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Stomatal Density as a Paleo-Barometer: Analyzing Fossilized Leaves to Estimate Atmospheric CO_2 from Millions of Years Ago

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Abstract

The most informative terrestrial proxies to the reconstruction of the ancient carbon dioxide (CO2) in the atmosphere are stomatal characteristics that are preserved in fossil leaf cuticles. Since stomata trade-off carbon gain and water loss, it is common in plants to decrease stomatal abundance when cultivated in high CO2 conditions, and this developmental signal may remain in fossilized epidermal tissue millions of years later. The paper will review the biological basis, analytical workflow, and key quantitative methods that support stomatal paleo-CO2 reconstruction with a particular focus on the distinction between stomatal density and stomatal index, the significance of species-specific calibration, and the increased use of mechanistic leaf gas-exchange models. The modern inverse stomatal response to increased CO2 was determined by classic experiments and herbarium studies and the method has been applied to much of the Phanerozoic in fossil applications. Synthesized case studies indicate that stomatal proxies allow the support of a general Paleozoic drop in atmospheric CO2, middle and high latest Cretaceous and early Paleocene concentrations, current-day-like values in part of the early Paleogene and middle Miocene in certain lineages, and >400 ppm estimates in early Miocene tropical assemblies. Meanwhile, the proxy is not a one-to-one meter: uncertainty is affected by light environment, water stress, leaf expansion, taxonomic misidentification, taphonomic damage, and statistical calibration choices. The key finding is that stomatal characteristics serve optimally as a paleo-barometer when fossil cuticles are well-preserved, counts are strictly replicated, lineage-specific calibrations and stomatal evidence are interpreted in combination with independent stratigraphic and geochemical constraints.

Keywords: *paleo-CO2, stomata, fossil leaves, stomatal index, stomatal density, paleoclimate, leaf gas-exchange model*

Introduction

One of the key issues in paleoclimatology is the reconstruction of ancient atmospheric CO2 since carbon dioxide is the first-order control of radiative forcing, global temperature, hydrologic intensity, and long-term carbon cycle. Most of the history of the Earth has no direct atmospheric measurements, and scientists have to rely on proxies that are preserved in sediments, minerals, isotopes, and biological tissues. Fossil leaves have a special position in these archives since they were formed at the interface between the atmosphere and the plant body. As a leaf grows, the CO2 concentration that the plant is exposed to is incorporated into the epidermis of the leaf, and this data can be stored in the frequency, size and pattern of stomata that are stored on the cuticle (Beerling and Royer, 2002; Royer, 2001).

The deep geologic record can only be measured using proxies that are indirect in nature, whereas ice cores only give direct measurements of the last fraction of Earth history. Fossil leaves are particularly useful in that regard since they are a sample of the continental environments that are frequently underrepresented in marine archives. Marine proxies may be very strong, although they may rebuild surface-ocean chemistry and then atmosphere with further assumptions. The stomatal proxies act in the reverse way: they start with an anatomical structure created in direct contact with the air. Such straightforwardness does not eliminate doubt, but it is the reason why stomata continue to be the focus of discussion of deep-time greenhouse climates.

Stomata are minute apertures that are surrounded by guard cells. They control the exchange of gases by allowing the diffusion of CO2 inwards to be used in photosynthesis and water vapor to be lost. Due to this coupled carbon-water economy, plants tend to modify stomatal development with a fluctuation in CO2 in the atmosphere. Woodward (1987) demonstrated in a seminal study that leaves of contemporary plants cultivated under increasing CO2 concentration had significantly fewer stomata per unit area and that herbarium samples across the industrial period had the same direction of change. That discovery was vital since it transformed a physiological reaction into a possible geologic proxy: in the event that living plants record atmospheric CO 2 in epidermal anatomy, fossil plants could record it too.

The term stomatal density as a paleo-barometer is handy yet a little bit deceptive. The most intuitive measure is certainly the stomatal density (number of stomata per unit area of the leaf), but it is not necessarily the strongest. Most studies instead use stomatal index, which is a ratio of stomata to all epidermal cells and partially compensates the difference in cell expansion and leaf development (Royer, 2001). In the meantime, more recent mechanistic models combine stomatal density with pore geometry in the first principles estimation of leaf gas exchange and atmospheric CO2 (Franks et al., 2014). What is obtained is a family of related methods as opposed to a single proxy.

The thesis of this paper is that stomatal characteristics can be viewed as a taxon-sensitive paleo-barometer, which is biologically based. The discussion is based on the developmental foundation of the stomatal signal, the practical workflow of fossil cuticle analysis, and the primary quantitative models that transform stomatal measurements into paleo-CO2 estimates. It then compiles representative case studies of the Paleozoic to the Miocene and assesses the constraints which make the proxy powerful, equivocal and why good visual and statistical practice are important as much as the underlying biology.

Biological foundations of the Stomatal CO2 Signal

The balance between the need of the plant to gain carbon and the need to save water controls the stomatal development. At elevated CO2 levels in the atmosphere, a leaf may be able to sustain sufficient photosynthetic uptake at a lower stomatal pore count and, thus, lower transpirational water loss and construction expense. High CO 2 may also modulate stomatal aperture, epidermal cell growth, and leaf thickness and this implies that the fossil signal is not simply anatomical but developmental. Modern experiments have been reviewed to indicate that CO2 has an effect on both short-term stomatal behavior and long-term stomatal formation, although the strength of the effect is altered by light, temperature, humidity, soil moisture, and nutrient status (Xu et al., 2016). This is the reason why the stomatal characteristics have atmospheric information but are not regulated by CO2 alone.

Another reason why the stomatal signal is biologically plausible is that it represents development across the lifetime of a leaf, and not a transient environmental variation. The ultimate epidermal pattern of a leaf is a result of cell division, differentiation and expansion in growth. Consequently, the abundance of stomata indicates the environment during which the leaf was developed rather than the day in which the leaf was fossilized. One of the reasons why herbarium series and controlled experiments are so informative is that it can be demonstrated that leaves that have grown in various atmospheric histories can still retain different stomatal signatures despite being stored as non-living specimens decades or centuries later.

There are two related measurements that are of particular importance. The density of stomata is known as stomatal density (SD), and it is the number of stomata per square millimeter of the epidermis. The percentage of stomata in an area of epidermal cells counted is called stomatal index (SI). SI is usually expressed as SI = [stomata/stomata + epidermal cells) x 100]. This difference is important since SD may vary when epidermal cells merely grow bigger or smaller, although stomatal differentiation may remain the same. SI equalizes stomatal abundance to epidermal cell count and is thus not usually sensitive to leaf expansion, irradiance and other non-CO2 effects as compared to raw density alone (Royer, 2001). On this basis, most traditional paleo-CO2 models take SI as the input variable of choice.

With that said, stomatal density cannot be ignored. The highest stomatal conductance is determined by the number of stomata in an area and the geometry of each pore. Mechanistic reconstructions thus directly use density and pore size, as opposed to SI being the only parameter of interest (Franks et al., 2014). Practically, SD and SI are complementary. SI can be better used as empirical calibration in a lineage, whereas SD is the focus of conductance-based models that seek to be generalized over a larger range of taxa.

The current correlation between the CO2 in the atmosphere and stomata is not a simple mathematical relationship. The various plant groups have varying baseline epidermal architectures, varying developmental pathways and varying ecological tolerances. The choice of ginkgo and metasequoia as calibration taxa is due in part to their long geological histories, and extant representatives or close analogs of the taxa that allow modern calibration of CO2 histories (Royer et al., 2001; Royer, 2002). High CO2 levels of useful sensitivity also persist in conifers in the Araucariaceae, proving that the proxy does not necessarily saturate in greenhouse atmospheres (Haworth et al., 2011). These instances highlight a fundamental fact: stomatal proxies are not universal masters, but rather taxon-specific biological sensors.

This is a taxonomic peculiarity that is both a strength and a weakness. It is an advantage since the signal is based on actual plant developmental physiology; it is a limitation since the fossil has to be recognized with confidence and be compared to a suitable modern analog. A false identification of a fossil leaf, or a fossil that is placed in a wide morphotype, which obscures several biological species, can cause a shift in the entire calibration. Similarly, the noise of mixing sun leaves and shade leaves, adaxial and abaxial surfaces, etc., can contribute to noise that is not related to atmospheric composition. Rigorous sampling design is thus a must in the biology of the stomatal signal.

Fossil Leaf to CO2 Estimate: Analysis Workflow

A stomatal paleo-CO2 reconstruction is not calculated until long before a number. The former is the existence of fossil material that has sufficiently preserved cuticle to allow identification of epidermal cells and stomatal complexes. Compression fossils, mummified leaves, cuticles separated out of sediments, and unusually well-preserved macrofossils may all be appropriate, but the portion of a fossil that can be used is often only a fraction of the specimen. Areas to be chosen by analysts should be free of veins, margins, tears, fungal attack, and mechanical distortion. Even when only fragments are available, the anatomical side and the leaf area should be recorded since the abundance of stomata often differs across the lamina (Beerling & Royer, 2002; Royer, 2001).

The workflow no longer includes documentation and reproducibility as a supplemental feature. Good quality studies store photomicrographs, provide the magnification and counted area, specify whether counts were made on abaxial or adaxial cuticle and the number of leaves and fields that were used to arrive at the final mean. Such detail is not a bureaucracy. Since the number of stomata is prone to observer bias and field-selection bias, a reconstruction can only be assessed separately when subsequent workers can view what was counted and the definition of the measurement window.

Maceration, bleaching and careful mounting of cuticles are usually used to isolate them to be examined under light microscopy or scanning electron microscopy. This is aimed at exposing the epidermal architecture without artificial stretching or shrinking of cells. Samples are then taken at various fields of view, various positions on each leaf, and preferably various leaves of the same stratigraphic horizon. It is bad practice to report a single count of a single image since stomata are spatially heterogeneous even within a single leaf. Replication is necessary to estimate the mean as well as to measure uncertainty and determine outlier fields due to damage or preparation artifacts.

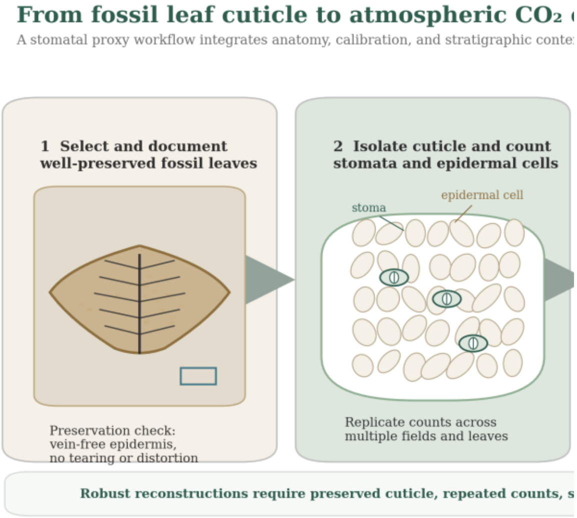

Figure 1

Workflow for converting fossil leaf epidermis into a paleo-CO_2 estimate.

Note. Author-generated schematic based on methods synthesized by Beerling and Royer (2002), Royer (2001), and Franks et al. (2014).

After counts, SD, SI or both are calculated by the analyst. The values are then transformed into atmospheric CO2 by one of three broad frameworks: an empirical calibration of a modern relative, a stomatal ratio method that compares the fossil with a reference taxon, or a mechanistic gas-exchange model that relates stomatal geometry to conductance and carbon assimilation (Franks et al., 2014; Royer, 2001). The selection is determined by the fossil lineage, the quality of preservation, availability of living analogs and the time interval of interest.

Contemporary calibration is normally the determining factor. The series of herbarium samples that have been collected in the past two centuries give us leaves that have grown in the previously known atmospheric CO2 levels, and the greenhouse experiments are able to extend the calibration to the higher CO2 levels under controlled conditions. In case these modern leaves are counted using the same counting protocol as the fossils, the analyst can obtain a species-specific transfer function. An example of such a calibration of Ginkgo biloba, one of the most well-known examples in the literature, is presented in Figure 2. The fact that it has a strongly nonlinear response is a reminder that proxy sensitivity is not always constant throughout the CO2 range.

The stratigraphic context is equally important as anatomy. An old cuticle of a badly dated bed provides a badly constrained estimate of the atmosphere in the past. The best studies thus combine sedimentology, magnetostratigraphy, radiometric dating, palynology, or mammalian biochronology in such a way that stomatal data can be put into a safe time scale. In effect, a reconstruction of the stomata always brings together 3 different pieces of evidence: anatomical fidelity, biological calibration and stratigraphic control. The weakness of one of the three is propagated through the final CO2 estimate.

Paleo-CO2 Reconstruction using quantitative methods of stomatal reconstruction

The most basic and the most popular framework is the empirical transfer function. In this case, fossil SI or SD is placed in a calibration curve that was developed using living leaves that were grown or collected in the known atmospheric CO2. This approach is appealing as it is open and can yield comparatively accurate estimates in the case when the fossil is a member of a well-calibrated lineage. An example is Royer et al. (2001), who reconstructed modern Ginkgo and Metasequoia data to obtain CO2 in the atmosphere during the last Cretaceous, Paleogene, and middle Miocene. The empirical approach has the advantage of being realistic: it does not need to explicitly model all the physiological parameters of a plant lineage to obtain the actual integrated response.

Another significant aspect of empirical calibration, as shown in Figure 2, is that the sensitivity varies along the curve. In Ginkgo, the steepness of the response varies with portions of the low-to-moderate CO2 range, but levels off at other portions, thus the same error in analysis in SI will result in vastly different uncertainty in reconstructed CO2 based on the location of the fossil. This is the reason why the coefficient of determination should not be used to judge the empirical calibrations. The practical question is the extent to which atmospheric uncertainty is associated with the measured fossil value considering the form of the curve and the dispersion of the training dataset.

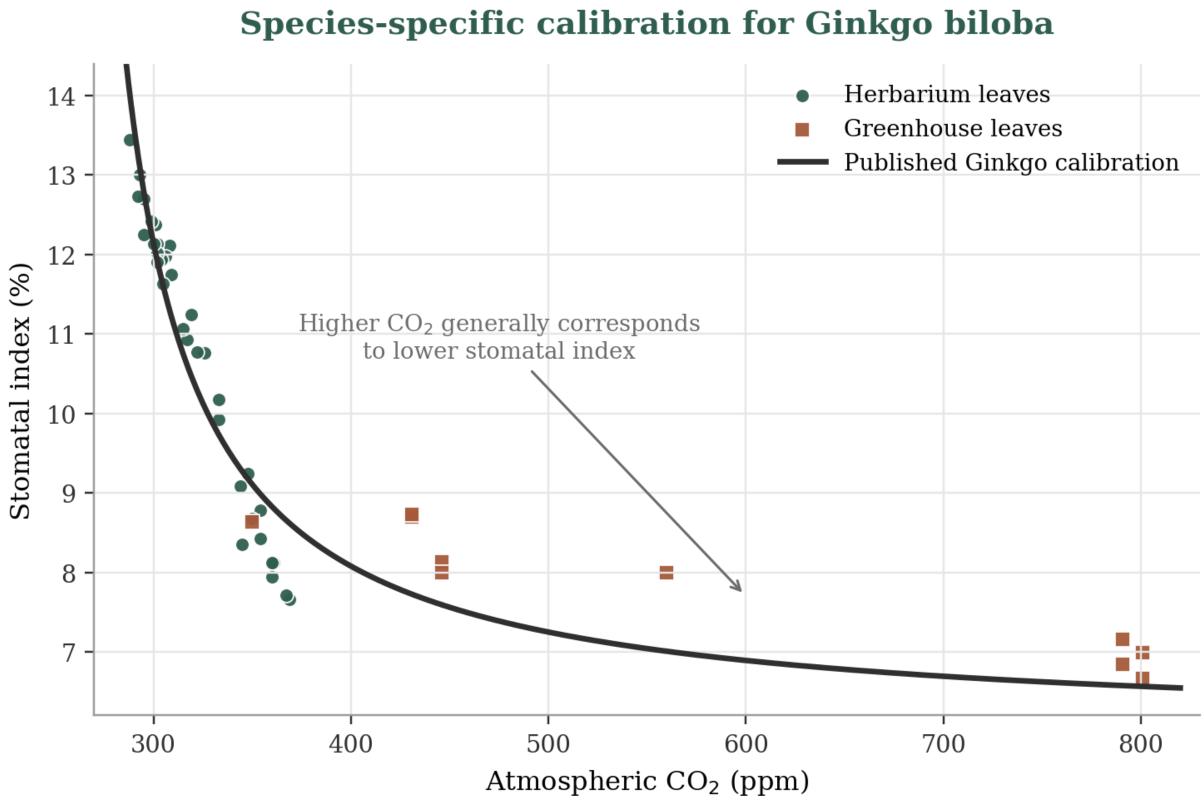

Figure 2

Species-specific Ginkgo biloba calibration relating stomatal index to atmospheric CO_2.

Note. Points represent modern herbarium and greenhouse calibration data compiled from the Royer Ginkgo dataset; the curve follows the published Ginkgo transfer function summarized by Royer (2002).

Its weakness is also quite evident. This is a lineage-specific relationship and is usually nonlinear, and therefore cannot be casually transferred across unrelated taxa. It is also as good as the modern calibration range. In the event that the fossil value is at or above the calibration cloud boundary, the uncertainty grows rapidly. The second difficulty is that empirical fits can seem accurate due to the fact that they average out environmental heterogeneity in the contemporary training set. The calibration in Figure 2 works well for Ginkgo because both historical herbarium material and controlled growth leaves exist, but many fossil taxa do not have that luxury.

The second method is the stomatal ratio method where the fossil stomatal value is compared to the related modern taxon and the ratio is then used to scale atmospheric CO2. This method may be useful in cases where the absolute calibration is constrained, but the method is based on the assumption that the fossil and reference taxon are proportional to CO2. Franks et al. (2014) described the ratio method as semi-quantitative and warned that the assumptions are not always justified. Consequently, stomatal ratios should be considered as supplementary evidence instead of the one that can be used to construct a high-confidence reconstruction.

The most ambitious is the mechanistic leaf gas-exchange model. Rather than fitting a transfer curve for a single taxon, this method relies on stomatal density, pore dimensions and diffusion theory to estimate maximum stomatal conductance, operational conductance, and the atmospheric CO2 concentration compatible with plausible photosynthetic demand

(Franks et al., 2014). Since the model is based on plant physiology, it can be used more flexibly to fossil taxa which do not have strong empirical calibrations. Milligan et al. (2022) demonstrated that the technique is capable of forecasting current-day CO2 with mean errors of the order of 5%-14% in validation exercises, which is promising when using the technique on deep-time scales.

Mechanistic models, however, substitute one set of constraints with another. They need additional anatomic measurements, more guesses regarding fossil physiology, and cautious extension of uncertainty using parameters like pore size, assimilation and internal-to-ambient CO2 ratio. Conifer leaf, broad angiosperm leaf, and morphotypes that are now extinct may vary in a manner that has physiological implications. Liang et al. (2022) demonstrated that the optimization of conifer-specific physiological parameters can significantly change reconstructed CO 2, which demonstrates the potential of the approach but also its dependence on the parameters used. Practically, the most effective studies tend to compare empirical and mechanistic findings, not to be based on one of them.

Table 1

Major analytical approaches for stomatal paleo-CO_2 reconstruction

Approach	Core inputs	Main strengths	Key limitations and best use
Empirical transfer function	Fossil SI or SD plus a modern calibration set from herbarium leaves, greenhouse experiments, or living relatives.	Transparent and often precise when the fossil belongs to a well-calibrated lineage.	Strongly taxon-specific and risky to extrapolate beyond the calibration range. Best for fossils with close living relatives such as Ginkgo or Metasequoia.
Stomatal ratio approach	Relative difference between fossil stomatal values and those of a modern reference taxon.	Useful as a first-pass estimate when a full calibration is unavailable.	Assumes fossil and reference taxa respond proportionally to CO_2; best treated as supporting, semi-quantitative evidence.
Mechanistic leaf gas-exchange model	Stomatal density, pore size, guard-cell geometry, and defensible assumptions about conductance and photosynthesis.	Physiologically grounded and more extendable to taxa lacking direct empirical curves.	Parameter-rich and sensitive to anatomical measurement quality and physiological assumptions. Best when geometry is preserved well and uncertainty is propagated explicitly.

Note. All three approaches remain useful; the most defensible choice depends on lineage, preservation quality, and the availability of calibration data.

Due to that reason, mature application of stomatal proxies is comparative and pluralistic. The gold standard is still empirical calibrations in the event that a close living relative with a long training set is available. Mechanistic models extrapolate the proxy to taxa and periods of weak or missing empirical curves. Ratio techniques may still be of use in giving first-pass information. The question of which method is more generally good is not one to be answered, but which method is the most justifiable in a particular fossil, lineage and stratigraphic problem.

Case Studies over Geologic Time

The Paleozoic provided one of the earliest and the most powerful fossil evidence. McElwain and Chaloner (1995) demonstrated that stomatal density and SI of fossil plants followed significant changes in atmospheric CO2 during the Devonian and Carboniferous suggesting a long-term decrease in stomatal density and SI between about 10-12 times current values in the Early Devonian and the end of the Carboniferous. The survival of all the numerical detail of those early estimates later in recalibration is less significant than the conceptual success: fossil stomata were able to record the change of carbon-cycle on an atmospheric scale during tens of millions of years, and could do so in a way broadly consistent with independent geochemical arguments.

The field was then transferred to finer time resolution by cenozoic studies. Calibrated Ginkgo and Metasequoia stomatal indices were used by Royer et al. (2001) to estimate latest Cretaceous, Paleogene and Miocene atmospheric CO2. Their main finding was provocative: a large part of the interval sampled gave values in the modern range as opposed to the very high concentrations that had frequently been supposed on the early Cenozoic. The dissertation by Royer (2002) extended this framework and indicated that numerous estimates between approximately 66 and 53 Ma were concentrated between approximately 300 and 450 ppm with a significant high estimate near the Paleocene-Eocene Thermal Maximum (PETM) and a few latest Cretaceous estimates above 500 ppm. What is significant about these reconstructions is that they did not make the debate more certain that it was before, but rather they re-framed the discussion on how warm climates can exist at CO2 levels that are moderate in geological terms.

Further effort around the Cretaceous-Paleogene boundary supported the usefulness of stomata as well as exposing complexity. Steinthorsdottir et al. (2016b) also used Southern Hemisphere fossil leaves to reconstruct pCO2 values of approximately 300-460 ppm in boundary sections throughout New Zealand, which was interpreted to mean that atmospheric CO2 was high, although not necessarily high everywhere, at the end of the Cretaceous. In comparison, Milligan et al. (2022) modeled a Franks-style leaf gas-exchange model on early Paleocene Platanetes leaves of the San Juan Basin and provided much greater estimates, around 665-1143 ppm. It is not that one study has to nullify the other. They sample various ages, various floras, various basins, and partly various analytical frameworks. They all show that stomatal reconstructions are able to reproduce real temporal and spatial variation as well as reveal the sensitivity of the proxy to taxon, method and calibration.

A second example of stomatal proxies that can be used to answer major paleoclimate questions is the Eocene-Oligocene transition. Steinthorsdottir et al. (2016a) found that the fossil plant stomata show a decrease in atmospheric CO2 before the boundary, which is in line with the hypothesis that the drawdown of greenhouse gases contributed to the shift of the climate system toward the Antarctic glaciation. The stomatal archive is again relevant here since it is terrestrial and atmospheric in its reasoning. It supplements marine records which can combine various residence times, reservoirs or local effects. As these pieces of evidence are brought together, it becomes more likely that the history of CO2 being inferred represents the global atmospheric change and not a local artifact.

Miocene uses indicate that the same proxy can produce moderate and high reconstructions in different settings and methods. In Royer et al. (2001), middle Miocene Ginkgo and Metasequoia leaves yielded values of about 310-396 ppm which is generally comparable to subsequent Cenozoic estimates. However, with a Neotropical early Miocene fossil assemblage and a leaf gas-exchange model, Londono et al. (2018) found a number of taxon-specific estimates that exceeded 400 ppm, with some near or over 900 ppm. Liang et al. (2022) then optimized conifer physiological parameters in gas-exchange models and estimated one conifer case study at 326 ppm at the beginning of the Miocene. These experiments do not mean that the proxy is ineffective, but demonstrate that when the fossil ecology, plant functional type and model parameterization are carefully matched, there is an increase in the quality of atmospheric reconstruction.

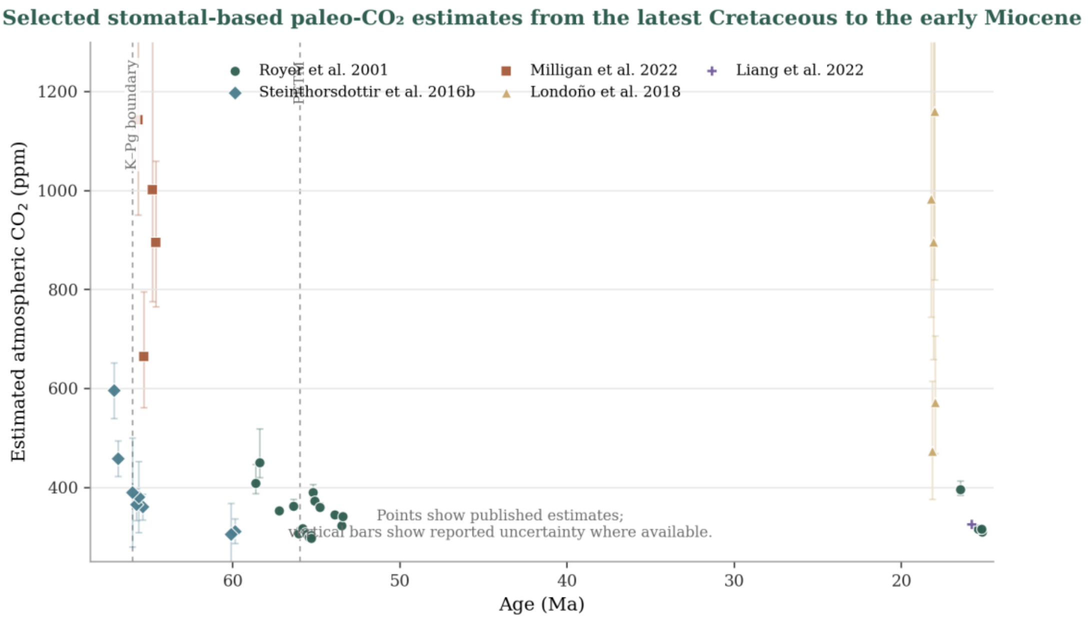

Figure 3

Selected stomatal-based paleo-CO_2 estimates from the latest Cretaceous to the early Miocene.

Note. Data were compiled from Royer et al. (2001), Steinthorsdottir et al. (2016b), Milligan et al. (2022), Londoño et al. (2018), and Liang et al. (2022). Vertical bars indicate published uncertainty where reported.

The case studies combined imply three general lessons. To begin with, the stomatal proxies can quite adequately identify large-scale secular trends, including the Paleozoic drop in CO2. Second, they are able to distinguish moderate background conditions and short-term high-CO2 events, albeit only in cases where stratigraphy is accurate, over shorter periods in the Cenozoic. Third, scientific disagreement between studies is frequently scientifically fruitful since it points to the area of uncertainty: in the history of climate itself, in the biological calibration, or in the extrapolation of a technique between one plant lineage and another. Figure 3 is a synthesis of a part of published estimates of the most recent Cretaceous to the earliest Miocene and visualizes that pattern.

Uncertainty and Best Practices Sources

Taxonomy is the first and the most obvious source of uncertainty. The only way a stomatal calibration can be valid is when the fossil is actually in the lineage that was used to construct the modern response curve. Even minor mistakes may be important as epidermal architecture is different in close relatives. Best practice is therefore conservative identification, explicit photographic documentation of diagnostic features and careful discussion of whether the modern analog is a true nearest living relative or simply a convenient ecological look-alike (Beerling & Royer, 2002; Royer, 2001).

Temporal resolution is also of concern. A fossil leaf captures the CO2 environment at the time the leaf developed during the season or years in which it developed, while some geochemical proxies have longer residence times or larger spatial reservoirs. Observed disparity between stomatal and marine estimates can thus be due to varying time filters and not analytical breakdown. The most persuasive reconstructions are those that match proxy choice with the timescale of the question - using stomata for example to test short-lived excursions or well-dated terrestrial intervals where leaf-level sensitivity is an asset rather than a complication.

The second source of uncertainty is environmental confounding. High CO 2 is not the sole cause that changes stomatal abundance. The intensity of light is of particular interest since leaves in the shade and in the sun can be different in systematically varying cell size and stomatal frequency. The development and stomatal functioning also depend on water availability, humidity, temperature, and nutrient supply (Xu et al., 2016). This is among the reasons why SI tends to do better in empirical reconstructions than SD: it to some extent normalizes the impact of epidermal cell expansion. Yet normalization is not immunity. The fossil leaves still need to be compared to the contemporary leaves of the same ecological position and similar leaf surface and analysts should not confuse microhabitats in a single calibration set.

Taphonomy brings in a third category of uncertainty. Fossil cuticles can be shrunken in burial, broken in maceration or only retain remnants of the original epidermis. Compression may cause distortion of cell outlines, elongation or collapse of epidermal cells. Organic residues and mineral overgrowth can cover pore margins. The solution is not to give up difficult material but to make the quality of preservation explicit, exclude dubious regions and report the number of counts, fields and specimens that underlie each estimate. A reconstruction from twenty carefully screened counts is more informative than one based on a single spectacular image.

It is also important to treat statistically. Calibration curves are frequently nonlinear, i.e. the same change in SI can suggest a minor change in CO2 in one section of the curve and a very large change in another. The propagation of uncertainty should be therefore done through the curve and not added later as an afterthought. Asymmetric confidence intervals are being reported in published studies more and more, and this is correct in both nonlinear transfer functions and mechanistic models. Mean values that are not accompanied by uncertainty limits are not useful since they give an illusion of accuracy.

Lastly, the best stomatal evidence is that which is combined with other archives. Independent age control discourages the urge to match stomatal excursions with climate events after the fact. Independent CO2 proxies, such as boron isotopes, paleosols, or estimates based on alkenones, offer an external test of plausibility. Consistency between archives cannot be requested in a naive manner since various proxies have different averages of time and conditions, yet convergence is desirable. A modern best practice philosophy therefore uses fossil stomata not as isolated numbers, but as part of a multi-proxy, stratigraphically explicit reconstruction of the ancient atmosphere.

Conclusion

The stomatal characteristics are worthy of the metaphor of a paleo-barometer since they are direct results of the interaction of the plant with the atmosphere. There are not many proxies that are as intuitively related to air composition: the surface of the leaf literally restructures itself in response to the availability of carbon. That biological understanding has over the past forty years developed into a strict paleoclimate instrument. The inverse developmental response to increasing CO2 was defined by Woodward in modern observations; this response was later turned into empirical calibrations and physiologically realistic models by Royer, McElwain, Franks, Steinthorsdottir, Milligan, Londono, Liang, and others, which could be applied over deep time.

The greatest thing that this literature has taught me is that there is no single stomatal metric or model that always prevails. Rather, the discipline has demonstrated that sound reconstruction requires the appropriate method to material. The empirical variable that is usually cleaner is stomatal index since it partially adjusts epidermal expansion. Stomatal density is also necessary where conductance and pore geometry are modeled in a mechanistic manner. Well-calibrated lineages like Ginkgo and Metasequoia can provide surprisingly constrained estimates, but other taxa need to be interpreted with caution. In all instances, the preservation of fossils, their copying and stratigraphic association make a stomatal estimate convincing or only suggestive.

Used with caution, fossil leaves do provide a coherent long-term picture: very high Paleozoic CO2, heterogeneous but often moderate-to-elevated values through the latest Cretaceous and early Paleogene, evidence for declining CO2 into major Cenozoic climate transitions, and continuing debate over how high CO2 was during warm Neogene intervals. It is not just a record that is of historical interest. It offers one of the most direct terrestrial archives for testing Earth system sensitivity to sustained greenhouse forcing. In that regard, not only are stomata a paleo-barometer of ancient air, but they are a connection between plant developmental biology and the vast history of planet climate.

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