Coprinopsis cinerea

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What is* Coprinopsis cinerea*? *Coprinopsis cinerea* is a small mushroom that has attracted the attention of geneticists and cytologists — and also, apparently, taxonomists, as the species has undergone five name changes in the last 30 years, most recently from *Coprinus cinereus* to *Coprinopsis cinerea*. It lies at the larger end of the 100,000-fold size range in the fungal kingdom, members of which include the microscopic yeasts and the giant toadstools. Even mushrooms of more modest size are capable of exerting forces sufficient to displace overlying flagstones, forces which Reginald Buller measured in his wide-ranging investigations in the early 20th century. Unlike the vast majority of mushrooms, *C. cinerea* is easy to culture in the laboratory on defined media. Thus it is not surprising that it has emerged as the model organism for large multicellular fungi — the larger members of a kingdom which has provided deep insights into eukaryotic biology through work on budding yeast *Saccharomyces cerevisiae*, one of the key 'genetic model systems' of modern biology.

What is interesting about its life history? *C. cinerea* can complete its life cycle on a simple medium in just two weeks in the laboratory. Haploid spores germinate, and grow as filaments (hyphae). If filaments of compatible mating type fuse, a new growth pattern is established, the 'dikaryon', in which a single haploid nucleus from each parent is maintained in every cell. The dikaryon can respond to light and nutritional cues to form fruit bodies (Figure 1). Meiosis and spore formation occur in the gill tissue — folded sheets of tissue in the mushroom caps. In addition to his studies of flagstone-lifting fungi, Buller wrote extensively about the light requirements for and synchrony of spore production in the genus *Coprinus*. He noted that the expansion and deliquescence of the cap facilitated spore discharge from the formerly vertical gills and also enforced the synchronous development, as all the spores would need to ripen before deliquescence is completed. The resulting black puddle of spores can actually be used as ink (a nice touch in a thesis dedication, for example), and thus mushrooms of this type are known as 'ink caps'.

What experimental advantages does it offer? The light regulation of synchronous development means that incubator lights can be timed to produce any desired stage (meiotic prophase, for example) at any particular time of day. Meiotic prophase occurs synchronously in the 10^7–10^8 basidia on the surfaces of the double-sided gills. This facilitates analyses of differential transcription during meiosis. The genome size (37 Mb) and chromosome number (13) make high-throughput sequencing as well as conventional cytology attractive. The extensive and accessible dikaryotic stage is also advantageous for analysis of meiotic mutants. Strains with mutant mating factors produce haploid spores, which can be further mutagenized. These strains form dikaryons and fruit bodies that are homozygous for the newly induced mutations. Meiotic mutants can be recognized as they lead to no spores (or dead spores). The desired dikaryon can then be mated to a compatible monokaryon to recover the recessive meiotic mutants in conventional genetic backgrounds for complementation testing and further analysis.

What have we learned using this model organism? *C. cinerea* is one of a small number of species in which whole-genome shotgun sequencing has enabled the production of telomere-to-telomere sequence assembly for the majority of the chromosomes. This analysis together with a high-resolution genetic map and improved sequence annotation revealed sub-telomeric hot spots for meiotic recombination that coincide with regions where meiotic synapsis initiates. The *C. cinerea* genome also exhibits a striking enrichment for single copy orthologs in regions with low rates of meiotic recombination, and an enrichment for paralogous multicopy genes in regions with high rates of meiotic recombination.

Figure 1. Mature *Coprinopsis cinerea* mushrooms fruiting in a deep Petri dish. (Photo courtesy of Hajime Muraguchi.)

Genes involved in meiosis are differentially transcribed, and there is a remarkable conservation of meiotic expression profiles in very distantly related fungi. Analyses of meiotic genes have revealed conserved functions, but also novel behaviors. For example, chromosome synapsis ordinarily occurs after DNA replication and depends upon the activity of Spo11 and Rad50, which are required to form and process DNA double-strand breaks; however, if DNA replication is prevented by the introduction of an additional mutation (*spo22*), extensive synapsis occurs. Such conditional dependence of synapsis on double-strand breaks has not been observed in other systems. *C. cinerea* has also contributed to our understanding of the genetic regulation of mating — particularly the structure and function of the mating type genes and their downstream targets — and mechanisms of photomorphogenesis, including analyses of genes involved in sensing blue light.

What else is being investigated? The *C. cinerea* genome project (http://genome.semo.edu/ccin) uncovered a family of protein kinases (FunK1) which is found only in multicellular fungi. Remarkably, 59 of the 133 FunK1 family genes are found in a single subterminal region, overlapping a recombination hot spot. The FunK1 kinases are differentially expressed at discrete stages of the life cycle. The genome project also revealed two genes for DNA methyltransferases of the DNMT1 family, which convert selected cytosines within the CpG sequence context to

5-methylcytosine. This specificity is unusual in fungi, but was predicted in prior studies of *de novo* methylation of repeated genes in *C. cinerea*. Recent attention has been focused on an additional modified base, 5-hydroxymethylcytosine, and its role in 'poised chromatin' in stem cells. The *C. cinerea* genome contains 38 copies of a gene predicted to carry out this conversion, and 5-hydroxymethylcytosine has been detected in the *C. cinerea* genome. It appears that this beautiful model organism will continue to contribute to our understanding of important problems in biology, as well as serving as a tractable system to facilitate desired manipulations of other basidiomycetes important for wood biodegradation (responsible for the return of 80 billion tons of carbon per year to the atmosphere), and perhaps bioremediation and alternative fuel production.

Where can I find out more?

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Q & A

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Rebecca Safran is currently an Assistant Professor at the Department of Ecology and Evolutionary Biology at the University of Colorado. She received her undergraduate degree from the University of Michigan (Ecology) and her graduate degrees from Humboldt State University (MS, Wildlife Biology in Mark Colwell's lab) and Cornell University (Ph.D., Ecology and Evolutionary Biology in David Winkler's lab). She worked with Daniel Rubenstein as a postdoc at Princeton University from 2005 to 2008. Her work on barn swallows examines interactions between phenotype and behavior and how these shape patterns of variation. She is particularly interested in the role of sexual selection in adaptation and reproductive isolation.

Why did you become a biologist? I think I have been a biologist all my life — I just didn't know it for sure until I was an undergraduate student. I had previously intended to be a writer — I spent much of my childhood dedicated to writing and peddling a magazine of my own creation around the neighborhood. My first independent purchase was a typewriter so that I could set up a little office in my bedroom. The true wake-up calls came much, much later, during my undergraduate days at the University of Michigan. The first 'a-ha' moment was a plant taxonomy class which was absolutely mind blowing! Through this class and its weekly field trips, my eyes were opened to biological diversity in a different way than they had been before. I became quite passionate about identifying plants and eventually all living things. An entire summer spent at the University of Michigan's Biological Station sealed the deal. Here, there were biologists of all flavors — each more passionate and excited than the next. The next question then became: yes to biology, but what kind? I think I am still working on an answer to that question — because I love so many different aspects of scientific inquiry. Still, I don't feel that my time spent on the art of writing was a waste. Contrary to popular stereotypes, I think of science as a very creative endeavor. Thinking outside the box is rewarded and designing elegant experiments requires being resourceful and innovative. Perhaps this is why I so much enjoy a course I teach at the University of Colorado on film-making and the science of climate change.

If you were so excited by plants initially, why study birds? Aren't they really difficult to work with? I will admit to being fairly pragmatic in my choice of study organism for my dissertation work. Before starting my Ph.D. (and not knowing where I would end up), I thought about a system that was common, tractable, typically abundant, in which it would be easy to measure relevant features of ecology, behavior and evolution. I had a checklist of 'key features' and several field guides to thumb through. Everything kept pointing to the birds. Their nest sites are discrete locations where a lot of important measurements can be collected quite easily and they are more tractable than one would think, especially if you are interested in reproductive behavior.

What is the best advice you've been given? Something my graduate adviser David Winkler once said to me has stuck: "*biology is 24–7, not 9–5, so you have to really live and breathe it.*" This came from someone who works hard and has also never lost a child-like curiosity for all things biological. I took this advice to mean something like: embrace this job as your lifestyle. If it feels like 'work' or a 'job', it is probably time to think about doing something else. I still feel like I learn something new and fascinating about biology each week; and things that I have now experienced dozens of times — marked birds returning to breed in the same locations year after year — never cease to fascinate me.

Speaking of lifestyles, what about work–life balance? As a female scientist with two young children, I get asked this question a lot. Lately, I have come to realize that there is no such thing as a work–life balance. You do what needs to get done. Sometimes that means picking up a sick child from school and canceling work plans and sometimes that means staying late at work to meet a