produce 64 spores per sporangium, whereas apomicts produce only 32 unreduced spores per sporangium, allowing us to establish reproductive mode easily for *P. quadriaurita*. For this study, we examined 225 specimens of *P. vittata* and 154 specimens of *P. quadriaurita*. For each specimen, mature sporangia were located (Fig. 2a) and removed to a drop of glycerol on a prepared microscope slide. Sporangia were then dissected (Fig. 2b) using mounted insect pins. Spores were separated from the sporangial wall fragments and then imaged on Leica DM4 B compound microscope. Per-sporangium spore number (Fig. 2c) and mean spore diameter (Fig. 2d) were calculated using LAS X software.

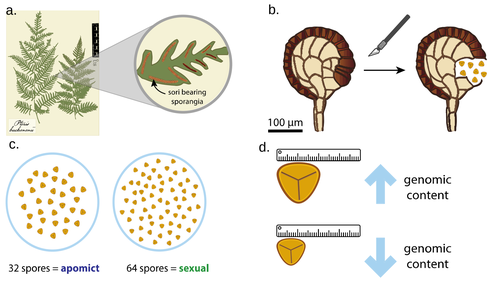

Figure 2. Spore work flow diagram: (a) locate fertile tissue on herbarium specimen; (b) open the closed/in-tact sporangium to glycerol drop on prepared microscope slide; (c) count the spores per sporangium; (d) calculate mean spore diameter for spores

Results

Pteris vittata

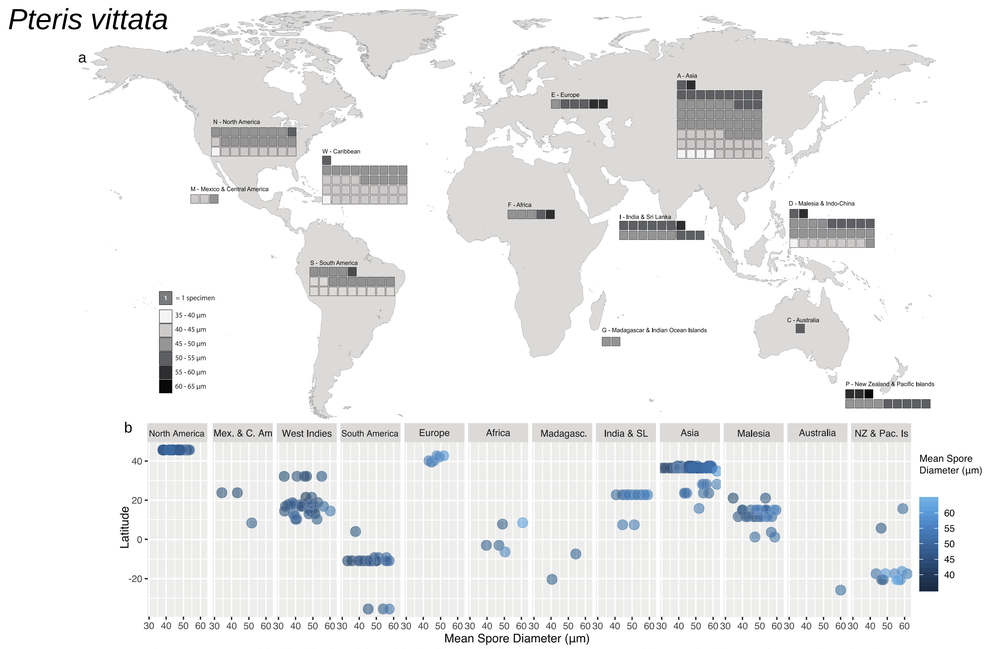

Figure 3. Geographic distribution (a) and latitudinal variation (b) of mean spore diameter in 225 specimens of *Pteris vittata*.

Pteris quadriaurita

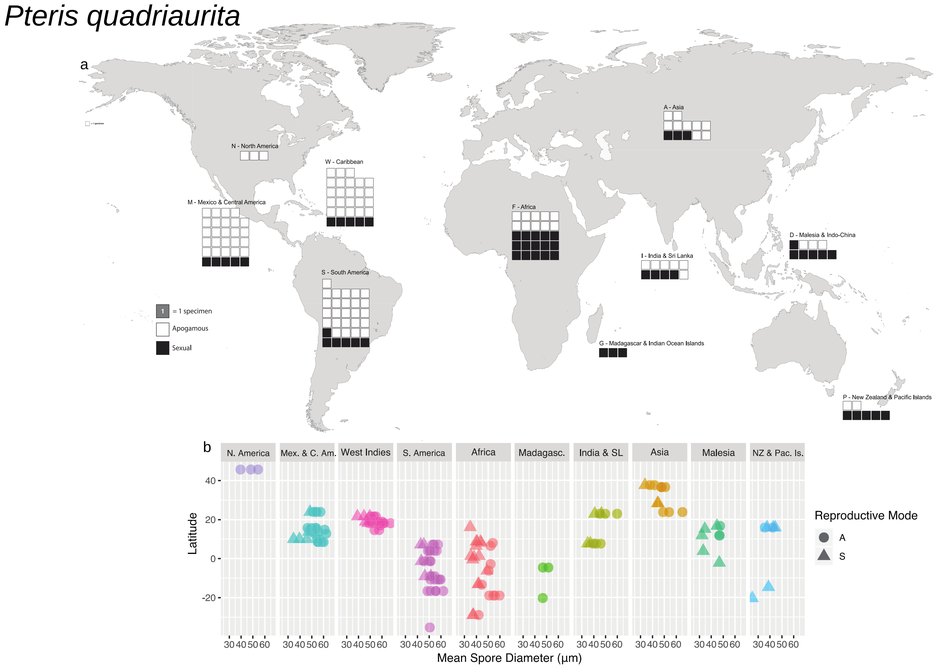

Figure 4. Geographic distribution of reproductive mode (a) and latitudinal variation in mean spore diameter and reproductive mode for 154 specimens of *Pteris quadriaurita*.

Results

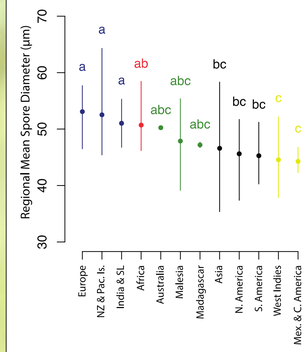

Figure 5. Mean spore diameter by ecoregion for *P. vittata.* Regions that do not share letters have significantly different spore sizes ($p < 0.05$).

- In *Pteris vittata*, the most common range of mean spore diameters was 45-50µm (44% of specimens), with most specimens (75%) falling between 40 and 50µm (Fig. 3a).
- Mean spore diameter varied both within and among regions in *Pteris vittata* (Fig. 3a).
- For well-sampled regions, Europe had the greatest mean spore diameter (53.1µm) and the West Indies had the lowest mean spore diameter (44.5µm) (Fig. 3b).
- In *Pteris vittata*, mean spore diameter is significantly larger in Europe, New Zealand & Pacific Islands, and India & Sri Lanka than in the New World (Fig. 5).
- In *Pteris quadriaurita* 108 specimens (69%) were apogamous while 48 specimens (31%) were sexual (Fig. 4a).
- For well-sampled regions the highest rates of apomixis were in Mexico and Central America (83%), the West Indies (82%), and South America (81%) (Fig. 4a).
- The incidence of apomixis increased toward the equator (Fig. 4b).

Discussion

- Our findings suggest that both reproductive mode and ploidy level vary by region, but it remains unclear which environmental factors shape these processes in *Pteris*. Future studies incorporating regional climatic data will provide additional insight.
- Because we lack chromosome counts for the *Pteris vittata* specimens examined, we cannot definitively assign particular ploidy levels to given mean spore diameters. Nevertheless, the well-documented ploidy-level variation in this species leads us to conclude that the observed differences in mean spore diameter among ecoregions reflect underlying ploidy-level differences.
- Although the incidence of apomixis is thought to increase with distance from the equator, we found the opposite to be true for *Pteris quadriaurita*.

Acknowledgements & References

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