Cell size in *Ginkgo* and the paleo-CO_2 proxy

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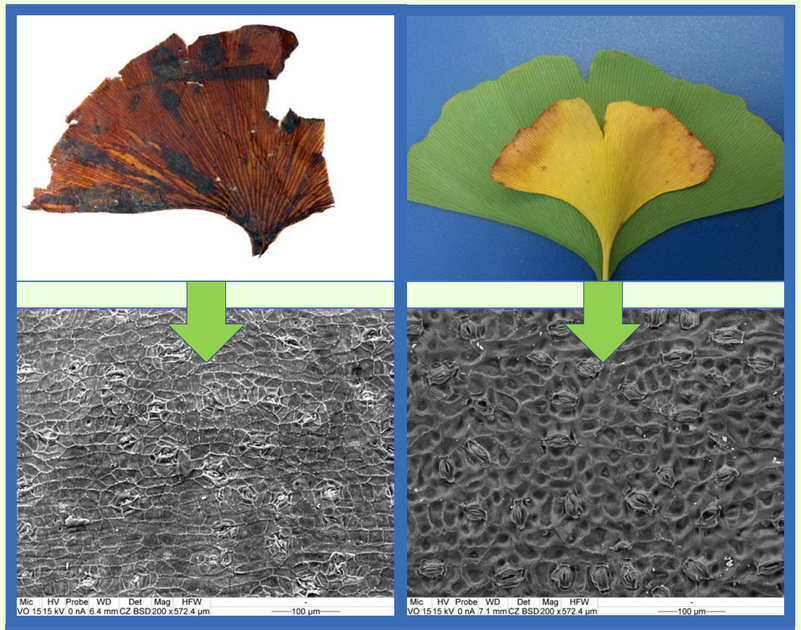
Introduction

Knowing how much CO_2 there is in the atmosphere is central to understanding climate, but it can be difficult to assess for climates prior to ice core records. One common method for estimating atmospheric paleo-CO_2 concentration is the stomatal index (SI), the proportion of stomata (pores in the leaf used for gas exchange) out of all epidermal and stomatal cells[1]. The SI is sensitive to changes in CO_2 level because it is most advantageous for the plant to have as few stomata as possible, to reduce water loss, while still taking in enough carbon for growth and reproduction[2]. The organism of choice for this proxy has been *Ginkgo*, the only extant genus of ginkgophyte gymnosperm. *Ginkgo* has been used in large part because Cenozoic fossil leaves, seeds, and fertile shoots assigned to *G. wyomingensis* are very similar to modern *G. biloba*. This study's purpose was to compare leaf epidermal cell size in *G. wyomingensis* and *G. biloba*.

Figure below: Leaf and SEM image of epidermis (200X) of fossil *G. wyomingensis* (left) and modern *G. biloba* (right).

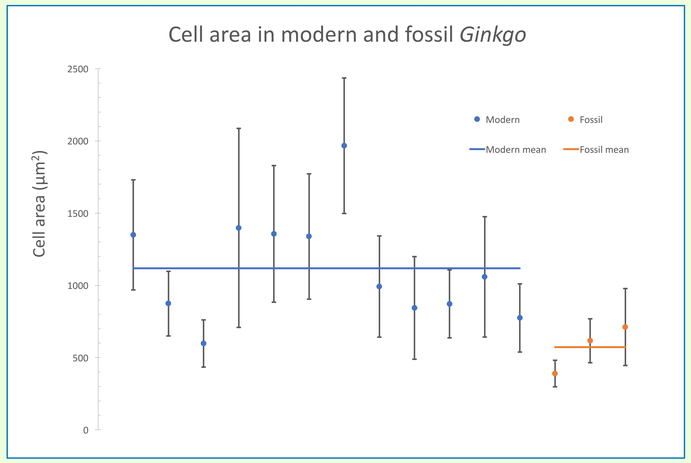


Materials & Methods

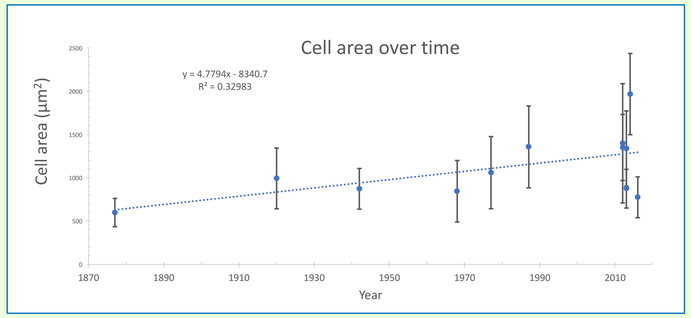
We examined three fossil cuticle specimens of *G. wyomingensis* from the early Eocene (55.8 Ma) Willwood Fm. of the Bighorn Basin, WY, USA. We compared these with 12 leaves of *G. biloba* from herbarium collections made 1877–1987 and living trees in experimental farms. All leaves were macerated with Cr(VI)O_3 to separate the upper and lower cuticles, and the internal side of the lower cuticles were imaged with a Zeiss environmental SEM. The NIH software ImageJ was used to measure the area of cells. We randomized the choice of cells with the Grid Overlay plugin by Wayne Rasband. Preparation methods and stomatal pore length data are taken from Barclay and Wing (2016).

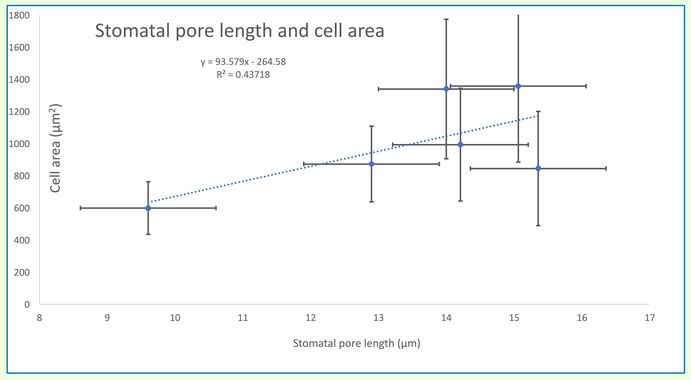
Results

The *G. biloba* epidermal cells are about twice as large as those of *G. wyomingensis*, with the former having a mean cell area of 1119 µm^2 and the latter a mean cell area of 573 µm^2. The difference is significant with $p < .00001$ (Student's t-test, $t = 5.75948$, df = 176).



G. biloba cell area may have increased over historical time as well, although the relationship is highly influenced by the small size of the oldest specimen. The length of *G. biloba* stomatal pores is correlated with non-stomatal epidermal cell area, but this requires more data to confirm.





Discussion

Cell size in *Ginkgo* has not been constant since the early Cenozoic. Although these are preliminary results, we do not think taphonomic effects could have caused this magnitude of difference. The size and density of stomata are negatively correlated[3], which affects stomatal density as a proxy for atmospheric CO_2, and may affect SI models as well. Theory suggests that stomatal pore length should be correlated with cell area, but the linear fit does not explain the majority of the variation, suggesting that more samples should be added. If *G. wyomingensis* had smaller epidermal cells and stomatal pores, as well as fewer pores[4], its evapotranspirative and photosynthetic rates would be lower. This in turns means that the relationship between CO_2 and SI observed in *G. biloba* may not be directly applicable to fossil *G. wyomingensis*.

Conclusions

The results show that modern *G. biloba* and fossil *G. wyomingensis* are distinct in their epidermal cell size. This not only belies the notion that *Ginkgo* can be treated as a perfect "living fossil", but, more importantly, impinges on the models used to reconstruct paleo-CO_2 from stomatal indices. More work on epidermal cell size and morphology is required to establish if ancient CO_2 levels can be inferred by using SI data from *G. biloba* and applying it to *G. wyomingensis*.

Figure above: experimental open-top CO_2 chambers with *G. biloba*.

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References

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