Minireview

The genome sequence of *Podospora anserina*, a classic model fungus

Mathieu Paoletti and Sven J Saupe

Address: Laboratoire de Génétique Moléculaire des Champignons, IBGC UMR CNRS 5095, Université de Bordeaux 2, rue Camille Saint Saëns, Bordeaux, F-33077 France.

Correspondence: Sven J Saupe. Email: sven.saupe@ibgc.u-bordeaux2.fr

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Abstract

The completed genome sequence of the coprophilous fungus *Podospora anserina* increases the sampling of fungal genomes. In line with its habitat of herbivore dung, this ascomycete has an exceptionally rich gene set devoted to the catabolism of complex carbohydrates.

Fungi represent a vast and highly successful branch of the Eukaryota. Yet the fungal kingdom is invariably overshadowed by the animal and plant kingdoms in the minds of the general public and scientist alike. All too often, even biology students are uncertain about the evolutionary position of the Fungi, which for gastronomic reasons are sometimes equated with plants. Direct contact with the fungal world, in the form of a brightly colored poisonous mushroom or a moldy crust of bread, more often inspires disgust than actual interest. Genomics, however, is one field in which fungi do very well. The discipline started with the pioneering effort on the yeast *Saccharomyces cerevisiae*, and, with more than 40 fully sequenced species, fungi have the greatest number of sequenced genomes of any branch of the eukaryotes [1]. This densely knit network of sequences provides a unique opportunity for comparative genomics and holds great promise in helping to understand how sequence defines phenotype and how evolutionary events shaped the organisms that make up our biosphere.

Podospora anserina: a classic model fungus for genetics

In this issue of *Genome Biology*, Espagne *et al.* [2] publish the genome sequence of *Podospora anserina*, a joint effort between the *Podospora* research community and Genoscope, the French National Sequencing Center. *P. anserina* is one of the most recent additions to the constantly growing collection of fungal genomes [2], but it has been around as a fungal genetic model for quite a while, having been introduced in the 1930s by the late French geneticist Georges Rizet. *Podospora anserina* is a coprophilous fungus inhabiting the dung of various herbivores such as rabbits, goats or horses. In contrast to other popular fungal models such as *Aspergillus* and *Neurospora*, it lacks asexual reproduction and it is strictly dependent on the sexual cycle for production of the resistant form, the ascospore. The presence of an appendage on these ascopore spawned the name of the genus: *Podospora*, spore with a foot. The sexual cycle can be completed in as little as a week and typically produces bunches (rosettes) of four-spored asci (Figure 1). The ascospores are heterokaryotic (that is, they contain nuclei of different genetic constitution) and invariably contain nuclei of opposite mating-types; as a consequence, the mycelium germinating from an ascospore is self-fertile. The early work of Rizet on *P. anserina* introduced a particular emphasis on nucleo-cytoplasmic interactions and cytoplasmic inheritance. The discovery of the senescence syndrome, a cytoplasmically transmitted aging process, probably represents the first described example of cytoplasmic inheritance in fungi. Currently, *P. anserina* is used as a model species in the study of mating type, aging, cell death, genome conflicts, conspecific and heterospecific non-self recognition, and prion biology and structure.

Within the ascomycetes, *P. anserina* belongs to the sordariomycetes, a group that also includes *Neurospora crassa*, the rice pathogen *Magnaporthe grisea*, and the wheat pathogen *Fusarium graminearum*, all of whose sequences have been published [3-5]. The genome of an even closer relative, *Chaetomium globosum*, is also publicly

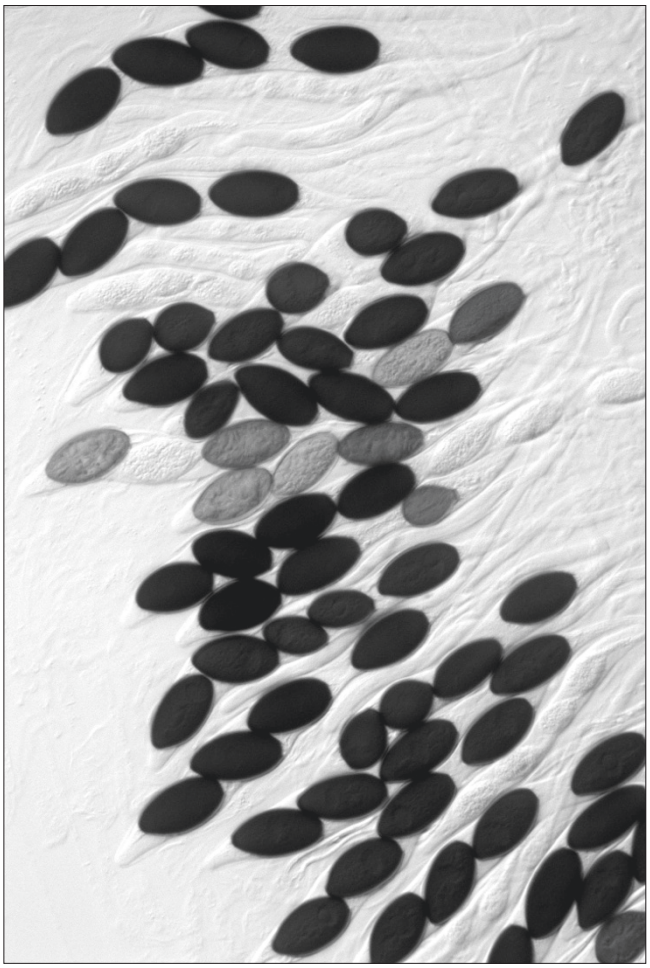

Figure 1
Ascospores of *Podospora anserina*. A micrograph of a bunch of *P. anserina* asci is shown. The asci contain four large ordered ascospores featuring a hyaline appendix that led to the designation of the genus. Ascospores constitute the resistant form of the fungus. Photograph courtesy of Henk Dalstra.

available but has not yet been published. The genome sequencing of *P. anserina* was prompted both by its phylogenetically interesting position as a close relative of *N. crassa* (the red bread mold) and the existence of a body of fundamental research on its biology spanning seven decades. *Podospora* never attained the general popularity of *Aspergillus* or *Neurospora*, but has been extensively studied as a fungal model, especially in France and Germany. It is also the first coprophilous fungus for which the genome is available, and as such, might serve as a proxy for many other species of this extremely diverse and ecologically important group.

Markers of fungal evolution

A basic trend revealed by comparative fungal genomics is the high divergence in sequence of species that appear morphologically or even phylogenetically closely related. For instance, the genomes of the three *Aspergillus* species *A. nidulans, A. fumigatus* and *A. oryzae*, revealed only 68% average sequence identity between any species pair [6]. Even more strikingly, extensive sequence differences can be found between two isolates of the same species [5,7]. The genome published by Espagne *et al.* [2] illustrates the trend towards divergence. The authors mainly emphasize the comparison with *N. crassa*. On average, sequence conservation of orthologous genes between *N. crassa* and *P. anserina* is only 60.5%, and roughly a quarter of predicted proteins in *P. anserina* lack orthologs in either *N. crassa, M. grisea or A. nidulans*. These numbers serve to remind us that the evolutionary distance separating these seemingly closely related organisms is on the order of the distance between fishes and humans.

Another feature of fungal genome evolution is the important and frequent chromosomal rearrangements that occur through random breakages [1]. Analysis of syntenic blocks of orthologous sequences between *P. anserina* and *N. crassa* again highlights this tendency. Short blocks of synteny are more frequent than long ones, fitting with the model of random breakage. A surprise, however, is the fact that rearrangements in *P. anserina* and *N. crassa* appear mostly intrachromosomal, as revealed by the high conservation of chromosomal gene content. This is in contrast with previous observations in *Aspergillus* genomes, where syntenic blocks are spread over all chromosomes between the three compared species [6]. As Espagne and co-authors point out, this observation may relate to the heterothallic life-style of *N. crassa* and *P. anserina*, which requires the pairing of homologous chromosomes from different nuclei; this would not suit well with interchromosomal rearrangements. Significantly, a single syntenic block encompassing 37 orthologous gene pairs stands out from the random-breakage model. This is the largest syntenic block shared by *P. anserina* and *N. crassa* and is centered on the mating-type locus, which controls sexual development. This supports the idea that recombination is restricted around sex-controlling loci, as noted in *Aspergillus* and *N. tetrasperma* [6,8]. In the latter species, recombination is actually suppressed over most of the chromosome carrying the mating-type locus. This peculiarity appeared after the split between *N. crassa* and *N. tetrasperma*, and it is suggested that in *N. tetrasperma* we might be witnessing the early steps in the evolution of a proper sex chromosome [8].

Overall, repeated sequences are rare in filamentous fungi compared with plant or animal genomes. This might in part be due to the existence of genetic mechanisms aimed at the inactivation of mobile genetic elements, such as repeat-induced point mutation (RIP), meiotic silencing of unpaired DNA (MSUD) and 'quelling', all originally described in *N. crassa*. RIP operates during the sexual cycle and heavily mutates and methylates both copies of any repeated sequence as long as it is longer than about

800 bp [9]. *P. anserina* displays the full fungal arsenal against mobile genetic elements, as all the genes required for RIP, MSUD and quelling appear functional. So far, however, only RIP has been demonstrated in *P. anserina* in laboratory conditions [10]. Despite this equipment, the *P. anserina* genome does have repeated sequences. Gene families generated through duplications have evolved in *P. anserina*, despite the fact that these duplications could be potential targets for RIP mutagenesis. It would thus appear that the *P. anserina* genome evolved through a history of transpositions, duplications and gene losses, accompanied by a low level of RIP that preserved it against transposons (as most are inactivated by RIP) while possibly increasing the divergence of copies of duplicated genes [11].

Adapting to the environment

As a coprophilous fungus, *P. anserina* grows exclusively on herbivore dung. This is an ecologically rich microcosmos where, alongside dozens of fungal species, bacteria, animals and plants are also represented. Coprophilous fungi typically appear in a phylogenetically determined succession during dung degradation. Typically, zygomycetes come first, followed by ascomycetes, which finally leave the last crumbs of the feast to the basidiomycetes. One study on game animal dung from the Kruger National Park in South Africa identified a succession of 106 species belonging to 23 genera over a period of 112 days [12]; thus, competition must be fierce for both resources and territory. *P. anserina* appears to be one of the last of the ascomycetes to reach its ecological peak (the time when it becomes predominant) in this habitat, and by then the simple carbohydrates are depleted. Espagne *et al.* [2] show that to exploit the limited resources, *P. anserina* possesses formidable enzymatic tools for degrading complex biopolymers, including enzymes that potentially can degrade cellulose/hemicellulose, xylan and even lignin. The authors report that the ability of *P. anserina* to grow on media containing different complex carbon sources is in line with the existence of this complex enzymatic tool-box. At the same time, *P. anserina* has lost the enzymatic potential to degrade 'easier' carbohydrates such as sucrose. This is in sharp contrast to the enzymatic equipment of the ectomycorrhizal basidiomycete *Laccaria bicolor*, which has lost many enzymes that degrade plant cell walls, presumably to avoid harmful damage to its plant host during symbiotic development [13]. The *P. anserina* enzymatic equipment is unique among the ascomycete genomes sequenced. In certain aspects, *P. anserina* even rivals basidiomycetes of biotechnological interest, such as the wood-degrading fungus *Phanerochaete chrysosporium*, which causes white rot [14]. It thus appears that *P. anserina*'s life-style on dung promoted the development of a copious assortment of enzymes to degrade complex biopolymers. This rich gene repertoire might potentially turn *P. anserina* into a viable alternative or complement to the white-rot basidiomycete fungi in biotechnological applications such as bioremediation or industrial biomass processing [15].

For better or worse, we depend for much of our biological knowledge on a handful of model organisms. While the benefits of *S. cerevisiae* for modern cell biology are undisputable, it is now clear that it is a very peculiar organism and - in some aspects - not that good a model for other fungi or for eukaryotes. It is very fortunate that the field of fungal genetics has entertained a variety of models over the years rather than relying only on one superstar. The value of this diversification now comes to full bloom with the progressive entry of the field into the genomic era. The fundamental impact of comparative genomics is, and will certainly continue to be, considerable. The amusing surprise here with the work by Espagne *et al.* [2] is that *P. anserina*, originally selected as a tool for formal genetics, might in the longer run make an unexpected career in biotech.

Acknowledgements

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