DNA repair as a possible evolutionary advantage of sex in the green alga, *Chlamydomonas reinhardtii*

Deborah E. Shelton[1], Harris Bernstein[2], and Richard E. Michod[1]

(1) Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ

(2) Department of Cell Biology and Anatomy, University of Arizona, Tucson, AZ

1. Background

Sex is an evolutionary puzzle

- Sex, or genetic recombination between distinct individuals, is nearly universal
- Dramatic costs of sex exist, e.g.:
  - o Lowered relatedness to progeny
  - o Time/energy to find/secure a mate
  - o Risk of exposure to pathogens (Michod and Levine, 1988)
- The evolutionary benefits of sex are still debated, in spite of much scientific attention to the problem
- Facultatively sexual species (FSS) are thought to have sex when it is adaptive to do so (Williams, 1975). Thus, the benefits of sex in FSS are relevant to the adaptive value of sex

DNA damage is a problem that sex can solve

- DNA damage has negative consequences on fitness, e.g.:
  - o Terminated transcription
  - o Impaired replication
  - o Decreased cell survival (Bernstein and Bernstein 1991)
- A potential **direct benefit** of sex is repair of DNA damage through recombination of homologous chromosomes (Bernstein *et al.*, 1985)
- Stressful conditions, which can cause DNA damage, often induce sex. Sex can be understood as a response to stress (Nedelcu, 2005)

2. Hypothesis, predictions, and objectives

We hypothesize that sex is an adaptation for coping with DNA damage

Sexual reproduction in *C. reinhardtii*

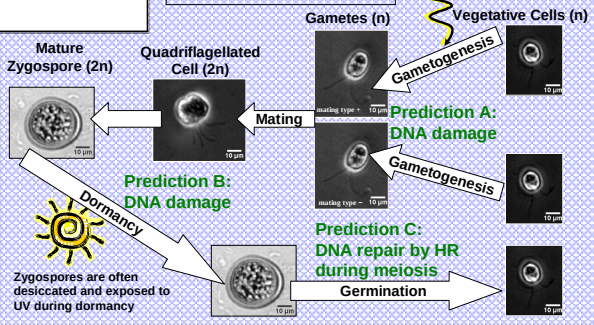
Stress induces gametogenesis

For *C. reinhardtii*, predictions based on our hypothesis include:

(A) DNA damages could be a cue for signaling asexual individuals to become gametes (gametogenesis)

(B) DNA damages accumulate in zygospores

(C) Homologous recombination (HR) during meiosis repairs DNA damage prior to germination of zygospores



Our objectives are to:

(1) Confirm the utility of the Comet Assay for detecting DNA damage in *C. reinhardtii* vegetative cells by showing a dose-dependent response to H_2O_2

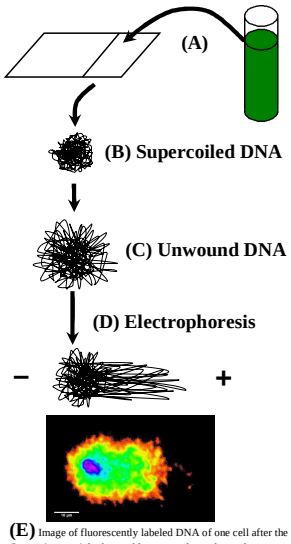
(2) Test **Prediction A** by assaying for DNA damage in gametes

(3) Modify Comet Assay conditions as necessary for use with zygospores

(4) Test **Predictions B and C** by assaying for DNA damage in zygospores and in newly germinated vegetative cells

3. Methods

We used the Comet Assay to detect DNA damage



(E) Image of fluorescently labeled DNA of one cell after the Comet Assay. A look-up table was used to indicate the intensity of fluorescence in color (highest intensity is violet; lowest is red)

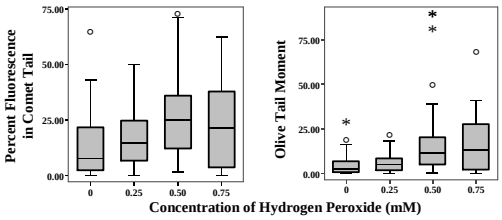
- The Comet Assay:
  - o Detects the level of DNA damage in a treated population of cells by measurements made on single cells (Singh *et al.*, 1988)
  - o Is commonly used on mammalian cells, but requires technical adjustments to be used with *C. reinhardtii* (Erbes *et al.*, 1997)
- The procedure includes:
  - **(A)** Suspended cells are embedded in agarose and spread in a thin layer on a slide
  - **(B)** Cells are lysed to produce nucleoids (supercoiled loops of DNA)
  - **(C)** Slides are incubated at high pH to partially unwind the DNA
  - **(D)** Slides are electrophoresed at high pH, creating "comets." Comet tail (DNA migration) is proportional to the level of DNA damage
  - **(E)** DNA is stained and comets are viewed on an epifluorescent microscope and photographed
  - **(F)** Comet metrics are calculated and compared among treatment groups (see right panel)

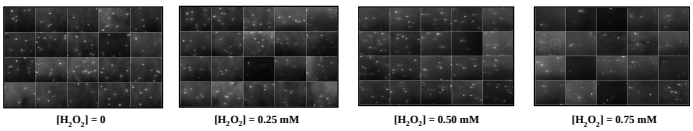
4. Preliminary results

Objective 1: Show that the Comet Assay effectively detects DNA damages in *C. reinhardtii*

- Vegetative *C. reinhardtii* cells were exposed to H_2O_2 for 2 hours at room temperature
- We used a Trevigen® Comet Assay kit with some modifications to the standard protocol, notably different cell lysis conditions
- DNA damages are indicated by higher "Fluorescence in Tail" and "Olive Tail Moment" values. Box plots show medians, IQR, and outliers. Number of cells scored per treatment was: 53, 58, 39, and 34, in order of increasing $[H_2O_2]$
- For both metrics, means were significantly different ($\alpha = 0.05$) from the control mean for $[H_2O_2]$= 0.50 and 0.75 but not for $[H_2O_2]$= 0.25 (Dunnett's Test)

Images were taken with a Nikon Eclipse TE300 epifluorescent microscope and Photometrics CoolSnap *fx*™ CCD camera using ImagePro™. Images were scored for several metrics using CometScore™. Statistics and graphs were produced using SPSS 12.0.1.





5. Conclusion and future direction

Our results indicate:

- The Comet Assay is effective for detecting DNA damages in *C. reinhardtii* vegetative cells
- Distinct lysis conditions are necessary to use the Comet Assay with algal rather than mammalian cells
- *C. reinhardtii* is a useful model organism for testing the DNA repair hypothesis for the evolution of sex

What's next?

- Objectives 2-4: assaying for DNA damages during various life stages of *C. reinhardtii*
- Technical challenges will include lysis of mature zygospores and developing methods to standardize Comet Assay results from spores and vegetative cells. Suggestions on these topics would be greatly appreciated

6. References

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7. Acknowledgements

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