Grindle, M. and D. O. Woodward. Variations among extrachromosomal mutants of N. crassa.

The mi mutants of N. crassa have been characterized by their slow growth, excess cytochrome c, absence of cytochmmes a + a3 and maternal inheritance of the factors responsible for these phenotypic traits (Mitchell and Mitchell 1952 Proc. Natl. Acad. Sci. U.S. 38: 205; Mitchell, Mitchell and Tissieres 1953 Proc. Natl. Acad. Ssi. U.S. 39: 606). Some of the mi strains analysed recently in this laboratory, however, differ in both phenotype and breeding behavior from the strains described in the 1952 and 1953 reports. In view of this, the following data should be useful to anyone using mi strains in research programs or for clan demonstrations.

We obtained cultures of mi-1 A, -1a, -3A and -3a from the Fungal Genetics Stock Center in 1964 and additional cultures of mi-1 A and -1a from the FGSC in 1966. Cultures of mi-2a, -4A, -5A, -6A, -7A and -8a were obtained from Mary Mitchell in 1966. Each of the strains was subcultured on Vogel's minimal medium (M) and conidia were plated on Vogel's N agar medium containing 1% sorbose and 0.1% sucrose (MS) and on MS supplemented with 0.5% bacto-peptone and 0.5% yeast extract (MSPY). Most of the strains were crossed both as protoperithecial and conidial parents to the wild types STA4, 74-OR23-1A and 74-OR8-1a and to auxotrophic and morphological nuclear mutants with the genetic background of these wild types. Ascospores from individual perithecia were plated on MS and MSPY (MSPY permitted growth of both prototrophic and auxotrophic

progeny and, since vegetative growth was more rapid on MSPY than on MS, progeny could be scored earlier).

Asexual (conidiol) progeny of the various mi strains could be classified into 4 main types of colonies after 4 days at 34°C on MS medium; Type I "tiny" -- 0.5-1.0 mm. diometerwith sparse myceliol growth; Type II "compact" -- approximately 2.0 mm. diameter with considerable myceliol growth; Type III "large" -- opproximotely 4 mm. diameter with considerable myceliol growth; or Type IV "diffuse" -- opproximotely 6.0-8.0 mm. diameter with sparse myceliol growth. Progeny on MSPY medium grew more rapidly but the relative morphological ond growth-rote differences were maintained. Colonies of mi-1A and -1a (1964 stocks) were types I and IV, those of mi-1 A ond -1a (1966 stocks) and also those of the wild types were type III, those of mi-2a, -4A, -5A, -6A, -7A ond -8a were type 1, and those of mi-3A and -3a were types I ond II. In flasks of liquid M, the rote of growth of types II and III were similar and types I ond IV grew at about 30-50% the rote of the wild types. Although mycelia from flask cultures of mi-2a, -30, -4A, -5A, -&A, -7A ond -8a were deficient in cytochromes a + a_3, cytochrome spectm of mi-1A, -1a and -3A were almost identical to those of the wild types with appreciable absorption for cytochromes a + a_3 at 605 mμ. Cytochrome spectm of the 1964 mi- 1 stocks were not determined.

Progeny from crosses of tiny, compact or large mi strains x prototrophic or auxotrophic normal strains consisted of tiny, compact ond large colonies (i.e., types I, 11 and III) plus those with various intermediate phenotypes (the 1964 stocks of mi-IA and -1a were not tested). That is, progeny of types 1, II ond III were recovered fmm all sexual crosses involving mi strains as protoperithecial parents irrespective of the phenotype of the mi parent. Progeny from the reciprocal crosses, normal x mi, were also heterogeneous but no tiny colonies were recovered. That is, the factors responsible for the tiny phenotype were apparently not transmitted to sexual progeny when the mi strains were used as the conidial parents in crosses. The proportions of tiny progeny varied considerably among different mi x normal crosses and among different perithecio of the some cross, but most perithecio gave 30-70% tiny progeny. In contrast, tiny progeny were very rarely recovered from crosses involving pairs of normal stmins. Progeny with the tiny phenotype were deficient in cytochromes a + 03; those with the compact or large phenotype were not deficient in cytochromes a + a_3. Thus, regardless of the phenotypes of our mi cultures, we hove derived stmins from them whose properties are similar to those of the original Mitchell strain.

Cultures of mi strains that do not give phenotypically heterogeneous progeny when crossed to normal strains con be obtained by selecting tiny f_1 progeny and backcrossing these repeatedly to a normal strain such as 74-OR&lo. When tiny strains of mi-1A derived in this manner by Patricia St. Lawrence were crossed as protoperithecial parents to normal strains with the 74-OR8-1 a genetic background, virtually all of the progeny hod the tiny phenotype.

All of the mi stmins carry some extrachromosomal factor or factors that ore inherited only through the maternal parent in a sexual cross. The expression of these factors is influenced by many nuclear genes such that the colonial morphology, growth-rote ond cytochrome spectrum of on mi mutant con be modified by associating its extrachromosomal constituents with a variety of genetic backgrounds. Our data indicate that, when modifying nuclear genes hove been eliminated from the eight mi strains by suitable backcrossing procedures, they hove very similar phenotypic ond genetic properties.

Due to the slow growth of mi mutants, there must be strong selection in favor of spontaneous nuclear mutations (and also, perhaps, of extrachromosomal mutations) thot result in more mpid growth ond conidiation and possibly also some risk of contamination by normal strains during routine transfer. It is not surprising, therefore, that mi stock, subjected to constant vegetative transfer in various institutions should differ from the original mi stmins by Mitchell and Mitchell. It moy be advisable to derive new mi strains from the existing stock. by repeated backcrossing to a standard wild type so thot all mi mutants would hove more similar genetic backgrounds. * * * Stanford University, Department of Biological Sciences, Stanford, California 94305.