Transmission of Mitochondrial Deoxyribonucleic Acid in 
Neurospora crassa Sexual Crosses

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Neurospora crassa mitochondrial deoxyribonucleic acid shows strict uniparental inheritance in sexual crosses, with a notable absence of mixtures and recombinant types that appear frequently in heteroplasmons.

By using restriction enzyme analysis to distinguish different types of mitochondrial (mt) DNA, we have been able to follow the behavior of Neurospora crassa mt DNA in heteroplasmons and in sexual crosses. In initial experiments, to identify appropriate parental strains, we screened EcoRI digest patterns of mt DNA from many wild-type strains and from several subcultures of poky, an extranuclear, cytochrome-deficient mutant (3, 6). mt DNAs from all wild-type strains examined gave one of the two previously described (2) EcoRI digest patterns (types I and II; Fig. 1). By examining the mt DNA of ancestral wild-type Neurospora strains, we were able to trace type I to Abbott 4A and 12a and type II to Lindegren IA and 25a (Fig. 2). Most poky strains were found to contain type II mt DNA consistent with the fact that poky is derived from wild-type Lein 7A (6; M. Mitchell, personal communication; see Fig. 2).

Significantly, all of the variant mt DNAs appear to be related to type II DNA (as judged by the positions of EcoRI bands 5 and 9), but contain additional EcoRI bands (Mannella and Lambowitz, manuscript in preparation). The gel pattern for variant type IIA DNA, shown in Fig. 1, is characterized by an extra band, a, and a shift in the position of band 10. Subcultures containing the variant mt DNAs remain phenotypically poky, but it is possible that the apparent instability of mt DNA is somehow related to the poky phenotype.

In a previous study, we analyzed the behavior of mt DNA in forced heterokaryons between a wild-type strain containing type II mt DNA and a poky strain containing type IIA mt DNA (5). Each of 10 independent heterokaryons that we examined became phenotypically poky after a period of subculturing. Mixtures of parental mt DNAs sometimes persisted through several subcultures, but generally one mt DNA species came to predominate after subculturing. In most cases, the predominant species was the type IIA poky mt DNA, but in the remainder the parental types were replaced by putative recombinant types. We have since verified that similar phenomena occur in heteroplasmons formed between wild-type parents containing types I and II mt DNA. Furthermore, it has been possible in such cases to demonstrate that the putative recombinant mt DNAs contain regions derived from each of the parental mt DNA species (Mannella and Lambowitz, manuscript in preparation).

The situation in sexual crosses was found to be markedly different. Reich and Luck (7) demonstrated previously that the progeny of interspecies crosses of Neurospora contain the mt DNA of the maternal (i.e., protoperithecial) parent as judged by profiles on CsCl density gradients. It is also well established that putative mt DNA mutations in Neurospora crassa are maternally inherited (see discussion, ref. 7). The family tree of Fig. 2 suggests that mt DNA inheritance is in fact strictly uniparental with a significant lack of recombinant types that are readily observed in heterokaryons. To obtain further evidence on this point, we examined 10 isolates from a cross between a wild-type strain containing type II mt DNA and a poky strain containing type IIA mt DNA, the poky strain being used as the protoperithecial parent. In each case, the isolate was examined as soon as possible after the cross (i.e., using a conidial inoculum obtained directly after ascospore germination). Each isolate was found to be phenotypically poky (as judged by grossly decreased ratios of 19S to 25S mt rRNA [3]) and, as shown in Fig. 3, each contained the poky parental type mt DNA (i.e., type IIA DNA; note that doublet band 5 characteristic of types II and IIA mt DNA is resolved in the gel of Fig. 1 but not in the gel of Fig. 3). We have also analyzed three random isolates from crosses between two wild-type parents with the same result; i.e., each isolate contained the mt DNA of the protoperithecial par-
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Fig. 1. EcoRI digests of different types of Neurospora crassa mt DNA. The bands labeled in the figure are those that vary with mt DNA type. The bands in type II DNA are assigned numbers sequentially on the basis of increasing mobility in agarose gels (according to the convention of Terpstra et al. [8]). The bands in types I and IIa mt DNA are assigned the number of the related type II DNA fragment. Type IIa is one of the variant poky mt DNAs described in the text. This variant is distinguished by an extra band, α, as well as an alteration in the mobility of band 10. Strains used to illustrate types I, II, and IIa mt DNA were, respectively, [+]/ Em 5256A, [+]/237A nic-1 al-2, and poky PP-6 a nic-1 al-2. Procedures for mt DNA isolation, treatment with restriction endonuclease, and electrophoresis were as described previously (5) except that the slab gels in this figure and in Fig. 3 contain a 4 to 10% polyacrylamide gradient. The arrow indicates the direction of electrophoresis.

Fig. 2. Genealogy of common laboratory strains of N. crassa (1, 4). The mt DNA type of each strain is indicated in brackets. Parental designations for each cross are based on mt DNA type of the progeny strain with the assumption that inheritance is strictly maternal. Abbreviations: Lind, Lindgren; ST, St. Lawrence.

ent with no indication of mixtures or recombinant DNA types. If these results can be generalized, the implication is that the mt DNA of the conidial parent is effectively excluded in sexual crosses, possibly as a consequence of the small amount of cytoplasm transmitted by the conidial parent (cf. discussion by Mitchell and Mitchell [6]).

Restriction enzyme analysis of mt DNA may be useful in strain identification, particularly for cases in which the strains contain no nuclear markers. For example, our Em 5256A strain and that of Bernard et al. (2) contain type I mt DNA, whereas the strain designated Em 5256B by Terpstra et al. (8) contains type II mt DNA. Our results imply that this discrepancy must be due to an error in strain designation and that Em 5256A most likely contains type I mt DNA.

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Fig. 3. EcoRI digests of mt DNAs of the parents and progeny of a sexual cross between [+]/56 A pan-3 aI-2 and poky PP-6 a nic-1 al-2. Note that the mt DNA of each progeny strain contains the marker bands α and 10 of the protoperithecial poky parent.
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