The effect of light intensity and temperature changes on the stomatal and epidermal morphology of *Quercus kelloggi*: implications for paleoelevation reconstruction

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Introduction

Recently a new method to reconstruct paleoelevation has been developed, based on the adjustment in number of stomata on leaves to the predictable decrease in CO_2 partial pressure with altitude (McElwain, 2004). The first time application of this method focuses on determining the altitude of growth of early Miocene *Quercus pseudolyrata* leaves from the northern Sierra Nevada. Calibration of the fossil stomatal numbers to altitude is based on the stomatal response of the modern day California black oak (*Quercus kelloggi*), whose leaves are indistinguishable from *Q. pseudolyrata* and grows in the same region.

The clear correlation between increasing stomatal density and index vs. increasing altitude of growth found in *Quercus kelloggii* can for a large part be attributed to the predictable decrease in CO_2 partial pressure. Many plants, among which several *Quercus* species, show this increase in stomatal frequency with decreasing CO_2 and CO_2 is the only environmental parameter that globally and predictably changes with elevation.

However, several other climatic variables can also change with elevation and potentially influence stomatal density. The most important two are light intensity and temperature. In a natural setting, the relative influence of all environmental parameters on the stomatal change over altitude transect is extremely difficult to pry apart. Therefore, saplings of *Quercus kelloggi* were grown under controlled conditions in growth chambers, varying the levels of light and temperature. We will present preliminary data on the effect of both light levels and temperature regimes on stomatal frequency and epidermal cell morphology of *Quercus kelloggii* leaves.

Material and methods

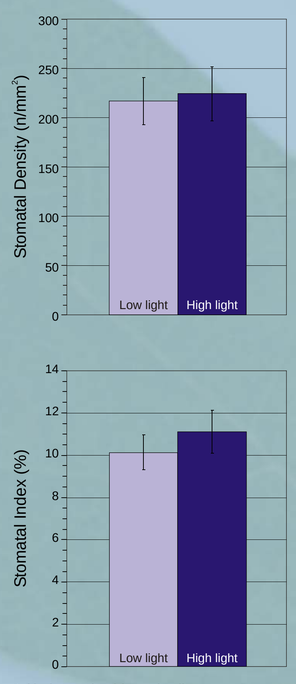
In the temperature experiment, two-year old sapling of *Quercus kelloggi* were grown in the spring/summer of 2004 in two growth chambers where light intensity, CO_2 level and temperature were controlled. Plants in both chambers were subjected to the same manual watering regime. Temperature followed a 24-hr cycle, where under the low temperature regime it ranged from 20°C (day) to 15°C (night). The high temperatures were 27°C (day) and 22°C (night). Light intensity was set at a relatively low level to mimic the natural understorey habitat of *Q. kelloggii*.
The light experiment was conducted on a different batch of *Q. kelloggi* saplings in four growth chambers during the spring/summer of 2006 (with controlled light, temperature and CO2, under relatively low light levels in two chambers and relatively higher light levels in the other two. Temperature in all chambers was set to resemble a typical 24-hr cycle in the northern Californian habitat (22°C during the day and 17°C at night). Watering occurred two times a week using an irrigation system. All chambers contain about 15 plants in separate pots.

Leaf samples were taken from two leaves per plant in the temperature experiment, and three to five leaves per plant in the light experiment. Small leaf disks were cut out using a holepunch, and the disks were treated with either 4% sodium hypochloride or a 50/50 mixture of concentrated glacial acetic acid and 30% hydrogen peroxide (both at ~50 °C for one to two days). After removal of the mesophyll, the cuticle was stained with saffranin, mounted in glycerin jelly on a slide and analysed using a Leica DMLB epifluorescence microscope (using transmitted light). Images were digitally captured using a SPOT camera and SPOT image analysis software.

Light

	Low light	High light	p-value
S.D. (n/mm^2)	217 ± 24	224 ± 27	0.56
E.D. (n/mm^2)	1926 ± 173	1803 ± 234	0.24
S.I. (%)	10.1 ± 0.8	11.1 ± 1.0	0.04*

Table 1: Means, s.e.m.'s and Student's t-test P-values for stomatal density and index and epidermal cell density of *Quercus kelloggii* leaves from low and high light treatments. The low light measurements are from 5 counting fields (0.068 mm^2) on 7 leaves from 3 trees in two chambers, the high light measurements from 13 leaves from 5 trees in two chambers.



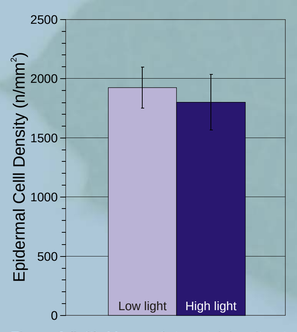


Figure 1 (left): Means of stomatal density, stomatal index and epidermal cell density of *Quercus kelloggii* leaves from low and high light treatments. Stomatal index was calculated as (SD/(SD+ED)*100). Error bars indicate the standard error of the mean.

Figure 2: *Quercus kelloggii* leaves from each of the four chambers in the light experiment.

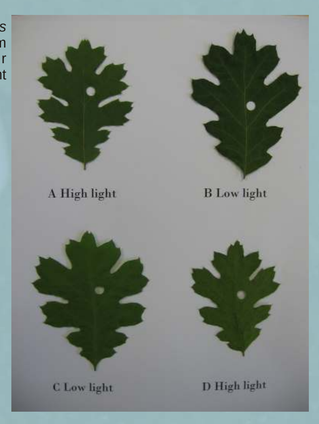
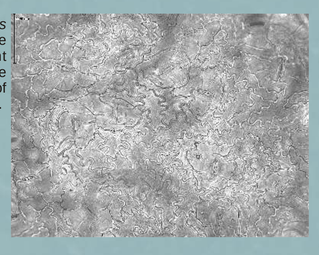
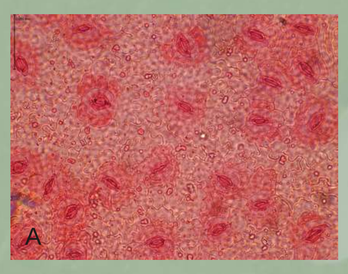


Figure 3: *Quercus kelloggii* cuticle from the high light treatment (chamber D). Note the prominent undulation of the epidermal cell walls.



In *Quercus kelloggi* stomatal density did not increase significantly with light intensity (but was slightly higher), epidermal cell density did not change significantly, but stomatal index increased by a small, but significant amount. This pattern can be found to varying degrees in most plant species, where light intensity during leaf growth results in adjustments in leaf anatomy and epidermal morphology, either in experimental or natural settings. In general, shade leaves are thinner and larger, show lower stomatal initiation (stomatal index) and due to larger epidermal cells have lower stomatal and epidermal cell densities (see Royer et al., 2001). This has also been reported for *Quercus petraea*, where typical shade leaves have lower stomatal densities and index and are also characterized by more undulating epidermal cells walls (Kurschner, 1997).
The relatively modest increase in stomatal index (and the very small change in density) could indicate that the high light settings in this experiment were still relatively low compared to the natural variation. The prominent undulation of the epidermal cells under the high light treatment (Figure 3), which is typical for shade leaves, suggests that the experimental light difference is well within the natural range, since more typical, straight-walled, sun leaves of *Quercus kelloggi* are encountered in natural populations.

Temperature



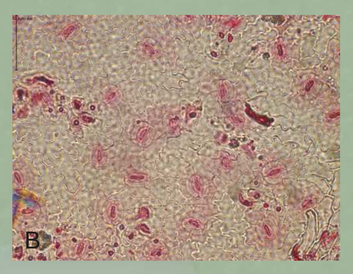
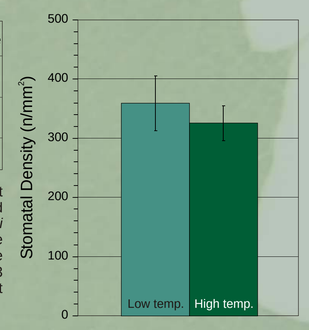
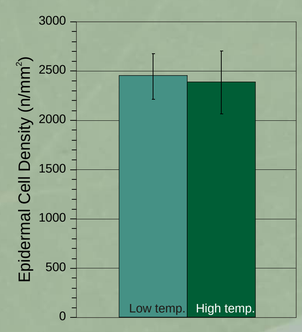


Figure 4: Counting fields on *Quercus kelloggii cuticle* from the high (A) and low (B) temperature

	Low temp.	High temp.	p-value
S.D. (n/mm^2)	360 ± 46	326 ± 29	0.30
E.D. (n/mm^2)	2450 ± 232	2387 ± 318	0.76
S.I. (%)	12.8 ± 1.5	12.0 ± 0.7	0.43

Table 2: Means, s.e.m.'s and Student's t-test P-values for stomatal density and index and epidermal cell density of *Quercus kelloggii* leaves from low and high temperature treatments. The low temp measurements are from 5 counting fields (0.068 mm^2) on 3 leaves from 2 trees, the high light measurements from 5 leaves from 3 trees.





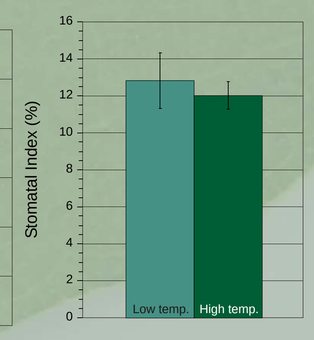


Figure 5: Means of stomatal density, stomatal index and epidermal cell density of *Quercus kelloggii* leaves from low and high temperature treatments. Stomatal index was calculated as (SD/(SD+ED)*100). Error bars indicate the standard error of the mean.

In this study, realistic changes in growth temperature did not have a significant effect on stomatal density and index or epidermal cell density of Quercus *kelloggi* leaves (Table 2; Figure 5). In other controlled growth experiments increased temperature has been shown to positively influence stomatal densities (Ferris et al., 1996; Reddy et al., 1998; Wagner et al., 1998) and stomatal initiation (Wagner et al., 1998). Other experimental studies, however, also show no influence of temperature on stomatal frequency (Apple et al., 2000). Even if there is a response in experimental settings, in natural habitats, the influence of temperature on stomatal initiation may be of no consequence, since most plants compensate for fluctuating temperatures by adjusting the timing of leaf development (Wagner, 1998). At higher altitudes leaf development will likely start later in the year as soon as similar temperatures are reached as the earlier growing season at lower altitudes.

Implications for paleoelevation reconstruction

The first results from growing *Quercus kelloggi* saplings under controlled light intensity and temperature in this study, indicate that stomatal density and index in this species do not respond to realistic temperature changes, and only slightly (stomatal index) to light intensity. The potential influence of light intensity might be underestimated in this study, as epidermal cell shape indicates that the high light treatment did not produce typical sun leaves. Future experiments may be performed under more enhanced light intensity. However, currently the stomatal density response to altitude that is used for paleoelevation reconstruction is based on shade leaves, so the light experiment does suggest convincingly that changes in light intensity with altitude should not be a main interference in the presently reconstructed paleoelevation estimates for the Miocene Sierra Nevada.
The lack of response of stomatal density and index to temperature suggests that under different climatic conditions in the past (such as the Miocene) the stomatal frequency-altitude relation was probably not significantly different because of temperature.

Light intensity and temperature are the main environmental factors, apart from CO_2 partial pressure, that change strongly with elevation. The reported change in stomatal density and index in *Quercus kelloggi* with altitude, that is now applied to reconstruct Miocene paleoelevation of the Sierra Nevada, may therefore reasonably be attributed to changes in CO_2 partial pressure with altitude. Since this decrease in CO_2 partial pressure is highly predictable, the lack of interference of light and temperature in determining stomatal density strengthens the accuracy of this new paleoelevation tool. Future experiments growing *Quercus kelloggii* under lowered CO_2 partial pressure (by either decrease the CO_2 mixing ratio or the air pressure) may confirm the domination of the CO_2 effect in stomatal response to altitude.

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Acknowledgements

J. McElwain gratefully acknowledges financial support of the National Science Foundation (grant EAR-0207440) and funding for L. Kouwenberg was provided by the Netherlands Organisation for Scientific Research (TALENT-fellowship) and the John Caldwell Meeker postdoctoral fellowship. We greatly appreciate the technical assistance of Rebekah Hines, Shannon Loomis and Madeleine McLeester.