High-throughput analysis of phenotypic variation between interfertile *Chlamydomonas* species

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Background

We are developing a high-throughput analytical framework designed to elucidate complex genotype-phenotype relationships across a wide array of species within the evolutionary tree of life[1,2]. To validate this approach, we focused on interfertile species of *Chlamydomonas*—specifically, *C. reinhardtii* and *C. smithii*—as our pilot test subjects. These unicellular green algae serve as an exemplary model system due to their clonal nature, amenability to high-throughput analysis, and diverse physiological traits[3,4,5].

To establish a foundational phenotype rubric, we performed an in-depth comparative analysis of the parental species across multiple dimensions. Our findings reveal that *C. smithii* cells, unlike *C. reinhardtii* cells, possess remarkable adaptability to various growth media—from ultrapure water to high-salt marine broth, are more prone to detergent-induced lysis, have thicker cell walls, and are more sensitive to magnetic fields. Intriguingly, *C. smithii* cells undergo bizarre morphological and flagellar changes when cultured in marine broth, suggesting potential defects in cell polarity and division.

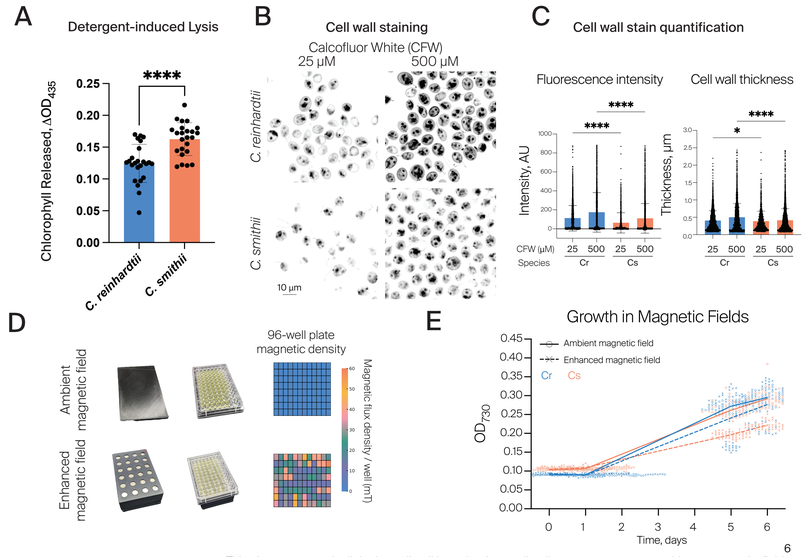
Leveraging these baseline phenotypes, we are currently investigating the phenotypic diversity in a library of more than 1,700 hybrid strains. These strains will be made publicly available through culture collections, serving as a valuable resource for the broader scientific community.
By integrating our phenotypic data with genotypic information, our high-throughput methodology enables a nuanced understanding of multifactorial genotype-phenotype associations, thereby laying the groundwork for future biological research.

For more, read the full project narrative: **research.arcadiascience.com/genetics**

Key Questions:

1. Are there easily distinguishable differences between these two species?
2. Can we develop methods to quantitiatvely measure these differences in high-throughput?
3. Can we develop genotype-phenotype matrices to map nonlinear relationships?

Cell wall function and morphology



This phenotype may be linked to cell wall integrity since cell wall mutants are more sensitive to magnetic field [6].

***C. smithii* has a weaker cell wall. (A)** Cells were vortexed with 0.05% Triton X-100 for 2 minutes and then centrifuged. The amount of chlorophyll released into the supernatant is represented here. **(B)** Cells were stained with the indicated concentration of calcofluor-white (CFW) for 15 minutes and then fixed in 4% PFA prior to imaging on a spinning disk confocal microscope. **(C)** Quantification of the CFW fluoresence intensity and the thickness of the fluorescence signal measured using linescans through the minor axis. **(D)** 3D printed custom MagBlock to generate magnetic fields in a 96-well plate. The magentic flux density for each well was measured and is represented as a heat map. **(E)** Optical Density (OD_{730}) readings over time representing the growth of *C. reinhardtii* (Cr) and *C. smithii* (Cs) in liquid TAP media grown on a non-magnetic control block (Ambient magnetic field) or on the MagBlock (Enhanced magnetic field).

Next Steps

As we continue to collect phenotypic data on the ACDC collection of strains, we're also generating genomes for each. These datasets will provide the foundation to test our genotype-phenotype non-linearity model with an ultimate goal of predicting genotype-phenotype correlations

I'd appreciate feedback on any of this work, but I'm especially curious about the following:

- What are some more interesting phenotypes that we could map next?
- We're always excited to improve our methods! Are there any other techniques we could develop to characterize these cells in highthroughput?

Check out Ryan's related poster right now! (B18 - New Technologies for Cell Biology)

Leave Feedback!

Comment on the pub:

Phenotypic differences between interfertile Chlamydomonas species

Post with #ArcadiaGenetics

bit.ly/chlamy-parents

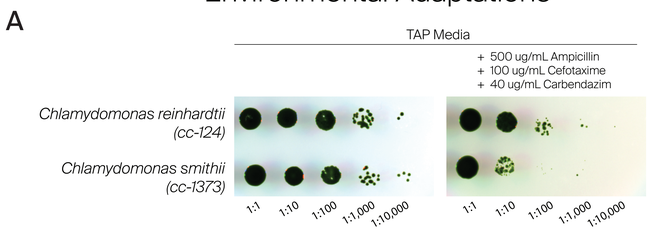
All other published work: research.arcadiascience.com

Check out Brae's poster right now! (B36 - Proteomics and Genomic Methods)

PHOTOGRAPHY ALLOWED

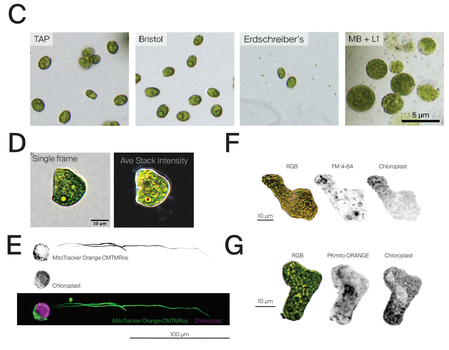
Phenotypic variation between *C. reinhardtii* & *C. smithii*

Environmental Adaptations



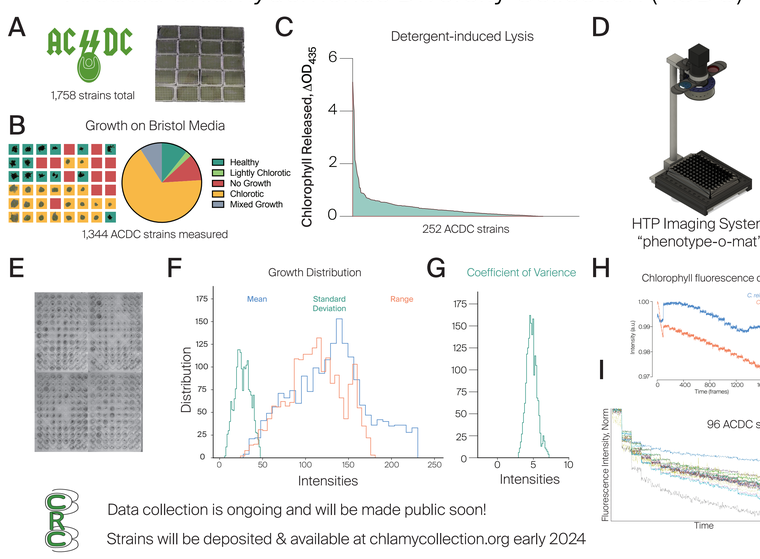
B

Species / Media	*C. reinhardtii*	*C. smithii*
TAP		
Soil extract		
Bristol		
Marine broth +L1 nutrients		
Erdschrieber's		
Kuhl's		
Ultrapure water		



***C. smithii* is highly adaptable to different environments. (A)** Serial dilution spot assays of *C. reinhardtii* and *C. smithii* grown on TAP media with or without a harsh antibiotic/fungicide cocktail. *C. smithii* is more sensitive to these drugs. **(B)** Summary graph of *C. reinhardtii* and *C. smithii* growth on various media formulas based on serial dilution spot assays. The number of cells indicates the species ability to grow on the media. 3 cells = healthy growth, 2 cells = okay growth, 1 cell = barely growing, 0 cells = no growth. Green cells indicate the colonies appeared healthy and green. Yellow cells indicate the cells were chlorotic **(C)** *C. smithii* cells taken off of 1.5% agar plates supplemented with the indicated medium, suspended in water, and imaged immediately. **(D–G)** *C. smithii* cells grown on marine broth had multiple unique phenotypes. **(D)** A multi-flagellated, large, amorphous cell. The average stack intensity shows the flagella are motile. **(E)** MitoTracker Orange CMTMRos stained cell with abnormally long flagella. **(F)** Amorphous cell stained with FM 4-64. **(G)** Amorphous cell stained with PKmito Orange with a clear separation between mitochondrial and chloroplast networks.

Arcadia *Chlamydomonas* Diversity Collection (ACDC)



Data collection is ongoing and will be made public soon!

Strains will be deposited & available at chlamycollection.org early 2024

We've generated a large collection of diverse strains with phenotypic variability (A) Our ACDC collection consists of 1,758 strains across 20 96-well plates. **(B)** We replica plated the first 1,344 strains in the collection to Bristol media. A representative 8 x 6 grid of colonies growing on 1.5% agar in Bristol media is shown. Our qualitative observations are reported here as a pie chart. **(C)** The first 384 strains of the collection were treated with 0.05% Triton X-100 or control buffer, vortexed, and centrifuged. The optical density (435 nm) of each well's supernatant was recorded. Values were normalized to the starting culture density (OD_{730}) and then subtracted the control values from the treated values. Negative values were removed and the remaining values were plotted from highest-to-lowest values. **(D)** Our bespoke "phenotype-o-mat" imaging system (~$400-500) used to collect high-throughput data. **(E)** Four representative 96-well flat-bottom plates containing ACDC strains in liquid TAP medium imaged using the phenotype-o-mat. **(F–G)** Statistical analysis of growth patterns of all 1,758 strains imaged. **(H)** Chlorophyll fluoresence decay of parental strains over time. **(I)** Chlorophyll fluoresence decay of 96 ACDC strains over time.

Conclusions/Summary

Although the strains used in this study share a similar history, we found these species to have substantial phenotypic divergence! These differences range from prefered nutrient availability to cell wall integrity. For more phenotypic datapoints including motility, gross morphology, and organelle morphology, check out and comment on our pub! **bit.ly/chlamy-parents**

When we crossed these strains, we found further instances of phenotypic divergence in the progeny!

A NOTE ON SHARING WITH US!

Part of our mission is to share as much useful research as we can.

If you choose to share a protocol or other useful information with us after viewing this poster, please understand that we may act upon this knowledge and share it when we publish our work. We publish quickly on an independent platform, so this may happen soon after you share, and we cannot wait for you to publish elsewhere.

If you decide to share anyway, yay! That's what science is all about. If your input is useful, we will include you as a contributor to the publication and explain that your role was in providing "Critical Feedback," likely with an additional description of what you shared.

tl,dr — If you're not ready for everyone to know about something, please refrain from sharing it with us.

Contributors (A–Z)

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Underlined contributors are also present at Cell Bio 2023!

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