Asci of bis X bis crosses for chromosome cytology

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Abstract
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frequency of mature ascospores failing to germinate.

The data obtained thus far indicate that selection has been effective in increasing growth rate. Figures 1 and 2 show the progress attained through selection in two intra- and two inter-strain crosses. As can be seen from Figure 1 (two inter-strain crosses), considerably more progress was made in the cross Hon. la/Hon. 3A than in 77a/74A. This is not surprising, since 74A and 77a are highly inbred and improvement in growth rate in these lines in contrast to the other cross would seem more dependent on favorable mutation and less dependent on favorable recombination.

Slightly more progress was attained in the inter-strain crosses as shown in Fig. 2. In most of the inter-strain crosses studied thus far, the unselected progeny means of the original crosses are greater than those of the intra-strain crosses. This is not entirely true, however, in the four crosses included in figure 1 and 2 at 18°C. In addition, lower progeny means for the unselected original crosses were obtained at 35°C in each case where 74A was used as either parent due to one or more temperature-sensitive genes evidently present in this strain. These genes appear to be eliminated following one cycle of selection at 35°C.

Statistical analyses of the data indicate a lower genetic component of variance among the progeny as the number of cycles of selection increases. The rate at which the variance is reduced appears to be directly correlated with the type of cross involved (inter- or intra-) as well as with the strains used in the original crosses. A reduced variation among progeny accompanied by no significant increase in mean growth rate for several consecutive cycles should offer some indication of the extent to which selection, in the manner outlined, can be continued. Although all of the data have not yet been statistically analyzed, there appear to be no real differences between reciprocal crosses. Therefore, extra-chromosomal effects on growth rate in this study are either absent or too minor to detect. ---Department of Plant Breeding, Cornell University, Ithaca, New York.

Perkins, D. D. Asci of bis X bis crosses for chromosome cytology. In perithecia of crosses homozygous for the group-V morphological mutant bis: biscuit (B6), developing asci become greatly swollen, and early in growth assume the shape of pears or balloons. In squashes of bis X bis material, asci at pachytene and later stages are easily ruptured, and nuclei from the ruptured asci are greatly flattened merely by the pressure of a coverglass, so as to give workable figures and well spread chromosomes far more frequently than are ordinarily obtained using the same procedures with standard materials. (Paraphysoid cells are also swollen in crosses homozygous for bis.)

Chromosome behavior appears to be normal in Prophase I of B6 X B6 crosses, and pairing was normal at the most fully extended pachytene stage in one nucleus where all seven bivalents could be traced. Ascospores are not linearly arranged in maturing asci, however, and spore formation is frequently irregular and abnormal. Eight-spored asci are most frequent, but asci are found with four spores, with odd numbers less than eight, and occasionally with more than eight spores. Ascospore size varies. Giants and dwarfs are found. In some asci, nuclear material remains outside the spore walls.

These observations were made with two B6 strains of 74A background, Fungal Genetics Stock Center numbers 277 and 278. Observations are now being extended to homozygous crosses and intercrosses involving all available presumed bis alleles, including B6, B12, D12, B30, D312, C-1670 and C-1810-1, and to B6 X wild. (In crosses heterozygous for B6, asci are presumably normal. B6 has been widely used as a marker for investigating linkage and crossing over, with no evidence of aberrant behavior.)

J. C. Murray (1960. Cornell Ph.D. thesis) and Murray and Srbl (1962. Canad. J. Bot.) have reported that mature asci are non-linear in crosses that are homozygous for group V mutants C-1810-1 or C-1670, called pk-I: peak-I and pk-2. Intercrosses between C-1810-1 and bis (B6) indicate that the two are allelic (no wild-type recombinants were observed among B181 segregants by W. N. Strickland, unpublished). Murray and Srbl do not mention any irregularities other than the absence of linearity.

In homothallic fungi, a homozygous effect on ascus morphology would immediately be apparent in every selfed perithecium. Mutants have been reported in the homothallic Sordaria macrospora that resemble bis in their effect on ascus development. (Heslot 1958. Rev. Cytol. et Biol. Veg.; Esser and Straub 1958. Zeits. Vererb.) Possibly these, like bis, may prove favorable for chromosome cytology. In heterothallic
fungi a special effort must be made to determine recessive homozygous effects on ascus development. Most of the known morphological mutants in Neurospora have never been examined in this respect. Some of them might well prove to have favorable cytological effects. Our prior knowledge of the observations of Murray and Srb made bis a natural first choice in the search for strains that would be technically superior for the observation of meiotic prophase chromosomes. ---Department of Biological Sciences, Stanford University, Stanford, California.

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Turian, G. The hexosemonophosphate shunt as an alternate metabolic pathway for conidial differentiation in Neurospora. Considerable early stimulation of conidial differentiation (3rd day) without impediment for subsequent protoperithecial formation (9th day) has been observed in Neurospora crassa grown in the Westergaard and Mitchell liquid medium containing, in addition to its usual nitrate 0.1% and sucrose 2%, a supplement of $10^{-2} \text{M}$ Na citrate or succinate.

Furthermore, with Na malonate ($10^{-1} \text{M}$) as the organic acid source, protoperithecial differentiation is inhibited and thereby exclusively highly conidiated cultures are produced (Turian, G., Abstr. VIII Intern. Congress Microbiology 1962, A2.9).

The conidiogenous effect of these organic acids is suppressed when a mineral NH$_4^+$ salt such as (NH$_4$)$_2$HPO$_4$ is substituted for the usual nitrogen source, KNO$_3$. Thus, the nitrate reduction processes are in some way necessary for the morphogenetic action of the organic acids in the presence of sucrose.

In Neurospora crassa, grown in the presence of other organic acids than acetate, the isocitratase and therefore the glyoxylate cycle is either repressed (with citrate or succinate) or only slightly, endogenously induced (in the presence of malonate). On the other hand, the Krebs cycle through the deficiency of its succinic dehydrogenase step is for the most part inactive in the conidia (Zalokar, M., 1959, Amer. J. Bot. 46, 555; Turian, G., 1960, Path. Microbiol. 23, 687), so that heavy conidial differentiation can occur in the presence of malonate. Therefore, another oxidative metabolic pathway must function in the conidial formation induced by the triple combination nitrate - sucrose - organic acid.

The dependability of this process on the nitrate reduction mechanism (using TPNH+---TPN) was suggestive of the functioning of the TPN-mediated direct oxidation of glucose (from sucrose) or hexosemonophosphate shunt, the whole process and especially its initiation being stimulated by the additional presence of an organic acid as H$^+$ donor.

Preliminary enzymatic measurements, using conventional spectrophotometric methods on cell-free extracts of N. crassa, have confirmed this view. Up to 9 times enhancement of the activity of the glucose-6-phosphate dehydrogenase has been measured in the extracts of the heavily conidiated cultures from the nitrate-sucrose-malonate medium compared to the low dehydrogenase activity of the nitrate-sucrose controls. A similar high activity of the glucose-6-phosphate dehydrogenase has been detected in the young (3-5 days) conidial extracts of the citrate cultures. In the extracts of older citrate cultures (6-9 days), however, the slight succinic dehydrogenase activity measured seemed compatible with the subsequent initiation of protoperithecial differentiation. ---Institute for General Botany, University of Geneva, Geneva, Switzerland.