CYTOFLUOROMETRY IN FUNGAL DIVERSITY AND TAXONOMY

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The purpose of this lecture is to provide some basic information on the Flow Cytometric analysis of nuclear DNA content and then a small review of the current status of the developing area in fungi.

Quantitative cytofluorometry, based on the application of DNA specific fluorochromes was introduced by Ruch (1966). By the late 1970s, Flow CytoMetry (FCM) was established as a more powerful technique for DNA content analysis. The major advantages over microdensitometry and static cytofluorometry are convenience, precision and rapidity. Tens of thousands of cells can be analysed within several minutes. The flow cytometer may be also equipped with a sorting facility allowing the possibility of selecting subpopulations of cells for further analysis or even culture. Such an instrument is often called a Fluorescence Activated Cell Sorter (FACS). During the early 1980s, FCM was introduced into the field of plant sciences and is now routinely used in many laboratories (Dolezel 1991, Marie and Brown 1993) and also by ORSTOM (Cros et al.1994).

FCM analysis of nuclear DNA content is based on the use of DNA-specific fluorochromes and on the analysis of the relative fluorescence intensity of stained nuclei. A typical flow cytometer consists of several basic components: a light source, a flow chamber and optical assembly, photodetectors and processors to convert light signals into analog electrical impulses and a computer system for the analysis and storage of digitalized data.

Fluorochromes such as DAPI (4',6 diamidino-2 phenylindole) and PI (Propidium Iodine) have many attractive properties as nuclear stain. DAPI binds selectively to AT-rich double-strand DNA. The fluorescence of DAPI-DNA is nearly proportional to the DNA quantity and only needs an UV light. PI is an intercalent dye and is fully proportional but needs a laser exitation. This dye also binds with double-stranded RNA and consequently needs a ribonuclease pretreatment. The determination of nuclear DNA content by FCM requires comparison with a reference standard.

For fungi, 3 majors uses were identified : 1. Ploidy level identification ; 2. Nuclear DNA content estimation; 3. Species identification and subpopulation separation.

1. Ploidy level identification. Martegani and Trezzi (1979) first mentionned the successful staining of nuclei in conidia and mycelium *of Neurospora crassa*. Staining with DAPI was investigated as an alternative to Feulgen staining procedure for zoospores of *Phytophthora infestans*. Isolates with approximately 4C values were shown to be tetraploid (Whittaker et al. 1991). The relative DNA content of different species and discussed ploidy levels were studied within the genus *Armillaria* (Motta et al. 1986). Three levels of ploidy were determined within *Pleurotus* (Bresinsky et al. 1987) and for thirteen species of *Coniophoraceae* (Meixner and Brezinski 1988).

2. Nuclear DNA content estimation. FCM and IP were used to estimate the relative nuclear DNA content of pycniospores of 85 collections of 13 species of rust fungi (Eilam et al. 1994). For each sample 10,240 fluorescent events were measured and the peaks were of good quality (CV<10%). DNA content, relative *to Puccinia graminis*, gave a large level of diversity : *P. lagenophorae* (53% - 67 Mbp), *P. recondita* (105%), *P. allii* (164%) and *Uromyces appendiculatus* (346% - 418 Mpb). It is also important to note that DNA content genetically

diverge witin the same species according to the host species (i.e. *P. hordeum* on different species of the genus *Hordeum*).

Fungi have been shown to contain some forms of repetitive DNA sequences other than ribosomal repeats. For example, the genome of *Bremia lactucae* and obligate fungal pathogen has a genome size of about 50 Mbp of DNA and 65% of the nuclear DNA is repeated (Francis et al. 1990).

3. Species identification and subpopulation separation. The light scattering properties in addition to the DNA contents of spores could also be useful. With the spores of five basidiomycetes, the forward and wide angle light scatter and the DNA amount are enough for discrimination. In addition sub-populations were evident in samples of *Megacollybia platyphylla* and *Fuligo septica* which would not have been detected using conventional techniques (Allman 1992). Bianciatto and Bonfante (1992) shown that *Glomus versiforme* and *Gigaspora margarita*, two arbuscular mycorrhizal fungi possess nuclei with significantly different diameters and DNA contents.

It seems that the FCM technology, when used in conjunction with standards of DNA, will provide an efficient method genetic analysis and systematics of fungi.

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