How to stain meiotic chromosomes using acriflavine.

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Background

Aceto-orcein staining is very good for pachytene chromosome analysis but does not stain spindles, spindle pole bodies, or the nucleolus satisfactorily. Hematoxylin is excellent for routine nuclear cytology of Neurospora and other fungi but is less useful than aceto-orcein for pachytene analysis because fine chromomere detail is not as clearly revealed or because the large dark-staining nucleolus may obscure underlying chromosomes. The Feulgen procedure using conventional Schiff's reagent has not proved useful for examining chromosomes of Neurospora and most other fungi, in part because of faint staining. Of various fluorochromes used by Raju for staining fungal nuclei (DAPI, Hoechst 33258, olivomycin, auromine O, and acriflavin), only acriflavin is especially suitable for staining meiotic chromosomes (Figures 1 and 2). Because acriflavin is DNA-specific, the nucleolus is transparent and strands of chromatin can be seen extending through it to the satellite (Raju 1986; Perkins *et al*. 1995).

Procedure

The protoperithecial culture is fertilized after 5 days incubation at 25º C on synthetic cross medium (2% sucrose, 2% agar) thinly poured in a petri plate. Between 4 and 6 days after fertilization, strips of agar bearing developing perithecia were cut out and the unfixed samples were processed for staining with acriflavin. The staining protocol, adapted from Tanke and van Ingen (1980), resembles the Feulgen pararosaniline-SO_2 procedure. Unfixed intact perithecia on the thin agar strips are hydrolyzed in 4 N HCl for 20-30 min at 30º C, rinsed once in water, and stained in a solution containing acriflavin (Sigma. 100-200 μg/ml) and $K_2S_2O_5$ (5 mg/ml in 0.1 N HCl) for 20-30 min at 30º C. The stained perithecia are washed three times (3-5 min each) in a concentrated HCl-70% ethanol mixture (2:98 v/v at 30º C), which removes the non-covalently bound stain from cells, and twice in distilled water. Perithecia are dissected in a drop of 10% glycerol and the asci are squashed under a cover glass as described under '*How to use hematoxylin for cytological studies*'.

The stained asci are examined with an epifluorescence microscope employing an HBO 100 mercury lamp or a quartz-halogen lamp. The excitation filter/dichroic beam-splitter/barrier-filter combination was chosen for excitation at about 450 nm and emission at 540 nm. Observations are made at a magnification of 500× or higher. General information on fluorescence microscopy, and photography is given in Raju (1982). See also "*How to use GFP-tagged proteins in cell biology*".

References

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Tanke, H. J., and E. M. van Ingen. 1980. A reliable Feulgen-acriflavin-SO_2 staining for quantitative DNA measurements. J. Histochem. Cytochem. 28:1007-1013.

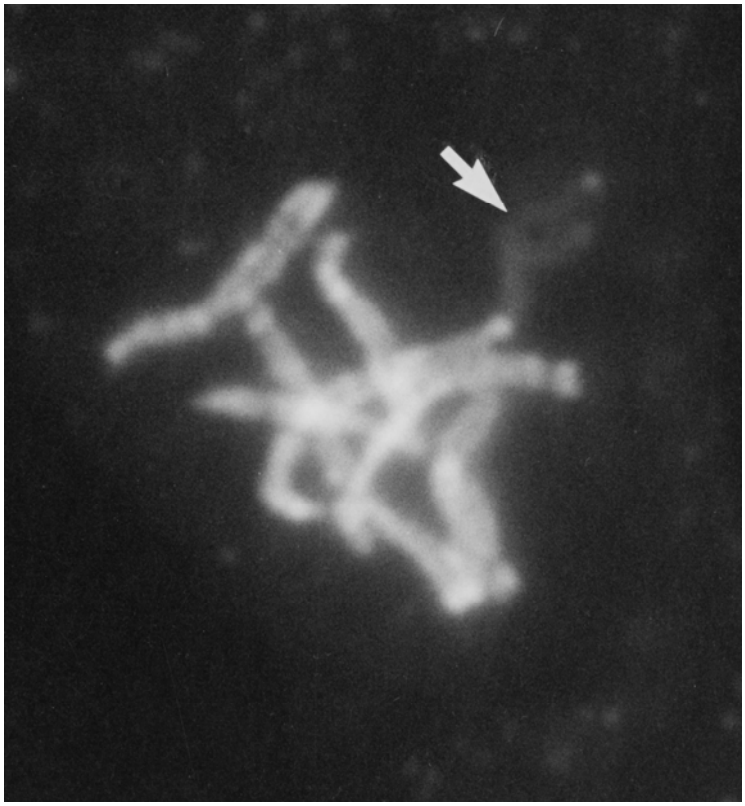

Fig 1. *N. crassa* x *N. intermedia*. Pachytene chromosomes made visible with the DNA-specific fluorochrome acriflavine and fluorescence microscopy. The ribosomal RNA- and protein-rich nucleolus does not fluoresce at all, but the nucleolus organizer (rDNA) is clearly seen passing through the “unstained” nucleolus. Photo credit: N.B. Raju.

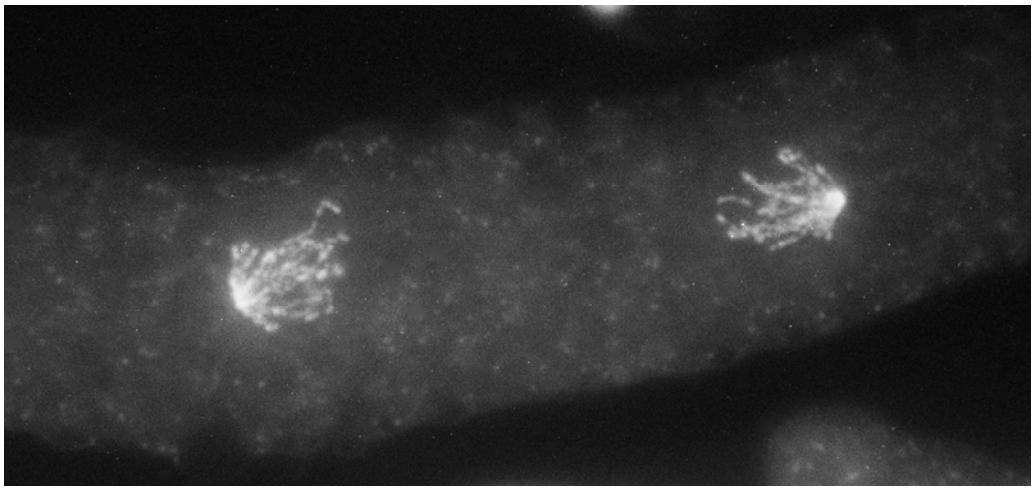

Fig 2. *N. crassa* wild type. Interphase I nuclei following meiosis I; chromosome strands are clearly resolved. Photo credit: N.B. Raju.