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Homologies of *Neurospora* homothallic species using repeated and nonrepeated DNA sequences[1]

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Summary. DNA:DNA hybridization studies of the homothallic species of *Neurospora* showed that the repeated DNA sequences provided no means of distinction among them. Hybridization with nonrepeated DNA sequences, however, showed that the *N. terricola* species was quite unlike the others. These studies suggest that heterothallism might have evolved from homothallism in *Neurospora*.

Morphologically and physiologically, the homothallic species of *Neurospora: N. dodgei* Nelson and Novak, *N. africana* Huang and Backus, *N. lineolata* Frederick, Uecker and Benjamin and *N. galapagosensis* Mahoney and Backus present little or no distinguishing characteristics. *N. terricola* Gochenaur and Backus, another homothallic species with one germ pore instead of two, however, is somewhat different. Unlike the heterothallic and pseudohomothallic species of *Neurospora*, they do not produce conidia but produce perithecia from a single strain. DNA characterizations of these *Neurospora* species were conducted by Williams et al.[2] and Dutta et al.[3] at the molecular level. No distinguishing DNA characteristics between or within the 3 broad *Neurospora* groups were detectable although DNA:DNA homology studies were useful in identifying DNA sequence differences within heterothallic and pseudohomothallic species[3,4]. These latter hybridization studies involved the use of nonrepeated (unique) DNA sequences, but not repeated DNA sequences.

It has been proposed that repeated DNA sequences play a vital role in evolution[5,6]. Very little is known regarding DNA:DNA hybridizations of repeated DNA sequences of *Neurospora* species[7].

In this report we have tried to distinguish between the homothallic species of *Neurospora* by measuring DNA homologies of repeated and unique sequences. The genomes of 2 homothallic species, *N. lineolata* and *N. galapagosensis* were labeled with ^{32}P-isotope and repeated and nonrepeated sequences separated. DNA:DNA hybridization studies were then performed, independently, on an excess of unfractionated unlabeled DNAs of the other 3 homothallic species, *N. dodgei, N. africana, N. terricola* and on themselves, to form hetero- and homo-duplexes, respectively.

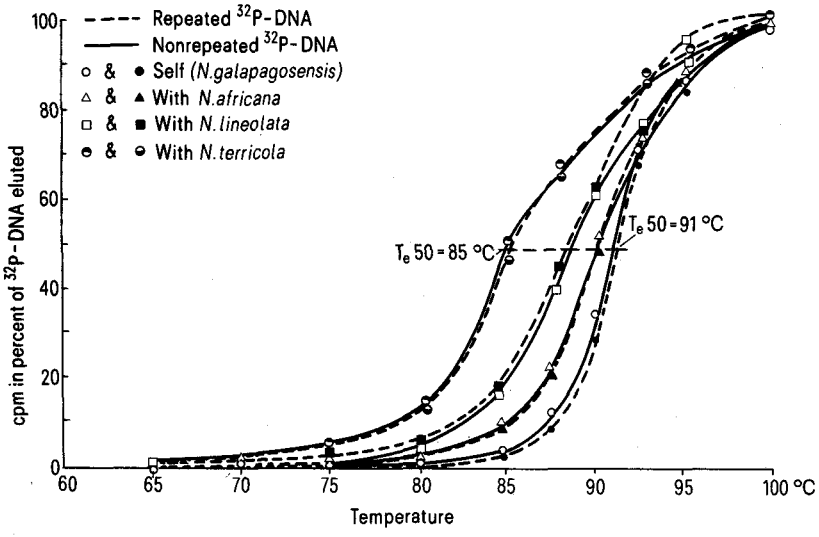
Materials and methods. The homothallic strains: *N. africana* (FGSC 1740) *N. lineolata* (FGSC 1910) and *N. dodgei* (FGSC 1692) and the heterothallic species *N. crassa* (FGSC 987) used were obtained from the Fungal Genetics Stock Center (FGSC), Humboldt, California, USA. Cultures of *N. terricola* and *N. galapagosensis* were obtained through the courtesy of Dr Lafayette Frederick of this Department. All homothallic strains of *Neurospora* were cultured 6–7 days under aeration in minimal medium containing 2% sucrose, using small amounts of mycelial fragments as inocula as described by Dutta et al.[3]. Growth of cultures were terminated when heavy deposits of melanin were visible near the mouth of the 4-l flasks in which they were grown.

The modified urea-phosphate method[8] for *Neurospora* DNA isolation was adopted. Cultures meant for ^{32}P-labeled DNA source were grown in phosphate-free medium[9], containing 2 μCi ^{32}P-isotope (New England Nuclear Laboratories) put into 100 ml of growth medium, cultured for 7 days

Compilation of normalized DNA:DNA hybridizations among DNA sequences of *Neurospora* homothallic species

^{32}p-labeled DNA fragments	Unlabeled DNA fragments	Normalized hybridization (%) Nonrepeated	Repeated
N. lineolata	*N. lineolata*	100	100
	N. galapagosensis	97 ± 0.7	96 ± 0.8
	N. africana	91 ± 1.9	97 ± 0.6
	N. dodgei	91 ± 1.4	93 ± 1.1
	N. terricola	83 ± 1.2	87 ± 0.7
	N. crassa	78 ± 1.3	80 ± 0.6
	E. coli	< 0.07	– –
N. galapagosensis	*N. galapagosensis*	100	100
	N. africana	100	97 ± 0.5
	N. dodgei	98 ± 0.7	95 ± 1.6
	N. lineolata	94 ± 1.4	96 ± 1.2
	N. terricola	81 ± 1.8	67 ± 2.1
	N. crassa	53 ± 0.6	60 ± 1.5
	E. coli	< 0.03	< 0.05

All experiments were repeated at least 3 times. Average values ± SD are shown. The basis for the estimation of normalized data is explained in the 'results' section. Average specific activity of repeated ^{32}P-DNA sequences at the time of reactions was 15,000 cpm/μg DNA and that for non-repeated ^{32}P-DNA sequences at the time of reactions was 2,000 cpm/μg DNA.



Thermal stability profiles of homoduplexes of repeated and nonrepeated ^{32}P-DNA sequences of *N. galapagosensis* and heteroduplexes between *N. galapagosensis* ^{32}P-DNAs and unlabeled DNAs from other homothallic species. Experimental details of these studies are given by Dutta et al.[3] and are briefly described in the text. The minimum radioactivity of ^{32}P-labeled DNAs of *N. galapagosensis* was 2000 cpm/µg DNA. Approximately 0.05 µg/ml of ^{32}P-DNAs were reacted with more than 200 µg/ml of unlabeled DNAs.

while shaking at room temperature. Unlabeled DNA was isolated from lyophilized mycelia. Repeated and nonrepeated (or unique) nucleotide sequences were first sheared to 400 nucleotide pairs[4] and separated from ^{32}P-labeled DNA preparations of *N. lineolata*, and *N. galapagosensis* as described by Dutta[10]. The C_0t-value was calculated as the product of the optical density at 260 nm and the incubation time expressed in h. Labeled repeated and unique DNA sequences were hybridized independently with total unlabeled DNA of each species. All measurements of DNA: DNA hybridization were made by radio-isotope counting in a liquid scintillation counter. The procedure for estimation of DNA: DNA hybridization is described earlier[3].

Results and discussion. Comprehensive summary of normalized data on the DNA:DNA hybridizations between the ^{32}P-labeled repeated and unique nucleotide sequences of 2 homothallic species *N. lineolata* and *N. galapagosensis*, with unlabeled DNAs of other homothallic species is given in the table. It shows that, except for *N. terricola*, the degree of hybridizations within the homothallic species varies very little. The heterothallic species, *N. crassa* and the bacterium *E. coli*, were used as controls. The normalized percent hybridization and T_e50 (temperature at which 50% of hybridized DNA dissociated) were calculated. For normalization the homoduplex of ^{32}P-labeled DNA of *N. lineolata* or *N. galapagosensis* formed by hybridizing with unlabeled DNA of the same species was used as 100% hybridization. The percent DNA:DNA hybridizations of *N. lineolata* homoduplexes were 90 for repeated ^{32}P-DNA and 89 for unique ^{32}P-DNA sequences. For homoduplexes from *N. galapagosensis* these values were 86% (for repeats) and 83% (for nonrepeats). These DNA:DNA hybridization data and the thermal stability profiles data (fig.) of the reassociated homoduplex of *N. galapagosensis* and heteroduplexes with other homothallic species show that DNAs of homothallic strains *N. galapagosensis*, *N. lineolata* and *N. africana* reacted similarly with each other's DNA when compared to reactions with *N. terricola* DNA.

It appears that the genomes of homothallic species, except *N. terricola*, of the genus *Neurospora* are very closely related to each other at the molecular level. This is interesting when compared with our previous observations[3,4] with the heterothallic species where distinct species differences at the DNA level were obvious. The data compiled in the table suggest that repeated DNA sequences of all the *Neurospora* species tested except *N. terricola* followed similar patterns of reactions as were obtained using nonrepeated DNA sequences and were not useful in revealing species differences. These observations confirm our previous report[2] that the genome (both repeated and nonrepeated DNA sequences) of *N. terricola* is different from the genome of other *Neurospora* homothallic species. DNA: DNA reactions of homoduplex of both repeated and nonrepeated DNAs were always higher (at least 2–4%) than corresponding reactions of heteroduplexes. The consistency of these differences throughout all reactions suggest that homoduplex reactions are normally higher than heteroduplex reactions. DNA homology studies between closely knit homothallic species (*N. lineolata*, *N. galapagosensis*, *N. africana*, and *N. dodgei*) hybridized with pseudohomothallics to a greater degree than when these DNAs were hybridized with heterothallic species: *N. crassa*, *N. intermedia*, and *N. sitophila*[2,6]. Unlike homothallic species, heterothallic species show greater diversity in DNA sequences. It is possible, therefore, to predict an evolutionary sequence for these sexually differing forms, the heterothallic, the pseudohomothallic and the homothallic species. A key to the species of *Neurospora*[10,11] also shows 3 different categories clearly, the pseudohomothallics having 4-spored asci while the homo- and the heterothallic species have 8 spores per ascus, the latter differing from the homothallics by having an imperfect stage. We, therefore, believe that the evolutionary sequence of *Neurospora* species has been as: Homothallism → Pseudohomothallism → Heterothallism.

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