Parasexual Cycle in Dermatophytes

The work submitted presents for the first time proof of the existence of parasexual cycle in dermatophytes, fungi parasitic for man and animals. Our findings pave the way for solving a series of practically important problems by genetic analysis (genetic control of virulence, morphogenesis, etc.). The parasexual cycle includes: origin of heterokaryons; formation of heterozygous diploids; segregation and recombination at mitosis. Quoted hereinafter are brief proofs relating to all three phases of the cycle.

For the preparation of mutants we used the wild strain MG-155 (*a, cre*) of the dermatophyte *Microsporum gypseum* (Bodin) GUIART et GRIGORAKI 1928 (perfect st. *Nannizzia incurvata* Stockdale 1961). This strain is prototrophic and abundantly produces microconidia[1]. By means of UV-radiation we prepared 69 auxotrophs with colour markers[2,3].

With the help of a micromanipulator a direct proof of heterokaryosis was realized by isolating hyphal tips. Nutritional complementation on minimal medium and morphological complementation on complete medium (CM) was used to indicate indirectly the heterokaryotic constitution. Growing sectors originated more quickly during growth of the heterokaryons on minimal medium (MM). Data given testify to their diploid constitution.

The phenotype. Heterokaryons on MM are always noted for reduced growth rate and heterogenous morphology. Growth and morphology of diploids are identical both on MM and CM with the wild strain MG-155: cream and prototrophic colony with abundant microconidia. Heterokaryons easily dissociate into haploid components; the diploids, however, are constant.

Conidial size[4]. From each strain 100 microconidia were measured (Table). The length of the conidia is in all diploids significantly greater than in the haploid strain. The change in length, however, was not sufficient to allow the possibility of using it as a factor to determine ploidy in mitotic segregants. The width of the conidia remained unchanged.

Nuclear size. The nuclei were coloured by HCl-Giemsa[5]. The nuclear diameter was measured in twenty microconidia from each strain. As to diploids, the volume of the nucleus (calculated as volume of sphere) was 1.4–1.6 times greater than in the haploid strain (Table).

Mitotic recombinants. Heterokaryotic constitution is not transferred through microconidia. Only coloured colonies of both the auxotrophic components were found growing in conidial spreads. Most colonies with complementary phenotypes were formed in spreads from diploids. The frequency of segregants varies in accordance with the genotypes of diploids. From each of the 12 diploids under study, we obtained mitotic recombinants. On the examples in conidial spreads from diploids *yel bio/vio ino* (prepared from mutants 6/39 and 7/4) we isolated classes of segregants of the following phenotypes: 1. *vio*$^+$ *yel*$^+$ *bio*$^+$ *ino*$^+$; *vio*$^+$ *yel bio ino*$^+$; *vio yel*$^+$ *bio*$^+$ *ino*; 2. *vio*$^+$ *yel*$^+$ *bio ino*$^+$; *vio*$^+$ *yel bio*$^+$ *ino*$^+$; *vio*$^+$ *yel bio ino*; *vio*$^+$ *yel bio ino*$^+$; 3. the colonies of 2 new morphological types (marked m_1, m_2): m_1 *ino bio*; m_2 *ino bio*; m_2 *ino*$^+$ *bio*.

The occurrence of mitotic recombinants yields a convincing proof of diploid constitution. In *Microsporum gypseum*, heterokaryons and diploids very often originate under standard cultivation conditions. We surmise that this may be the case even with other species of these parasitic fungi.

Zusammenfassung. Die diploiden Stämme und die von ihnen gewonnenen mitotischen Rekombinanten beim Dermatophyten *Microsporum gypseum* werden beschrieben.

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Size of microconidia and nuclei in haploid and diploid strains

Genotype	Ploidity	Length of microconidia (μm)	Diameter of nucleus (μm)	Volume of nucleus (μm^3)
ino cre	*n*	3.7	1.1	0.73
bio och/ino yel	2 *n*	5.7	1.3	1.19
bio vio/ino bre	2 *n*	5.2	1.3	1.04
bio lye/ino bre	2 *n*	4.6	1.3	1.06
bio yel/ino cin	2 *n*	5.3	1.2	1.01
bio vio/ino lye	2 *n*	4.1	1.3	1.04
bio lye/ino cin	2 *n*	4.2	1.3	1.09

n, haploid; 2 *n*, diploid

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Stability of Tritiated Thymidine During Prolonged Labelling of Human Blood Leukocyte Cultures

Since 1960 tritiated thymidine has been used extensively for studying the replication of chromosomes[1]. The most widely used method of labelling chromosomes is a 20 min incubation with tritiated thymidine. Recently a prolonged labelling method in which cells are incubated for 50 to 72 h in medium with radioactive thymidine has been introduced[2–6]. The implication of studies using the prolonged incubation method is that little or no degradation of labelled thymidine occurred. However, to our knowledge, the only published data on stability of tritiated thymidine in cultures showed a rapid degradation of the label to tritiated thymine[7]. This study used suspensions of either dog bone marrow cells, dog thoracic duct lymphocytes or human leukemic blood in autologous se-