

NOTES AND COMMENTS

INTERALLELIC COMPLEMENTATION AT THE TRYPTOPHAN-3 LOCUS IN *NEUROSPORA CRASSA*

MAJEED AHMAD and NURUL ISLAM

Department of Botany, University of Dacca, Dacca, Pakistan

Received 1.vi.69

1. INTRODUCTION

AHMAD AND CATCHESIDE (1960) used 44 mutants to study the interallelic complementation at locus tryptophan-3 (*tryp-3*) in *Neurospora crassa*. Since that time 5 more *tryp-3* mutants have become available. Furthermore, it was felt that an improvement in the technique might reveal complementation in a larger number of mutants than was detected by Ahmad and Catcheside in 1960. Interallelic complementation at the *tryp-3* locus in *Neurospora crassa* was, therefore, reinvestigated.

2. MATERIALS AND METHODS

Forty-nine mutants were used, of which 44 (A16-A18, A21-A27, A29-A34, A36, A41-A46, A59, A68, A69, A72-A76, A78, A79, A88, A89, A97, A100-A102, A104, A111, A113, A120, A122) were the same as employed by Ahmad and Catcheside (1960). The remaining 5 mutants comprised 4 U.V. induced *tryp-3* mutants (A37, A40, A47, and A99) and a heterocaryon compatible isolate of C83, usually called *td-1* (Yanofsky, 1952). The heterocaryon compatible isolate of C83 was obtained by outcrossing C83 thrice with the Emerson wild type stock *Emerson A* (5296). Of the 49 mutants, 2 (A45 and A78) utilise indole or tryptophan (*IU*) while the remaining 47 do not utilise indole but grow on tryptophan (*NIU*). The 48 mutants which were induced by Ahmad were assigned to *tryp-3* locus on the basis of their complementation behaviour. The location of 21 of them (A16-A18, A27, A31, A34, A36, A45, A47, A59, A69, A72, A75, A78, A89, A97, A100, A102, A111, A113, A120) has been confirmed by linkage studies.

Complementation tests were carried out as described previously (Ahmad *et al.*, 1964). All heterocaryon tests as well as controls were duplicated. The sensitivity of the tests was increased through the use of: a solid as well as a liquid Vogel's minimal medium (V.M.) (Vogel, 1956); selected mutant isolates from backcrosses to *Emerson A* (5256); forced heterocaryon tests, and observation of heterocaryon tests for 30 days. The extent to which a heterocaryon grew was visually observed and was recorded by assigning arbitrary numbers ranging from $\frac{1}{8}$, representing the least growth, to 5, indicating the maximum growth.

3. RESULTS

The 49 mutants apparently belonging to *tryp-3* (*td*) locus were tested for complementation in all possible pairwise combinations (1176) and controls

TABLE 1
Order and distances of indole utilising mutants with respect to arom and A78

Cross	Number of ascospores				Order and map distance	Mean map distance (centimorgan)
	Germinated	Growing on arom medium	tryp.+ arom+	Pseudo- wild		
arom A78 × A45	24,779	3	0	0	A45	A78
arom A45 × A78	28,620	3	2	0	A45	A78
arom A78 × A303	63,974	0	0	0	A303 A78	
arom A78 × A 305	53,373	0	0	0	A305 A78	
arom A78 × A306	54,947	0	0	0	A306 A78	
arom A78 × A308	9,350	2	2	0	A78	A308
arom A78 × A644	30,127	5	2	0	A644	A78
arom A78 × A645	12,301	0	0	0	A645 A78	
arom A78 × A647	14,003	4	4	0	A78	A647
arom A78 × A668	44,046	5	0	1	A668	A78
arom A308 × A647	64,461	3	3	0	A308	A647
						0.022
						0.021
						0.043
						0.032
						0.057
						0.018
						0.009

for each mutant were maintained. During the tests, differences were seen between the responses of some original mutants and their isolates (table 1). A pair of mutants was considered heterocaryon positive if it gave a reproducible positive response in tests involving original mutants, isolates of mutants, or forced heterocaryons.

The heterocaryon tests of the 49 mutants gave a complementation matrix comprising 13 groups (fig. 1). Group-I includes those 31 mutants which do

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII					
A46 etc	113	32	73	99	42	43	69	89	29	74	47	18	102	68	45	78	72	75
46 etc	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
113	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
32	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
73	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
99	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
42	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
43	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
69	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
89	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
29	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
74	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
47	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
18	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
102	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
68	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
45	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
78	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
72	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
75	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

FIG. 1.—Complementation matrix of 49 *tryp-3* mutants. Thick continuous lines separate the different complementation groups. Thin discontinuous lines separate the mutants within a group. Solid circles = Complementation within 4 days. Inscripted dot = Complementation after 4 days. Open circle = No complementation. Numbers in the squares = Relative growth of heterocaryons.

not complement (A16, A17, A21-A27, A30, A31, A33, A34, A36, A37, A40, A41, A44, A46, A48, A59, A79, A88, A97, A100, A101, A104, A111, A120, A122 and C83). The remaining twelve groups comprised 18 mutants which complement in 56 pairs. The number of mutants in each of the twelve groups have been shown in fig. 1. Some pairs complement in 30 days even though they did not in 4 days (fig. 1). The intensity of complementation is not uniform (fig. 1).

The complementation map was made from the above data (fig. 2) assuming that mutants which do not complement have common defects. The map has 6 subunits or complons (A-F), arranged in a linear manner. The 2 *IU* mutants form a single group (XII) and occupy only one complon (A) while the 16 *NIU* complementing mutants occupy one or more of the 6 complons.

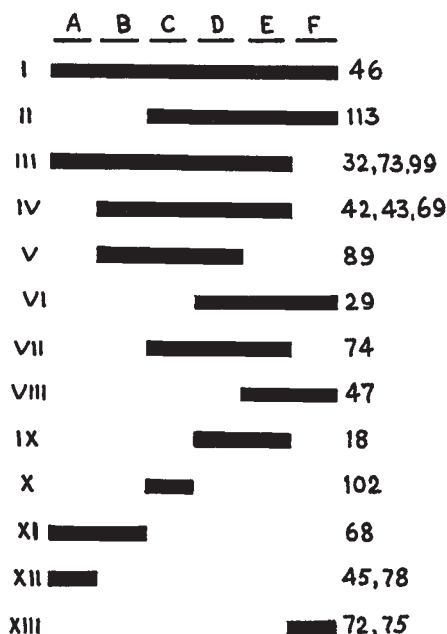


FIG. 2.—Linear complementation map of the *tryb-3* locus. Short bars A-F at the top indicate six complons. Roman numerals I-XIII on the left represent the thirteen groups of mutants. Mutants under each group are on the right. Functionally defective regions in each group of mutants have been represented by solid bars.

4. DISCUSSION

36.7 per cent. mutants tested have shown complementation in the present studies as against 22.7 per cent. and 24 per cent. reported by Ahmad and Catchside (1960) and Lacy and Bonner (1961) respectively. The complementation map obtained, suggests the presence of 6 complons (fig. 2) as against only 3 proposed by Ahmad and Catchside (1960) and Lacy (1965). The map also showed no polarity of any kind.

The variation in complementation response of the same pair of mutants in different genetic backgrounds has been an interesting observation in these studies (table 1). The fact that some pairs of mutants were heterocaryon negative when original mutants were tested together, but were heterocaryon positive when their isolates were tested (table 1, column 1), indicates that during irradiation there is a simultaneous change in heterocaryon incompatibility genes in these original mutants. This was also observed by DeSerres (1962). On the other hand, the observation that isolates of some pairs of mutants were heterocaryon negative, while the original mutants together were heterocaryon positive (table 1, column 2) demonstrates that

they can pick up heterocaryon incompatibility factors during back-crossing of mutants to the parental wild type. This obviously indicates that the two parental wild type stocks *EmA* and *Ema* differ in alleles of one or more genes controlling heterocaryon formation.

Owing to the operation of incompatibility factors it is necessary that, before a pair of mutants can be considered heterocaryon negative, we should test not only the original mutants but also their isolates, and their forced heterocaryons. Failure to observe this precaution can sometimes give misleading results. Thus at one stage when heterocaryon tests were made employing only isolates of some mutants, a matrix of interallelic complementation tests was obtained which gave a circular complementation map instead of a linear one for locus *trp-3*.

5. SUMMARY

1. Two indole utilising and 47 indole non-utilising mutants belonging to locus tryptophan-3 have been tested for interallelic complementation.
2. The 49 mutants fall into 13 groups.
3. The 13 groups can be arranged into a linear complementation map comprising 6 complons.
4. No polarity effect has been met with at this locus.
5. Variation in complementation response of the original mutants and their isolates has been found.

6. REFERENCES

- AHMAD, M., AND CATCHESIDE, D. G. 1960. Physiological diversity amongst tryptophan mutants in *Neurospora crassa*. *Heredity*, *15*, 55-64.
- AHMAD, M., KHALIL, MD., KHAN, N. A., AND MOZMADAR, A. 1964. Structural and functional complexity at the tryptophan-1 locus in *Neurospora crassa*. *Genetics*, *49*, 925-933.
- DESERRES, F. J. 1962. Heterokaryon—Incompatibility factor interaction in tests between *Neurospora* mutants. *Science*, *138*, 1342-1343.
- LACY, A. M. 1965. Structural and physiological relationships within the td locus in *Neurospora crassa*. *Biochim. Biophys. Acta*, *18*, 812-823.
- LACY, A. M., AND BONNER, D. M. 1961. Complementation between alleles of the td locus in *Neurospora crassa*. *Proc. Natl. Acad. Sci. U.S.*, *47*, 72-77.
- VOGEL, H. J. 1956. A convenient growth medium for *Neurospora* (Medium N.). *Microb. genet. Bull.*, *13*, 42-43.
- YANOFSKY, C. 1952. The effects of gene change on tryptophan desmolase formation. *Proc. Natl. Acad. Sci. U.S.*, *38*, 215-266.