

DNA damage accumulation in zygospores of a facultatively sexual green alga, *Chlamydomonas reinhardtii*

Deborah E. Shelton¹, Harris Bernstein², and Richard E. Michod¹

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

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

SIGNIFICANCE

- Sex, or genetic recombination between distinct individuals, is nearly universal
- Dramatic costs of sex exist, for example:
 - Lowered relatedness to progeny
 - Time/energy to find/secure a mate
 - Risk of exposure to pathogens
- What benefits drive the ubiquity of sex?
 - Production of highly variable offspring?
 - Homologous recombinational repair of DNA?
- In spite of much scientific attention, the benefits of sex and their implications for evolution are still debated

BACKGROUND

- Facultatively sexual species (FSS), such as *C. reinhardtii*, must gain a short-term benefit from sex¹
- Demonstrating the benefit of sex in FSS is relevant to the more general question of the adaptive value of sex
- The DNA repair hypothesis posits that the adaptive value of sex is homologous recombinational repair (HRR) of damaged DNA during meiosis²
- DNA repair can have a large impact on offspring fitness
- This project will test whether *C. reinhardtii* DNA damages fit the pattern predicted by the DNA repair hypothesis (i.e., accumulation of damage during dormancy of diploid zygospores and repair of damage by HRR during meiosis just prior to germination)

METHODS

- *C. reinhardtii* zygospores will be produced by exposing mature cells to nitrogen deprivation and mixing gametes of each mating type
- Zygospores of increasing ages will be assayed for damage
- Single-cell gel electrophoresis³ (comet assay) will be used to assess DNA damage

(1) Cells are embedded in agarose and spread in a thin layer on a slide

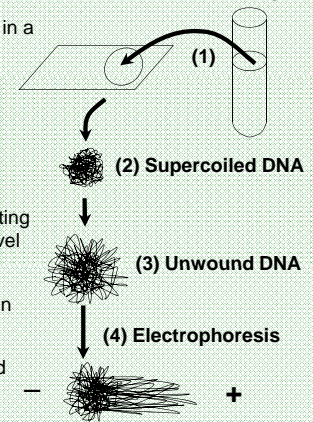
(2) Cells are lysed to produce nucleoids (supercoiled loops of DNA)

(3) Slides are incubated at high pH to partially unwind the DNA

(4) Slides are electrophoresed at high pH, creating "comets." Comet tails are proportional to the level of DNA damage

(5) DNA is stained and comets are viewed on an epifluorescent microscope and photographed

(6) Comet metrics are calculated and compared among treatment groups



RESULTS

- We are in the process of adapting the comet assay protocol for use with algal cells
- Preliminary data indicate that the method is applicable to algal cells, but requires further optimization based on the biology of these organisms

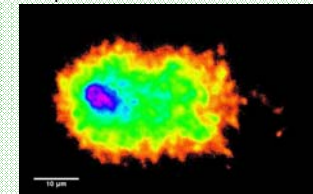


Image of a nucleoid produced by alkaline comet assay of a *C. reinhardtii* cell. A single-hue image was taken on a Nikon Eclipse TE300 epifluorescent microscope with a Photometrics CoolSnap fxTM CCD camera using ImageProTM. A fluorescein filter was used to view the slide, which was stained with a fluorescent DNA-intercalating dye, SYBR[®] Green I. To display the edge of the comet more clearly, grayscale levels were converted to use the full visual spectrum (with a look-up table) in TriTek CometScoreTM.

REFERENCES

1. Williams, G. C. (1975) *Sex and evolution*. Princeton University Press, Princeton.
2. Bernstein, H., H. C. Byerly, F. A. Hopf, & R. E. Michod (1985) Genetic Damage, Mutation, and the Evolution of Sex. *Science* **229**:1277-1281.
3. Singh, N. P., M. T. McCoy, R. R. Tice, & E. L. Schneider (1988) A Simple Technique for Quantitation of Low Levels of DNA Damage in Individual Cells. *Experimental Cell Research* **175**:184-191.

FURTHER READING

- Erbes, M., A. Wessler, U. Obst, & A. Wild (1997) Detection of primary DNA damage in *Chlamydomonas reinhardtii* by means of modified microgel electrophoresis. *Environmental and Molecular Mutagenesis* **30**:448-458.
- Michod, R. E. & B. R. Levin, eds. (1988) *The evolution of sex, an examination of current ideas*. Sinaur, Sunderland, MA.
- Nedelcu, A. (2005) Sex as a response to oxidative stress: stress genes co-opted for sex. *Proc. R. Soc. B* **272**: 1935-1940.

ACKNOWLEDGEMENTS

We thank M. Herron and J. Clarke for helpful advice and thank C. Crowley-Skillcorn and K. Dvorak for technical assistance.

C. reinhardtii Life Cycle

