Effects of differing light conditions on *Euglena gracilis* and chloroplast regeneration

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Abstract

Euglena are a well-studied protist of the euglenoids, often used in a variety of scientific experimentation. Although there are discrepancies in their classification, they are distinguishable by several characteristics, including the presence of chloroplasts, which gives them a green hue. *Euglena gracilis* Klebs, the subject of this project, has several chloroplasts containing multiple pigments in their makeup. It is this characteristic that leads to study into different subjects such as chloroplast loss and regeneration, the original goal of this project. However, throughout the project, other interesting developments in carotenoid and paramylon molecule build-up under different light conditions were observed.

Introduction

Regardless of being a well-studied protist, there have been ongoing arguments as to how to further classify euglena. Protists are a category of diverse eukaryotes, having distinguishing characteristics such as being unicellular and microscopic and sharing certain morphological and physiological characteristics with animals, plants, or both. Despite this, euglena have been classified as photosynthetic protozoa by zoologists, but according to botanists they are classified as algae.

Although there are discrepancies in classification of euglena, they are distinguishable by means of several characteristics, including having a pellicle, flagella, and a distinct, red eyespot known as the stigma (**Figure 1**). *E. gracilis*, have chloroplasts but are both autotrophic and heterotrophic, absorbing nutrients through their membrane when sunlight is not available. However, besides the chloroplast structures in euglena, there are other components contributing to their photosynthetic ability and survival. For example, euglena contain carotenoid pigments, as do other photosynthetic organisms such as plants, algae, cyanobacteria, and photosynthetic bacteria. Carotenoids often function in the absorption process of violet and blue-green light (450-475 nm) and help prevent photooxidative damage. Through several studies, it has been found that there are strong relationships between carotenoid development and light colors and intensities.

Figure 1: Euglena Anatomy

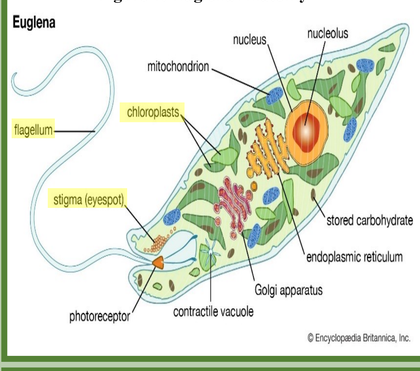


Figure 2: Abnormal *Euglena* (Left)

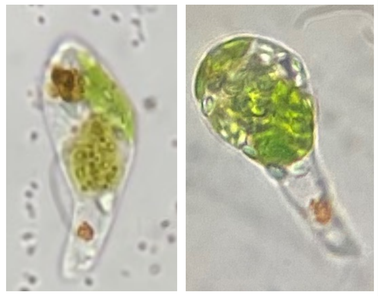


Figure 3: Normal *Euglena* (Right)

Methods

Cultures

- *E. gracilis* were sourced from Carolina Biological Supply and provisioned with Soil Culture Medium. They were maintained in light-proof boxes with various photoperiods and light conditions **(Figure 5)** with labeled vials and jars containing the cultures (**Table 1**).

Data Collection

- Wet mount microscope slides of the cultures were made and examined using a microscope. Each wet mount was visually inspected at 100X and the condition of the first 25 individuals was recorded. Individuals were labeled either abnormal, indicating presence of carotenoids **(Figure 2)**, or normal, indicating absence of carotenoids **(Figure 3).**
- Cultures were sampled weekly from early October to November, replicated 5 times for each culture. When the mounts were created, they were placed under a microscope and scanned. During this scan, individuals were tallied and categorized as normal/abnormal.

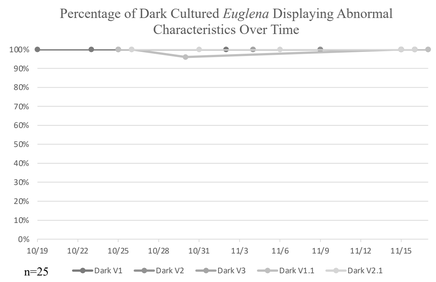
Table 1: Culturing Conditions

Box	Frequency	Cultures		Photoperiod
1	Dark	Dark Vial 1 Dark Vial 3 Dark Vial 2.1	Dark Vial 2 Dark Vial 1.1 Dark Jar	None
2	Full Spectrum	Control Vial 1 Control Vial 1.1 Control Jar	Control Vial 2 Control Vial 2.1	12:12
3	Red	Red Vial 1 Red Vial 3 Red Vial 2.1	Red Vial 2 Red Vial 1.1 Red Jar	12:12
4	Blue	Blue Vial 1 Blue Vial 3 Blue Vial 2.1	Blue Vial 2 Blue Vial 1.1 Blue Jar	12:12

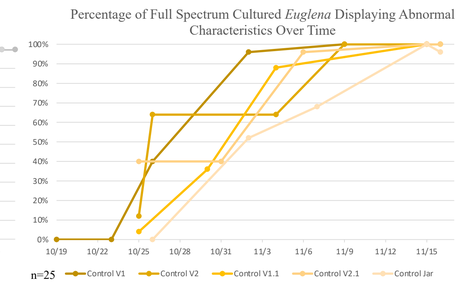
Figure 5: Culture Set-Up

Results

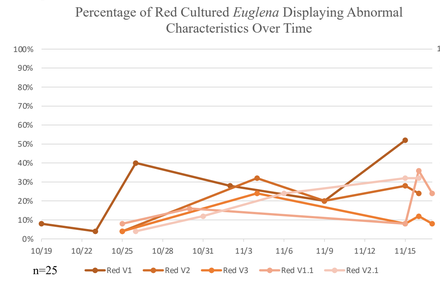
Graph 1



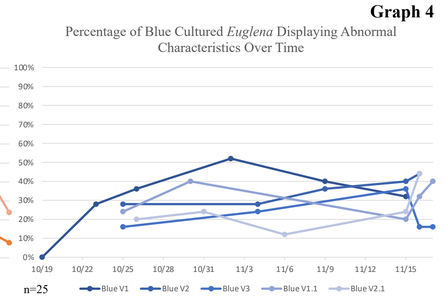
Graph 2



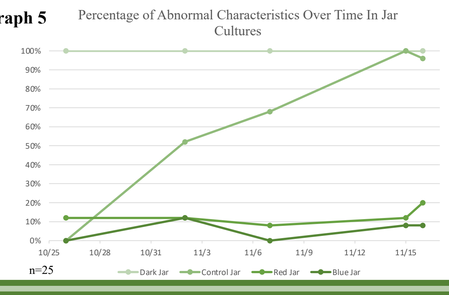
Graph 3



Graph 4



Graph 5



Discussion

In a study performed by Tanno et al., 2022, it was found that the carotenoid zeaxanthin accumulates in euglena in the dark. Zeaxanthin is also common in plants. This study also found that major carotenoid species in *E. gracilis*—including β-carotene, a red-orange pigment—accumulated corresponding to the duration of light during the light/dark cycle and was increased by low-intensity blue or red-light. This can be attributed to the fact that blue/red-light of certain intensities is similar to the light of dawn.

Because β-carotene aids in defense against strong sunlight and absorbs violet and blue-green light (450-475 nm), it corresponds that the range of light intensity shared between the light of dawn and low intensity blue or red-light would both cause an accumulation of β-carotene. β-carotene content in *E. gracilis* acclimates the cells to intense light conditions.

Another study, performed by Kato et al., 2017, reinforces the idea that carotenoid content increases in *E. gracilis* when exposed to bright light and leads to other changes in the cell. In this study it was found that under high light intensity conditions, euglena became pale green and with greater light intensities developed a yellow-orange or reddish-orange color, due to accumulation of carotenoids. This study concludes that increasing the intensity of light with *E. gracilis* causes an increase in carotenoid content and a decrease in chlorophyll content.

From these studies, it is evident that the findings according to the charts and graphs follow the expected natural reaction of *E. gracilis* cells to different colors and intensities of light. As can be seen in the **Graph 2**, instances of carotenoids increased with exposure over time to the bright light condition of the full spectrum control. The red and blue wavelengths also elicited some carotenoid pigment buildup **(Graphs 3 and 4),** but not to the extent seen in the full spectrum. The dark **(Graph 1)** had a constant of carotenoid presence and chloroplast loss, as to be expected in those conditions. It can be postulated that carotenoids such as zeaxanthin and β-carotene contributed to the carotenoid pigments found in the cultures.

Conclusion

While the original goal of the project was to determine if presence or absence of light and the frequencies of red and blue lights would have an effect on chloroplasts, our findings proved to be unexpected and interesting. The observation of cellular abnormalities was not anticipated. Investigation of additional indicated that these abnormalities were carotenoid pigments and their presence in some of the treatments was not unusual. Future studies could examine if light intensity in combination with frequency could have other effects on *Euglena* morphology.

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