Partial Complementation in *Aspergillus niger* Heterocaryon

Auxotrophic mutants require essential growth factors for normal growth on minimal medium. However, non-allelic mutants can usually complement each other in heterocaryons or diploids[1-3] under favorable conditions and show the normal phenotypic features of the wild type, or approach them, on the same medium.

Rarely 2 genetically dissimilar nuclei in 1 unit of cytoplasm do not complement each other but only do so if combined in a diploid nucleus[2,3]. In my case it has been found that 2 auxotrophic mutants will complement each other in a heterocaryon by showing growth on minimal medium which approaches wild type but it will not conidiate at all.

Yellow mutant Y_1 of *A. niger* was produced by exposure to 2537 Å UV-light and 2 auxotrophic mutants requiring histidine (hist) and hypoxanthine (hypox) respectively were induced in the same way. The optimal supplementation to minimal medium for histidine and hypoxanthine was 0.3 mg/ml and 0.1 mg/ml respectively. Both mutants grew alone and responded with similar type of growth at the same age and temperature but the hist Y_1 mutant did not conidiate as heavily as hypox Y_1 mutant. The mean spore size of the 3 strains did not differ significantly. A forced heterocaryon was made between the 2 auxotrophic mutants by taking a heavily sporing suspension of each mutant and putting equal amounts in a flask of liquid minimal medium supplemented with their growth factors. The flasks were incubated for 5 days at 25 °C. A mesh of submerged mycelium developed in the flask. It was isolated, spread on the surface of minimal agar and incubated for nearly a week at 30 °C. At various places on the plate mycelium grew vigorously after incubation. Such mycelium was cut out, transferred to minimal medium and incubated for a further 3–5 days at 25 °C. Despite effective hyphal growth on subsequent incubation for at least 20 days no conidia were seen.

Confirmation that the mycelium was heterocaryotic was provided by transfer to complete medium. After incubation heavy yellow conidiation developed; single spore isolations were tested for nutritional requirements and it was shown that both hist and hypox nuclei were present.

Forty milligrams of D-Camphor flower (natural) was sterilized in a petri dish for 5 lbs at 10 min and then sterilized minimal medium was poured on the top[4]. Plates so prepared had the heterocaryon inoculated on them and were left under a glass cover at room temperature for a month. Only 2 plates showed conidia at a few points; they were transferred to minimal medium, germinated satisfactory, grew and conidiated. These conidia were presumed to be diploid, were isolated and tested by single spore culture, the measurement of the conidial size and by the treatment of the conidia with *p*-flurophenylalanine[5]. The result showed that these were true diploid. The volume of the individual conidium was not quite double than that of the parents conidia[4].

The partial complementation in heterocaryon observed in these experiments could be caused by any of the following possibilities.

It might possibly be due to a disproportion of nuclear types in the heterocaryon which would lead to insufficient nutritional cooperation but, as soon as sufficient nuclei fuse together to make diploids, they would overcome the nutritional deficiency in a limited area and a diploid could resume full growth with conidiation. A second possibility might be a dilution effect on a secondary gene product arising by interaction in the cytoplasm. Here again in diploid nuclei due to the physically closer association of the genetic material, their products could interact before they had become diluted in the cytoplasm[6].

Zusammenfassung. Erzwungene Heterokaryen-Vereinigung zwischen Hypoxanthin und Histidin abhängiger Mutanten von *Aspergillus niger*, die auf minimalem Nährboden ohne Sporenproduktion wachsen. Das Phänomen wird als teilweise Ergänzung bezeichnet und als Gleichgewichtsstörung der Kerntypen im Heterokaryen oder als Verdünnungseffekt eines Genproduktes zweiter Ordnung im Zytoplasma gedeutet.

I. PRASAD

Department of Microbiology, Medical College of Virginia, Richmond (Virginia 23219, USA), 25 November 1968.

1 G. PONTECORVO, J. A. ROPER and E. FORBES, J. gen. Microbiol. *8*, 198 (1953).
2 D. APIRION, Genetics *53*, 935 (1966).
3 C. F. ROBERTS, Aspergillus Newsletter *3*, 6 (1962).
4 J. A. ROPER, Experientia *8*, 14 (1952).
5 P. LHOAS, Nature *190*, 744 (1961).
6 L. A. CASSELTON and D. LEWIS, Genet. Res. *9*, 63 (1967).

Sex Ratio Alteration in Litter of Parents Submitted to Experimental Neurosis

There are some data concerning the effect of stressful situations on sex distribution of children born in the 9–12 months following the stress. SNYDER[1] observed that military pilots of high performance have much more girls than boys after combat-fighting; the sex ratio diminished from 105.37 of controls to 59.32, which means that nearly $^2/_3$ of the children were females. (Sex ratio = number of males born related to 100 females born in a given population.) According to these data, it seemed worth while to test the effect of anxiety on sex ratio in animals.

Wistar albino rats, both males and females, were submitted to experimental neurosis lasting 3 months, which was accompanied by overt signs of anxiety. As anxiety always goes side-by-side with a large output of adrenomedullar hormones[2], both epinephrine and norepinephrine were administered to rats for 3 months, in order to test whether the effect of experimental neurosis might be attributed to the output of these hormones. This supposition seemed to be possible, because norepinephrine has an effect on progeny: it significantly reduces the litter size[3]. As control animals partly conditioned but not

1 R. G. SNYDER, Hum. Biol. *33*, 1 (1961).
2 L. LEVI, Psychosom. Med. *27*, 80 (1965).
3 A. SAI-HALÁSZ, Experientia *21*, 155 (1965).