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Abstract

Cytological techniques for meiotic chromosomes

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The following procedure has given satisfactory preparations of meiotic chromosomes of Neurospora crassa. Good results are obtained almost routinely and occasional excellent preparations are found. Procedures are described in detail because some of our techniques may be unorthodox or because we do not know in many cases whether a particular feature contributes to good results or whether it is irrelevant.

Crosses and timing: Crosses ore made by growing one parental strain (usually wild type) as protoperitheciol parent on 15 to 20 ml of synthetic crossing medium (Westergaard and Mitchell 1947) with 2% sucrose and 2% agar in petri Plates at 25° C. At 6 days the surface of the protoperitheciating culture is dusted with conidia (or scrubbed with hyphol fragments) from the fertilizing parent. To determine closely when to begin fixing material, a few perithecia may be picked from the cross beginning 3 days after fertilization and squeezed into a drop of water under a dissecting microscope to determine whether young asci are present. Usually, pachytene stages are first found 3 to 4 days after crossing, and condensed chromosome stages (diakinesis and later) are abundant from 4 days on. Material is fixed usually at 4, 5, and 6 days after crossing; for observations, the sample in which the desired stager are most abundant is wed.

Fixation: Strips of agar bearing perithecia are cut from the petri plates and immersed in freshly made fixative-d vials or IO X 75 mm tubes stoppered with No. 0 corks. The fixative consists of 6 parts absolute alcohol, 1 part glacial acetic acid, and 1 part 85% lactic acid. The fixed material is kept in a deep freeze or the freezer compartment of a refrigerator. Although some fixed material stored for two years has given good quality preparations, there is a suggestion of degeneration with time. For best results it is recommended that the fixed material not be stored more than 2 or 3 months.

Stain: Successful staining requires that fresh stain be made each day. It is convenient to make up the stain components in bulk. Liquid components of 47 ml glacial acetic acid, 20 ml lactic acid solution (1 ml 85% lactic acid in 24 ml distilled water), 5 ml 1 N hydrochloric acid, and 28 ml distilled water are mixed and stored at room temperature. Natural orcein (Edward Gurr, Ltd., 42 Upper Richmond Rd. West, London, S.W. 14, England) is weighed out into 10 X 75 mm tuber in 100 mg lots. For each 100 mg batch, 5 ml of the liquid portion of the stain is mixed with 100 mg orcein and refluxed (in a 50 ml beaker covered by a petri plate bottom containing two ice-cubes) for 4 minutes after beginning to boil over a low flame (alcohol lamp or Bunsen burner pilot). The stain is cooled for 2 hours in the melting ice-cube water in the petri bottom, and filtered through #1 Whatman filter paper into a small dropping bottle.

Dissection of perithecia; preparation of slides: Perithecia are taken from the fixative, scraped from the agar block, and placed in a drop or two of stain on the frosted end of a carefully cleaned slide (Aloe #58952) under a dissecting microscope (about 30X) with transmitted light from below. Mycelial fragments and remnants of agar are scraped with a pair of dissecting needles from about ten perithecia, and the perithecia are transferred to a fresh, small drop of stain near the middle of the slide. The contents of each perithecium are expressed through the ostiole into the stain using a slightly flattened and bent or chisel-shaped steel needle (about #1 embroidery needle). Perithecial walls and other debris are removed. Large clumps of asci, if present, are teased or chopped apart, or even removed. The best staining is found in separated and broken asci, and these are most readily obtained when the perithecial contents squirt through the ostiole rather than when a clump of asci emerges intact from a broken perithecium. A ring of

crystallized stain often forms during this operation, and later may cause difficulty by holding up the coverglass. We have used two methods to avoid the Problem: either the dried stain is put buck into solution by a rapid mixing with the needles, or fewer perithecia are used, the work goes foster, and the smaller amount of Precipitate does not interfere when the cover is lowered. A coverglass is dropped over the stain and permitted to flatten the asci without other external pressure. 18 mm square No. 1 coverglasses are convenient, and are less readily broken than No. 0.

When a small drop of stain has been used, and no debris or dust particles are present, a thin preparation results in which some asci moy even burst from the pressure when the coverglass is drawn down by surface tension as the stain spreads under it. If excessive numbers of bubbles are trapped under the coverglass, these are sometimes tapped out gently with the dissecting needle. The slide is heated over on alcohol lamp to increase contrast between cytoplasm and chromosomes. Underheating will give poor definition of chromosomes and "glassy" or "muddy" staining. Overheating results in boiling the stain and great contraction of the chromosomes, although it does also cause the asci to burst (a desirable event). Roughly, a slide is heated to the right degree if it stings when touched lightly to the back of the hand. After heating, the slide is sealed with dental wax (suggested by K.W. Cooper; preferable to other sealing compounds).

The temporary preparations may keep a week or longer if stored in the refrigerator when not under observation. If the preparation is too lightly stained, the intensity of staining may increase after a few hours or days at room temperature. Overstaining of a particularly good figure has sometimes been successfully corrected by gently heating the slide or by running a small quantity of the liquid component of the stain under the coverglass.

Observations; optical equipment and photography: Slides ore scanned under low power (100 or 120X). At this magnification, one soon becomes practiced at recognizing asci and ascus fragments containing nuclei worthy of study. For each such ascus, we then go directly from the 10X to the parfocal 90X oil immersion objective. (The high, dry lens is never used.) For critical observations, the slide is oiled to the condenser. A first-surface mirror is used. Critical alignment and illumination are essential.

Our observations have been mode with a Spencer research microscope equipped with 12X Leitz periplan oculars, apochromatic 10X and N.A. 1.3 90X objectives, and N.A. 1.3 condenser, or a Leitz microscope with 10X periplan oculars, apochromatic 12X and N.A. 1.4 90X objectives, and N.A. 1.4 condenser. 16X and 25X oculars and a 110X apochromatic objective have proved useful as accessories in working on the fine detail of pachytene chromosomes. Illumination is with a Bausch and Lomb model PR 27 illuminator with ribbon filament, using a Corning 4-68 filter in combination with a second filter, either 4-67 or 4-97. Lamp and scope ore fixed rigidly to a Bausch and Lomb 2-foot long metal base. Photographs are taken on 35 mm frame-numbered Kodak High Contrast Copy film M 135-36 using a Leica M2 camera with Leitz micro-mirror reflex attachment mounted on a Leitz Aristophot stand with 34 cm bellows length. Exposures require about 9 seconds at magnifications around 1100X.

Strain differences: It is likely that the spreadability and stainability of the chromosomes, and other factors which affect the quality of the preparations, ore highly dependent on strain differences. We hove used extensively the Oak Ridge wild types 74-OR23-1A and 74-OR8-1a and other strains with the Oak Ridge or St. Lawrence background. Some segregants from crosses give better cytological results than others, and we have had some success in finding superior strains by selecting among progeny from backcrosses of strains that are known to possess good qualities for cytology. The presence of several classes of mutant markers among 100 or more strains examined has had no apparent relation to the quality of cytological preparations, nor has the presence of heterozygous rearrangements.

Vegetative nuclei: The same stain also has been used successfully to stain nuclei in hyphae or macroconidia. Conidia and hyphae are immersed directly in the stain on the slide without Prior fixing, covered with a coverglass, heated, and sealed. Such conveniently mode preparations are adequate for counting nuclei in macroconidia or con be used, with some strains, to check numbers of nuclei in microconidia. Fixation of conidia and hyphae prior to staining may improve nuclear staining in some strains.

Acknowledgments: The methods described are not original, but hove evolved in the hands of Barbara McClintock, Jesse R. Singleton, Patricia St. Lawrence, and David D. Perkins, whose basic methods have been adopted and modified.

Previous accounts of some aspects of cytological techniques con be found in references 850, M5, S2, S108, and 5109 in Bachmann and Strickland (1965 Neurospora Bibliography and Index, Yale University press, New Haven).

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