HETEROKARYOSIS IN *NEUROSPORA SITOPHILA*

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HETEROKARYOSIS has been extensively used as a major tool in formal genetic analysis of both Mendelian and non-Mendelian genes and in establishing gene–enzyme relationships. So far it is the only way to perform genetic analysis of somatic cells in tissue culture (EPHRUSSI 1965; MINTZ and BAKER 1967). These aspects of heterokaryosis have been reviewed elsewhere (JINKS 1965; FINCHAM 1966; DAVIS 1966). Heterokaryosis was first described in Neurospora by DODGE (1935) and later its genetic control was reported by GARNJOBST (1953). In *Neurospora crassa* stable heterokaryons are formed between two strains only when they are isogenic with respect to *C*, *D*, *E*, and the mating-type loci (WILSON and GARNJOBST 1966).

During the present investigation the different strains of *N. sitophila* were found to form stable heterokaryons provided these strains had similar genetic backgrounds. However when strains of different genetic backgrounds (such as WHITEHOUSE and DODGE) were tested, they failed to form heterokaryons. Genetic evidence is presented for the presence of a gene controlling heterokaryon formation in *N. sitophila*. It was also found that in *N. sitophila* strains of opposite mating type form stable heterokaryons. Thus unlike *N. crassa*, the mating-type locus of *N. sitophila* was found to have no control over heterokaryon formation. This paper describes these aspects of heterokaryosis in Neurospora.

MATERIALS AND METHODS

During this study, morphological mutants were used for the following reasons. The heterokaryon between two morphological mutants can easily be scored visually as one with wild-type growth pattern. Further, unlike auxotrophs, no stringent selective conditions are required to maintain the heterokaryon formed between two morphological mutants. Besides, the gene-enzyme relations of some of these morphological mutants has been established (BRODY and TATUM 1967; MISHRA and TATUM 1968, 1970).

The strains of *N. sitophila* used were wild-type strains (W*A*, W*a*) ragged strains (*rg-1* (SFT-1) and *rg-2* (SFT-2)) and a double mutant strain carrying the marker genes crisp (*cr*) and yellow (*ylo*). The crisp yellow mutant strain was used as a tester strain to determine whether or not a particular strain could form a heterokaryon when tested in pairwise combination. These strains have been previously described (MISHRA and THRELKELD 1967).

The other strains used in the present studies were DODGE wild types (no. 412, 346, 414, 415 obtained from the Fungal Genetic Stock Center, Dartmouth College, Hanover, N.H., U.S.A.) and *rg-1* (346–7 and 412–9). The strains 346–7 and 412–9 were isolated as ragged progeny from the

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crosses SFT-1 × 346 and SFT-1 × 412. Thus these strains have genetic backgrounds different from that of the WHITEHOUSE strains. The cultures were grown on minimal medium (WESTERGAARD and MITCHELL 1947); the crosses were made on malt peptone medium (THRELKELD 1962). Conidial analyses were performed by standard procedures. Conidia were harvested in sterile distilled water, stirred on a vortex mixer to break the clumps of conidia, and then were filtered through glass wool to obtain a suspension of conidia. The conidial suspension was plated on minimal agar containing sucrose (2 mg/ml) and sorbose (4 mg/ml), in Petri plates. Prior to plating, the conidial suspension was diluted to give about 30 colonies per plate.

RESULTS

Heterokaryon formation between two ragged strains was evidenced by the wild-type growth pattern of the mixed cultures. The ability to form a heterokaryon was also shown by the wild-type growth pattern and pink color of the conidia of the culture when a particular mutant strain was tested in pairwise combination with the tester strain (50–2) having crisp and yellow phenotype. The heterokaryon was obtained as a single conidial isolate from the wild-type culture formed by two mutant strains or by mutant and wild-type strains inoculated in pairwise combination. Heterokaryon formation between two strains was confirmed by the recovery of the isolates with mutant phenotype following the conidial analysis of the phenotypically wild-type isolates.

The two complementing ragged strains (*rg-1* and *rg-2*) and also the progeny from the crosses SFT-1 × SFT-2 and SFT-1 × W*a* and SFT-2 × W*a* showed successful heterokaryon formation when tested in pairwise combination with the tester strain (crisp; yellow). However when the SFT-1 or SFT-2 was crossed with other wild-type strains (DODGE genetic background), some of the ragged progeny from such crosses failed to form heterokaryons with the tester strain. The data presented in Table 1 show that strains with the WHITEHOUSE genetic background differ from the other strain by at least one gene for heterokaryon incompatibility. The gene which controls heterokaryon formation in *Neurospora sitophila* is designated *het*.

TABLE 1

Inheritance of heterokaryon incompatibility among progeny of crosses involving strains derived from the same or different wild-type backgrounds

Cross*	Parental phenotypes†	Number of progeny in each class het+	het−	Total
SFT-1 × SFT-2	Both het+	50	0	50
SFT-1 × 346–7	het+ (SFT-1) and het− (346–7)	19	16	35
SFT-2 × 412–9	het+ (SFT-2) and het− (412–9)	21	25	46

* Strains SFT-1 and SFT-2 were derived from WHITEHOUSE wild-type background, whereas strains 346–7 and 412–9 were obtained from a cross between FGSC wild type with mutant (ragged) strains of WHITEHOUSE genetic background.

† het+ = ability to form heterokaryon.
het− = inability to form heterokaryon.
Heterokaryon formation was evidenced by the wild-type growth and pink color of conidia in pairwise combination of the individual isolate with the tester strain having crisp and yellow phenotype.

When *rg-1* and *rg-2* strains of either the same or opposite mating types were tested in pairwise combinations, there was always wild-type growth indicating successful heterokaryon formation. The mutant strains (*rg-1*, *rg-2*, and *cr*) were tested in over 1,000 pairwise combinations of opposite mating types; all of these were found to yield wild-type growth. Heterokaryon formation between *rg-1* + wild type or *rg-2* + wild type was always found to occur whether or not these strains were of the same mating type.

The heterokaryon between *rg-1 a* + *rg-2 a* and *rg-1 a* + *rg-2 A* (and also between *rg-1 A* + *cr; ylo a* or *rg-2 a* + *cr; ylo A*) showed similar wild-type growth on agar plates when scored for growth 48 hr after inoculation.

The existence of the nuclei carrying opposite mating-type genes (*A* and *a*) in the common cytoplasm of a heterokaryon formed between various strains (such as *rg-1* + *rg-2* or *rg-1* + wild type or *rg-1* + *cr; ylo*) was established in the following way. The heterokaryon-compatible (het$^+$) strains (*rg-1* + *rg-2* or SFT-1*A* + W*a*) of opposite mating types were inoculated together on an agar slope. After 4–5 days of growth conidia were harvested and plated on agar medium. The individual colonies were transferred separately to the crossing medium. On such conidial isolation, the colonies having wild-type phenotype always formed perithecia, and tetrad analysis of these asci showed 1:1 segregation for the *A*/*a* loci. Perithecial formation was never seen in the isolates having the mutant phenotype.

DISCUSSION

The data presented in Table 1 suggest that heterokaryon formation in *Neurospora sitophila* is genetically controlled. The strains of *Neurospora sitophila* were found to differ by at least one gene (*het*$^+$) for heterokaryon compatibility. Moreover, the function of the mating-type locus of *N. sitophila* seems to be different from that of *N. crassa* at least in its relation to heterokaryon formation. In *N. sitophila* all pairwise combinations of *rg-1* and *rg-2* (or *rg* and *cr* strains) of opposite mating types were found to have wild-type growth without any impairment in general growth or perithecial formation provided these strains had the same genetic background (WHITEHOUSE), i.e., these strains were het$^+$. In *N. crassa* BEADLE and COONRADT (1944) found that the heterokaryons between two strains of opposite mating types showed very poor growth. The authors attributed the slow growth of these heterokaryons to the increased production of perithecia and the direction of metabolic processes towards this end. GROSS (1952) explained these results on the basis of the alterations of the rates of synthesis of identical growth factors, due to changes in the nuclear ratios brought about by the presence of the *A* and *a* loci in a common cytoplasm. Apparently in *N. crassa*, *A* and *a* loci show an interaction similar to *C* and *c* loci (the genes controlling heterokaryon incompatibility) (WILSON, GARNJOBST and TATUM 1961).

Presumably in Neurospora, the mating-type locus has a function to restrict the existence of nuclei of the opposite mating types in close cytoplasmic vicinity. The role of such a function has been discussed by DAVIS (1966) in relation to the origin of secondary homothallism in *N. tetrasperma*. It is of interest to consider the evolutionary significance of such a function of the mating-type locus in the

three related species of Neurospora. In *N. tetrasperma*, the mating-type locus seems to have no control over the existence of nuclei of opposite mating types in any phase of the life cycle. In *N. tetrasperma*, no restriction to heterokaryosis is known to date. In *N. sitophila*, the mating-type locus does seems to restrict the existence of nuclei of opposite mating type in a common cytoplasm during ascus development, but nuclei of opposite mating type are allowed to exist in common cytoplasm of the vegetative mycelium. In *N. crassa* such restrictions on the existence of nuclei of opposite mating type prevail in all phases of the life cycle. In *N. crassa*, a heterokaryon formed between strains of opposite mating type is not only unstable but also leads to sterility of the heterokaryotic culture.

The mating-type locus of *N. crassa* seems to be a complex locus controlling several functions including heterokaryon incompatibility. However, the possibility that heterokaryon incompatibility may be controlled by another gene very closely linked to the mating-type locus of *N. crassa* has not been excluded. The nature of the mating-type locus of *N. sitophila* is different from that of *N. crassa*. The present studies lead to the conclusion that in *N. sitophila* the heterokaryon incompatibility function of the mating-type locus is either absent or is relegated to another unlinked gene. Possibilities regarding the nature of the mating-type locus in *Neurospora* could be resolved by experiments with strains carrying a *sitophila* mating-type locus in a *crassa* genetic background, or *vice versa*.

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SUMMARY

Genetic control of heterokaryon formation in *Neurospora sitophila* has been described. A gene controlling heterokaryon formation among different strains of *N. sitophila* is designated *het*. Strains of unlike mating types (carrying het^+) were found to form stable heterokaryons in *N. sitophila*. Thus the mating-type locus of *N. sitophila* seems to be different from that of *N. crassa* in that it does not show any heterokaryon incompatibility effect.

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