

## Leaf traits: Stomatal size and altitude

### What do you need:

For the computer component, you will need ImageJ. You can download it from the link below.

ImageJ: <https://imagej.nih.gov/ij///index.html>

You will also need three files. This instruction file; Using ImageJ to measure leaf area (but the same principles apply to measuring stomata) and *Ginkgo biloba* epidermal cell images

If you are also doing the lab component, you will need:

- Light microscope
- 1 – 5 *Ginkgo biloba* leaves
- Microscope slides
- Clear nail polish
- Clear sellotape

### Introduction

Stomata are the ‘breathing pores’ of plants. The stoma (plural stomata) is a microscopic pore found on the epidermis of leaves. It is surrounded by a pair of specialised epidermal cells called guard cells. Stomata are the site of gas exchange in leaves – that is stomata are where oxygen and carbon dioxide (and other gases) enter and leave leaf cells. Stomata are also where water vapour leaves the leaf in a process called transpiration. This means that stomata have a vital role in two of the most important physiological processes in plants: photosynthesis and transpiration.

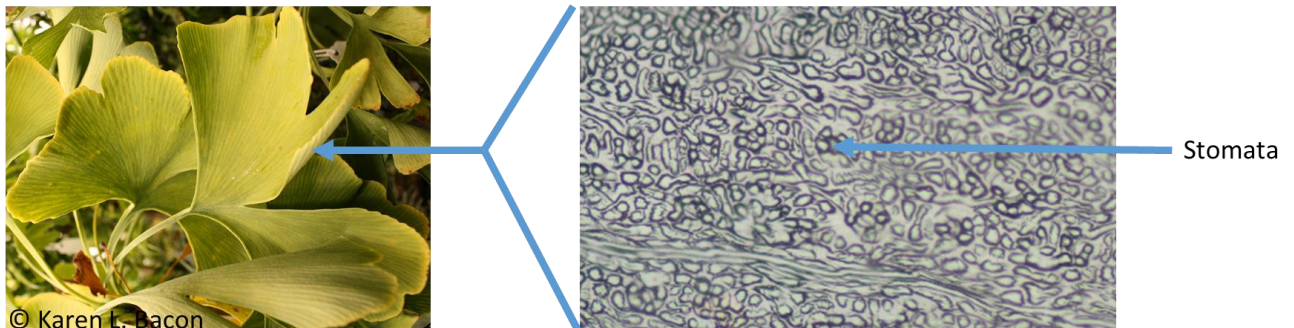


Figure 1: Example of *Ginkgo biloba* leaf and epidermal surface with stomata highlighted.

Stomata of some plants are known to have a relationship between the number of stomata and the level of atmospheric CO<sub>2</sub> in the atmosphere at the time the leaves grew. This is one way that scientists know that CO<sub>2</sub> levels have changed over geological time. One plant that has this relationship is *Ginkgo biloba*. This is a very interesting species with a very long fossil record, leading some people to refer to it as a “living fossil”.

As well as stomatal numbers changing over time, they can differ by location. In the images below, you can count stomata from 2014 from several sites in the United Kingdom. *Ginkgo biloba* is often common in Northern Hemisphere cities, so you may also be able to find a local one and make your own stomatal slides following the steps below to compare to the photographs provided.

Some images of the whole leaves are also provided and you can determine if the leaf size or shape changes based on location – what might be the environmental difference between Edinburgh and Exeter that could impact leaf growth?

You can find more information on *Ginkgo biloba* in the references below.

**Counting stomata.**

Using the photographs provided, you can count the number of stomata per leaf. Use the programme ImageJ to estimate the size of stomata (see instructions in separate file).

To count stomata, you ideally would create a running average per field of view until you get a smooth average between counts. This is not possible in the photographs but is if you make your own slides.

A running average works like this:

Table: Example of calculating a running average.

Field of view	Number of stomata per square unit	Average of this and previous FoV	
1	10	FoV 1 only = 10	
2	8	(FoV 1 + FoV 2)/2 = 9	You can see that roughly the same number (around 9) is being produced and therefore counting around 4 or 5 FoV will be sufficient to get a good average. Make sure that you do not change the focus between counts.
3	8	(FoV 1 + FoV 2 + Fov3)/3 = 8.6	
4	9	(FoV 1 + FoV 2 + Fov3+Fov4)/4 = 8.75	

You can then compare the number and size of stomata across the different sites. Remember to note the scale on the image as some are at a higher magnification than others.

You should calculate stomatal density and stomatal index:

Equation 1:  $SI = \frac{\#stomates}{(\#stomates + \#epidermal\ cells)} * 100$

Use the equation below to calculate the approximate CO<sub>2</sub> from the stomatal index of the leaves:

Equation 2:  $CO_2 = 205.7 + 13,630,000 \times SI^{(-5.224)}$ .

<https://scholarsbank.uoregon.edu/xmlui/handle/1794/20716> Conde 2016

SI is the proportion of stomatal cells over the total number of epidermal + stomatal cells (effectively the percentage of cells per unit area that are stomata rather than epidermal cells).

**Questions:**

What is the average stomatal density for *Ginkgo biloba* at each site?

What is the average size of stomatal complex for *Ginkgo biloba* at each site?

Look up the atmospheric CO<sub>2</sub> for 2014 – do you get a similar answer when you use Equation 2 on your counts? If not, why do you think that is the case?

If you prepared slides from modern samples, do you observe a change in stomatal numbers from today compared to 2014? Do you think this relates to changes in atmospheric composition or because your leaves are from a different location? Discuss.

### **Making your own stomata slides: Method**

1 Carefully coat some nail varnish over the under surface of the leaf and leave this to dry. This will create an impression of the leaf surface and the stomata.

2 Once the nail varnish is dry, carefully peel it from the leaf. You may need tweezers to begin this process. Be careful not to allow the nail varnish to curl.

3 Place the solid nail varnish on a slide.

4 Place a small drop of water on the impression. (This step is optional but often helps)

5 Gently cover the impression with a cover slide. To do this, place the cover slide at a 45° angle next to the impression and lower it slowly until the impression is covered. If you do this correctly, you should have no air bubbles and you may need to wipe excess water from the slide with a piece of tissue – be careful not to squash the impression.

You are now ready to count the stomata.

### **Using a light microscope:**

ALWAYS BE CAREFUL USING A MICROSCOPE – LENSES ARE EXPENSIVE AND EASILY DAMAGED

1 Pull the stage down and place the slide on the centre.

2 Move the x10 lens into position over the stage.

3 Gently bring the stage up as close as possible to the lens – but BE CAREFUL NOT TO CRUSH THE SLIDE!

4 Now make sure of the following before beginning the counts.

- The microscope should be plugged in and turned on
- The diaphragm should be closed
- The light source should be on full
- The lens should be directly over the slide

5 You can then move up to the x40 lens – be careful not to move the stage. You should only have to use the fine focus wheel to focus at the higher magnification if you have done the first four steps correctly.

You should now make sure that you can see the epidermal cells clearly – if you cannot, you need to gently move the course focus wheel so that the stage moves slowly away from you.

Once you can see some cell outlines, you should use the fine focus wheel to make the image clear.

You can then count the numbers of epidermal cells and stomatal cells within one field of view.

Once you have done this and noted the numbers, move the stage so that you get another field of view. You should do this for at least 5 fields of view for the impression. (see above for more detail)

Try to look at 3 – 5 impressions for 3 – 5 leaves. Use the equation above to estimate the CO<sub>2</sub> that the leave was growing in. You can compare this to reported atmospheric CO<sub>2</sub> values online – remember there is going to be some variation depending on location.

**Selected references:**

Beerling et al., (1998) Stomatal responses of the 'living fossil' *Ginkgo biloba* L. to changes in atmospheric CO<sub>2</sub> concentrations. *Journal of Experimental Botany* 49 (326) 1603-1609

Crane, P.R. (2018) An evolutionary and cultural biography of ginkgo. *Plants People Planet*.  
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Haworth et al., (2010) Differences in the response sensitivity of stomatal index to atmospheric CO<sub>2</sub> among four genera of Cupressaceae conifers *Annals of Botany* 105 411-418

Conde, G.D., (2016) [Stomatal index of \*Ginkgo biloba\* as a proxy for atmospheric CO<sub>2</sub> \(uoregon.edu\)](#)

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McElwain et al., (1999) Fossil plants and global warming at the Triassic-Jurassic boundary. *Science* 285 1386-1390

Retallack, G.J. & Conde, G.D. (2020) Deep time perspective on rising atmospheric CO<sub>2</sub>. *Global and Planetary Change*. 189: 103177 [Deep time perspective on rising atmospheric CO<sub>2</sub> - ScienceDirect](#)

Royer et al., (2003) Ecological conservatism in the "living fossil" *Ginkgo*. *Paleobiology* 29(1) 84-104

A lovely talk on *Ginkgo biloba* by Peter Crane: <https://www.youtube.com/watch?v=WBogvipUIK8>