# Long-Term Effects of $CO_2$ Enrichment on Plant Genome and Cell Size.


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## ABSTRACT


A major unanswered question in plant biology, ecology, and conservation centers on identifying the structural and functional characteristics of plants that ultimately determine species and community responses to environmental change. Of particular importance is the response of individual plants and communities to long term enrichment of atmospheric carbon dioxide ($CO_2$). In general, plant species respond to increased $CO_2$ by building leaves with fewer, large stomata and decreased overall surface conductance. However, the underlying cellular mechanisms for these observed changes is poorly understood, as are the larger scale effects on plant species distributions and community dynamics. Previous research suggests coordinated changes in cell size and genome size can occur in response to changes in atmospheric $CO_2$. Together these changes result in the down regulation of maximum potential leaf surface conductance to $CO_2$ and water vapor. Here we evaluated the influence of long-term atmospheric $CO_2$ enrichment on a native California grassland at Stanford University, conducted in collaboration with the Jasper Ridge Global Change Experiment. Plant samples were collected and analyzed from both experimental plots with sustained elevated $CO_2$ for 18 years and control plots with ambient $CO_2$ levels. The objectives of this study were to determine (1) the extent to which individual plant species' genome sizes change when subjected to long-term increased $CO_2$, and (2) species specific guard cell size changes in response of individual plant species' guard cell sizes when subjected to long-term increased $CO_2$. The results of this study will provide valuable insight into plant community responses to environmental change.


## BACKGROUND

Anthropogenic emissions and human perturbations of natural systems are causing the concentration of $CO_2$ in the atmosphere to rapidly increase at a rate far faster than in the past 20,000 years of geologic history, and the atmospheric $CO_2$ level is now beyond anything observed over the past 800,000 years (Ciais *et al.*, 2013; IPCC, 2014).

Over the past three decades the physiological effects of increased $CO_2$ on plants have been studied extensively but there is still some fundamental knowledge missing (Drake *et al.* 1997; Long *et al.* 2004; Norby *et al.* 1999; Stitt, 1991). Research has shown a general trend of decreased stomatal densities and increased stomata size in response to elevated $CO_2$ that lead to a lower overall stomatal conductance and increased water use efficiency (Franks & Beerling, 2009; Woodward, 1987). However, the underlying causes of these shifts is poorly understood and potential impacts of these shifts on plant dynamics and distribution remains relatively unexplored. We propose that these structural changes in stomata size and density are associated with changes in genome size.

Changes in genome size in response to changing environmental variables make sense to investigate because nuclear volume is coupled with cell volume in plants, meaning if genome size shifts, cell size also shifts (Beaulieu *et al.* 2008). This relationship is important because variation in cell size has important biophysical implications on plant morphology and physiology. For example, an increase in the size of the guard cells that regulate the opening and closing of the stomata can greatly increase water use efficiency and decrease carbon uptake (Franks & Beerling, 2009). Additionally changes in xylem (water conducting) cell size directly influences both freezing tolerance and drought tolerance of plants. These types of changes are expected to have massive implications for species' distributions and survival worldwide as climate changes.

In 2012 Franks *et al.* showed that when grown under elevated $CO_2$ for several weeks, the genome size of three distantly related plant species increased. These preliminary results are highly intriguing but due to the limited number of species studied, the brief growing time and growth chamber conditions (as compared to natural field conditions), it is difficult for these findings to be generalized and used to inform policymakers for real world climate change solutions. But if the proposed relationship between plant genome size and enriched $CO_2$ is substantiated on a longer time scale and under natural field conditions, the impacts could be significant and wide reaching.

## BACKGROUND (CONTINUED)

It is our objective to see whether this intriguing trend uncovered by Franks *et al.* (2012) on genome size and stomatal characteristics under increased $CO_2$ occurs on a much larger, longer scale within a native habitat. Overall plant community composition changes will also be examined on a genomic basis when exposed to long-term elevated $CO_2$.

## METHODS

Stanford University's Jasper Ridge Global Change Experiment (JRGCE) has had continuous elevated $CO_2$ from ambient (~370 ppm) to enriched $CO_2$ (at approximately twice the ambient level, or 700 ppm) in circular plots via a loop of emitters surrounding each plot, using mini-FACE (free air carbon dioxide enrichment) technology for 18 years. There are 8 plots with enriched $CO_2$ and 8 plots with no added $CO_2$ (controls). Overall this grassland ecosystem is dominated by annual grasses, but it also includes perennial grasses and many herbaceous forbs of both annual and perennial life strategies.

One of the plots during the collection period in June 2016 at the Jasper Ridge Global Change Experiment in Woodside, CA

Samples were collected at the JRGCE plots in the summer of 2016. Due to the invasion of nonnatives to our CA grasslands the 5 species we were able to sample widely across most plots were nonnative (shown below).


Top left: *Erodium botrys*

Top right: *Avena fatua*

Middle left: *Anagallis arvensis*

Bottom right: *Avena barbata*

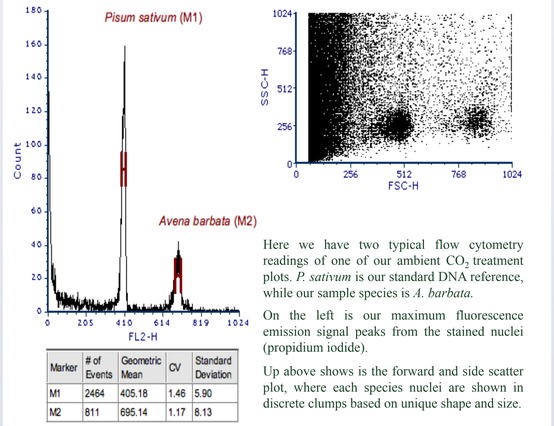
Bottom left: *Logfia gallica*

***Specific Aim 1*** Determine the extent to which individual plant species' genome sizes change when subjected to long-term increased $CO_2$. Genome size was measured of plant species co-occurring on experimental and control plots using flow cytometry, the most widely accepted method for quantifying genome size. Plants with known genome sizes were used, seeds provided generously from Dolezel (Dolezel *et al.* 2007), grown in San Francisco State University's climate controlled greenhouses as genome size standards. Data interpretation was performed on De Novo FCS Express.

## METHODS (CONTINUED)

***Specific Aim 2*** Determine the extent to which individual plant species' guard cell sizes change when subjected to long-term increased $CO_2$. Guard cell width and length of the same species sampled for genome size that are co-occurring on experimental and control plots will be measured. First using dental putty to make an impression of the leaf surface, then using a Nikon Eclipse 80i microscope and QCapture Pro software to examine the impressions. Measuring guard cell size is important because species with a larger genome possess a large nucleus and by extension large guard cells, which leads to lower stomatal densities and lower rates of stomatal conductance. Lower stomatal conductance is favored under $CO_2$ rich environments.

## RESULTS



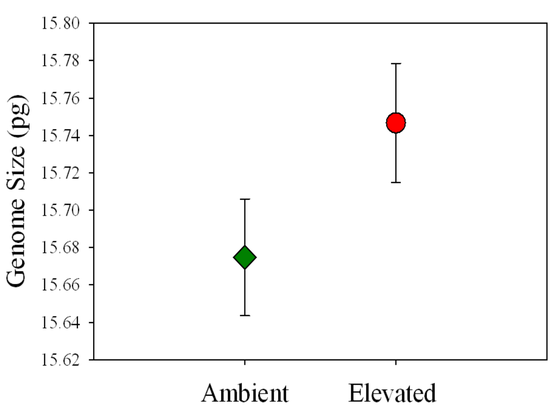

| Marker | # of Events | Geometric Mean | CV | Standard Deviation |
|---|---|---|---|---|
| M1 | 2464 | 405.18 | 1.46 | 5.90 |
| M2 | 811 | 695.14 | 1.17 | 8.13 |

Here we have two typical flow cytometry readings of one of our ambient $CO_2$ treatment plots. *P. sativum* is our standard DNA reference, while our sample species is *A. barbata.*

On the left is our maximum fluorescence emission signal peaks from the stained nuclei (propidium iodide).

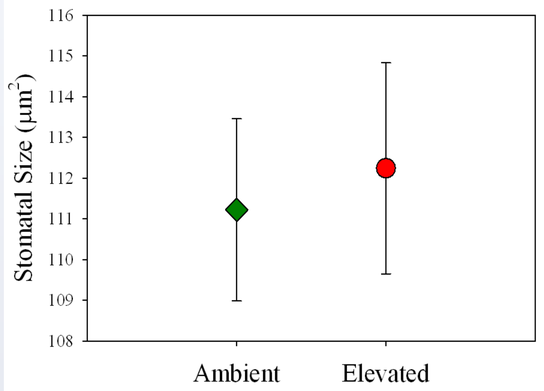
Up above shows is the forward and side scatter plot, where each species nuclei are shown in discrete clumps based on unique shape and size.

$$\text{Sample 2C value(DNA pg or Mbp)} = \text{Reference 2C value} \times \frac{\text{sample 2C mean peak position}}{\text{reference 2C mean peak position}}$$




Genome size (pg) of *Avena barbata* as influenced by $CO_2$ environment, ambient versus elevated $CO_2$ (ambient x2) treatments. Mean and standard error shown.

## RESULTS (CONTINUED)




Stomata Size (µm²) of *Avena barbata* as influenced by $CO_2$ environment, ambient versus elevated $CO_2$ (ambient x2) treatments. Mean and standard error shown.

## CONCLUSIONS

Our preliminary findings show both genome size (pg) and stomatal size are influenced by long-term elevated $CO_2$. Only one of the five sample species collected has been analyzed but results are promising thus far.

Notably, the elevated levels of $CO_2$ at the JRGCE are entirely within the range of possibility for California in the ensuing decades of the 21st century. This unique multi- generational analysis of community structure, plant carbon uptake and water use efficiency via stomatal characteristics and genome size in response to increasing atmospheric $CO_2$ concentrations will provide unprecedented data relevant to managing our natural lands. It will also provide important data for the knowledge base of plant physiology as a whole.

In the future, a study involving more diverse species as well as a higher sample size would be beneficial. Also looking at stomatal conductance rates under each treatment in a long-term $CO_2$ enrichment experiment would be valuable.

## LITERATURE CITED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