buffaloes, on the other hand, showed Tf-AA, Tf-DD and Tf-AD phenotypes, which were indistinguishable from the respective types in cattle[10]. However, the frequency of the gene Tf^A was only 0.27 while that of Tf^D was 0.73. The gene frequency for the 2 transferrin types Tf^A and Tf^B were found to be respectively 0.145 and 0.855 in Indian buffaloes. This shows a similar situation to that encountered in the Thai buffaloes, except that the transferrin types in Indian buffaloes differed from those of cattle Tf-AA and Tf-DD types in their mobility. Probably this difference is brought about by the use of KIRSTJANSSON's buffer as observed by STORMONT[8]. The exact relationship between the transferrin types of the African and the Thai buffaloes and the cattle will be clear if the study is repeated following the method of KIRSTJANSSON[11]. In general, transferrin polymorphism in buffaloes is less pronounced than in cattle. The exclusive prevalence of Tf-AA type in African buffaloes[9], and its significantly low frequency in the Thai[10] and Indian buffaloes, shows a differential geographical distribution of a genetical character from East to West provided the transferrin phenotypes in buffaloes are proved identical. The high frequency of Tf-BB (0.855) and very low frequency of Tf-AA type in the Indian water buffalo, in relation to natural selection and adaptability is a problem for future study[12].

Zusammenfassung. Bei 150 indischen Wasserbüffeln (*Bos bubalis*) wurden für das Transferrin 3 Phänotypen gefunden, die durch 2 codominante Gene determiniert werden.

S. N. NAIK, K. N. TANDON and P. V. NAIK[13]

Cancer Research Institute, Tata Memorial Centre, Bombay 12 (India), 24 February 1969.

[12] Acknowledgment. We thank Dr. L. D. SANGHVI, Epidemiology Division (Columbia), for his interest and encouragement in this work. We also thank Mr. K. G. SARAIYA and Mr. T. R. PATEL of Aadarsha (Dugdhalaya, Malad) and Dr. S. A. MALANDKAR from Bombay Slaughter House, for their kind cooperation.

[13] Asst. Professor of Anatomy, Veterinary College, Bombay 12 (India).

Some Aspects of Complementation with Carotenogenic *al* Loci in *Neurospora crassa*

The effect of conidial input ratios in heterokaryons on complementation of albino mutants has been previously noted[1]. A likely explanation is that a hybrid protein is formed similar to the situation in some glutamate dehydrogenase mutant pair heterokaryons[2] such that conformationally distorted polypeptide monomers with intact active sites may be structurally re-oriented by conformationally normal polypeptide monomers with non-functional active sites producing a functional multimeric protein. Previous complementation studies[3,4] have noted some positive complementation between the 2 genetically distinguishable subunits of the region[5] but never any positive complementation within either subunit. The discovery of new phenotypes[6] and the synthesis of new albino strains with linked nutritional markers (FM strains) necessitated a re-examination of the locus. Genetic studies[5] have localized rose and white 'albinos' in the first subunit and cream, lemon yellow, pale yellow and white 'albinos' in the second larger subunit. These complementation studies were begun in order to determine the number and extent of the cistrons present and to relate this information to the possible mode of gene function.

Inoculations were made with a concentrated ($>10^7$ conidia/ml) conidial suspension in a salt solution to prevent osmotic bursting. A small quantity (0.1 ml) of each parental strain was applied to a small tube then incubated at 25°C for 10 days under intense fluorescent lighting before scoring. Media, culture and classification methods have been described elsewhere[5]. In all cases a control heterokaryon was made with a wild type strain (74A-OR23-1A) to examine the possibility of suppressor activity. The original screening (Figure 1) was performed with the available mutant strains[7] in a simple mixed conidial inoculation in all pairwise combinations without the aid of forcing nutritional markers. Each test was repeated 4 times and any incidence of complementation was scored as positive complementation for that pair. Strains representing each phenotype and each complementary group (see '†' in Figure 1) were selected and forcing marker strains were synthetized.

The forcing markers used were either arginineless (arg-6 No. 2997) or lysineless (lys-3 No. 4545). Heterokaryons with forcing markers often produced poor or no growth at all, possibly because of various heterokaryon incompatibility gene[8] combinations, arising from the heterogeneous backgrounds of the strains used.

Further examples of positive complementation were discovered during the analysis of successive conidial isolates of apparent wild type prototrophs in a concurrent high resolution genetic study[5]. The apparent wild type prototrophs proved to be complementing heterokaryons either as a result of non-disjunction (pseudowild types[9]) or the hyphal inosculations of germling ascospores. The complementation results from the

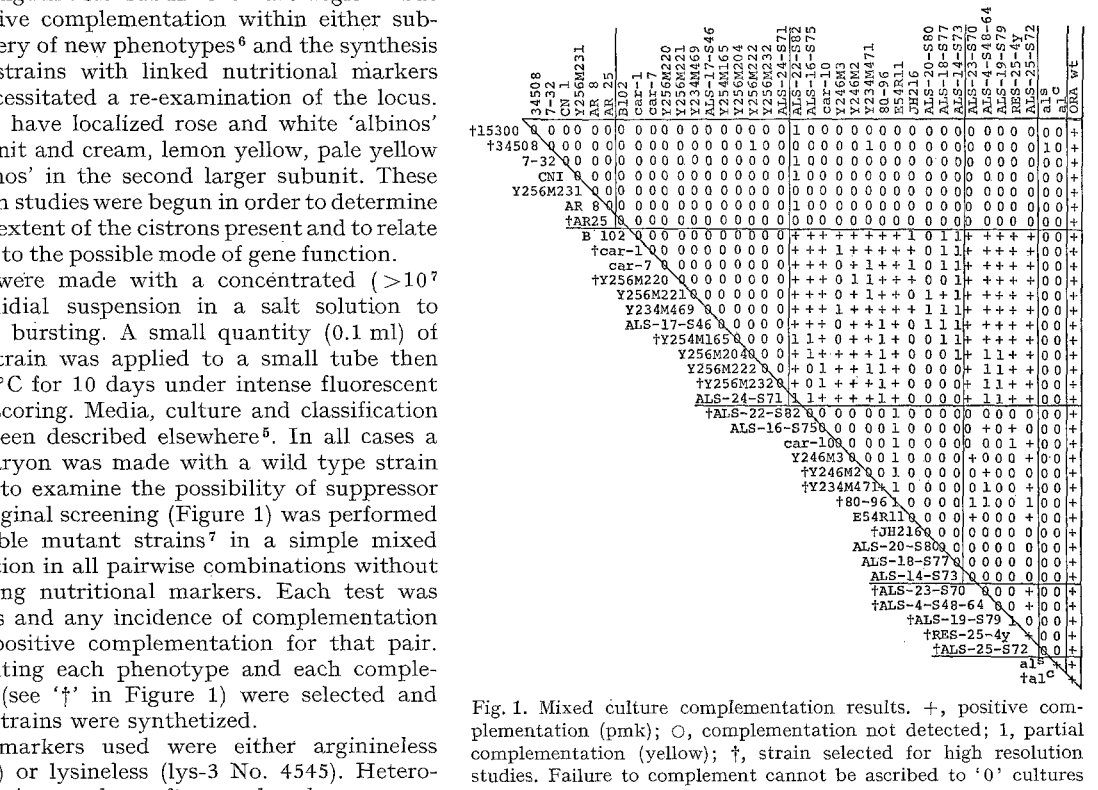

Fig. 1. Mixed culture complementation results. +, positive complementation (pmk); O, complementation not detected; 1, partial complementation (yellow); †, strain selected for high resolution studies. Failure to complement cannot be ascribed to '0' cultures as there is no proof of heterokaryon formation.

strains without forcing markers, with forcing markers and those from the recombination study are correlated in Figure 2.

Determining the point at which complementation occurs is often difficult. In some cases the value of the parameter used (burst size with phage[10] or growth rate with *Neurospora*[11]) may be only slightly higher in the heterokaryon than in one of the parental strains. Inconsistent and unsatisfactory results were obtained by analysis of absorption spectra of carotenoids from heterokaryon extracts to determine positive complementation. This was due probably to nuclear ratio variations. Visual classification which was used proved both consistent and reliable.

Heterokaryon 15300 arg-6 + Y234M471 lys-3 was successfully constituted, indicating clear complementation for the nutritional markers, but failed to complement at the al loci, while the same markers in a different combination, viz. 15300 lys-3 + Y234M471 arg-6, clearly demonstrated positive complementation. Strains al^s and al^c would only complement if the ratio of conidia was >2:1 respectively.

It is likely that, in the 15300 + Y234M471 heterokaryons, the nuclear ratio derived from the conidial input ratio was modified because of a selection for a ratio resulting in an optimal growth rate[12]. Hence the different ratios in the 2 heterokaryon combinations, one creating favourable conditions for carotenogenic complementation which are absent in the other. In the al^s + al^c mixed cultures, no selection for nuclear ratio based on nutritional requirements exists: hence the ratio should be constant.

With 2 specific exceptions (see Figure 3) all members of the first subunit complement with members of the second. The failure to complement in a heterokaryon cannot be unconditionally accepted as evidence of failure to complement because of previously described nuclear ratio effects in the forced heterokaryons and an inability to prove heterokaryosis in mixed culture (without extensive backcrossing or introducing new markers exclusive of their selective propensity).

Considering the second subunit as a discrete locus, subdivision into 3 cistrons seems warranted. Furthermore, it is likely that the gene product of the first subunit interacts with at least 1 cistron in the second subunit, and failure to do so results in identical phenotypes at either locus (see Figure 3). This would also be in agreement with the available biochemical evidence[13] and would accommodate the existence of the 2 aforementioned exceptions to the map (Figure 3).[14]

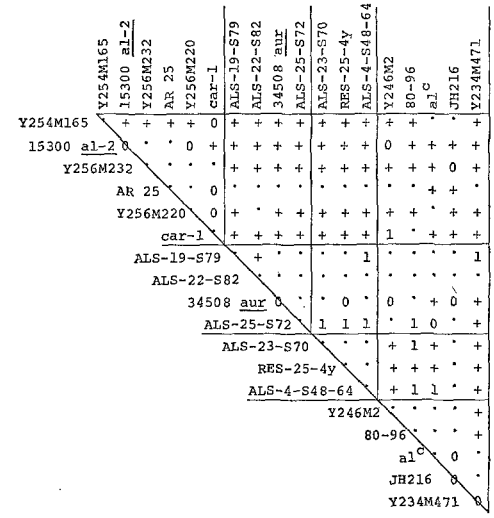

Fig. 2. Matrix of complementation data from mixed culture, pseudo-wild-type and forcing marker strain pair heterokaryons. +, positive complementation (pink); 0, no complementation observed in heterokaryons; 1, partial complementation (yellow); •, no complementation observed in mixed culture.

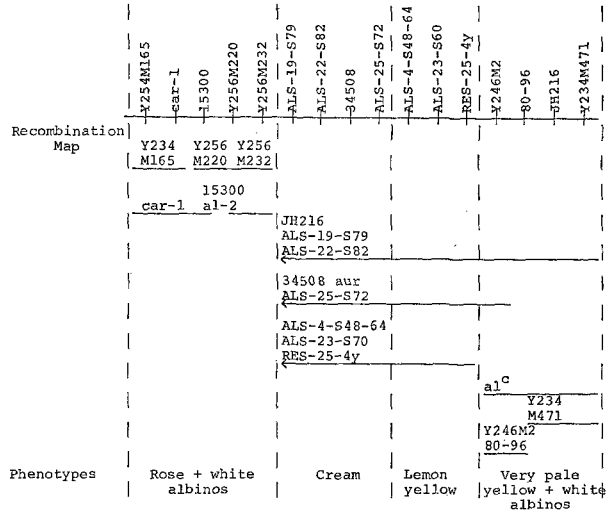

Fig. 3. Postulated complementation map[a].

[a] Constructed from negative results only from the heterokaryon tests (as opposed to mixed culture) and positive results from any source. Exceptions to the map

{ al-2 15300 + Y246M2 } failed to complement in a FM + FM
{ Y256M232 + JH216 } heterokaryon.

Résumé. Les résultats des tests de complémentation de souches mutantes albinos de *Neurospora crassa*, obtenus avec des cultures mixtes et des hétérocaryons forcés porteurs de différents marqueurs, ne sont pas nécessairement en accord. L'absence de complémentation positive à la suite de plusieurs tests ne permet pas de conclure nécessairement à l'incapacité de complémentation dans ce locus, conséquence d'effets de rapports différents des types nucléaires.

R. E. Subden[15] and S. F. H. Threlkeld

Biology Department, McMaster University, Hamilton (Ontario, Canada), 5 June 1969

[1] P. C. Huang, Genetics *48*, 894 (1963).
[2] J. R. S. Fincham and A. Coddington, J. molec. Biol. *6*, 361 (1963).
[3] M. V. G. Hungate, M. A. Thesis, Stanford University (1945).
[4] P. C. Huang, Genetics, Princeton *49*, 453 (1964).
[5] R. E. Subden and S. F. H. Threlkeld, Genetic Res., in preparation.
[6] R. E. Subden and S. F. H. Threlkeld, Can. J. Genet. Cytol. *10*, 351 (1968).
[7] Obtained with thanks from: Fungal Genetics Stock Centre, Dartmouth College, Stanford University Collection and Dr. R. Chalmers.
[8] L. Garnjobst, Am. J. Bot. *42*, 444 (1955).
[9] T. H. Pittenger, Genetics, Princeton *39*, 326 (1954).
[10] H. Bernstein, G. H. Denhart and R. S. Edgar, Genetics *51*, 987 (1965).
[11] A. Radford, Ph. D. Thesis, McMaster University (1966).
[12] D. O. Woodward, Ph. D. Thesis, Yale University (1959).
[13] R. W. Harding jr., Ph. D. Thesis, California Institute of Technology (1968).
[14] Financial support by the National Research Council of Canada is gratefully acknowledged.
[15] Present address: Laboratoire de Microbiologie générale de l'Université, 1211 Genève (Switzerland).