

## Genetic fine structure of the albino (*al*) region of *Neurospora crassa*

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### SUMMARY

By crossing a pair of albino strains each with a different adjacent nutritional marker and then crossing the same pair of albino markers with the nutritional markers transposed it was possible to order a series of *al* mutants with a resolution approaching that available for nutritional markers. A genetic map with approximated distances is provided demonstrating a grouping of mutant sites for a range of discrete carotenoidless phenotypes.

### 1. INTRODUCTION

With the exception of ascospore colour loci (e.g. Olive, 1956), high-resolution genetics has been avoided for morphological or visible mutants because of the lack of selective techniques by which the recombinant classes may be detected. The original low-resolution study of the albino (*al*) region in *Neurospora crassa* was performed by Hungate (1945), who divided the region into two complementary loci about 1-2 crossover units apart. The first unit was occupied by *al-2* (15300) type strains and the second by aurescent (34508) and *al-1* (4637T). Her results were later confirmed by Huang (1964), who indicated the potential of the outside marker transposition technique for ordering mutant sites, but he attempted only a preliminary study as suitable sets of transposition strains and a full range of phenotypes were unavailable.

Phenotypes ranging from a pure white 'albino' (Dodge, 1930*a, b*), through 'albinos' with a distinct rose or a yellow cast, to a bright lemon-yellow 'albino' (Subden & Threlkeld, 1968) are associated with the *al* region. This paper reports on a high-resolution study and the ordering of the mutant sites of 20 albino strains, which include representatives of each phenotype known to be associated with the *al* region.

### 2. MATERIALS AND METHODS

Crossing medium (Westergaard & Mitchell, 1947) was supplemented with 200 mg/l. arginine or 100 mg/l. lysine. The carbon source was sucrose for crosses (20 g/l.), glucose (20 g/l.) for ascospore isolates, and a combination of sorbose

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(20 g/l.), glucose (0.5 g/l.) and fructose (0.5 g/l.) for the selective medium. Lysine utilization was found to be inhibited by the presence of arginine (Doerman, 1944), so for crosses, the lysineless strain was always the protoperithecial parent. Optimal fertility was found with 10- to 12-day-old protoperithecial parents. The strains used are described in Table 1.

Table 1. *Strains used*

Description	Reference number	Allele or isolate number	Mutagen	Obtained from	
Pure white 'albinos'	{	1	46-11-68	—	R. Chalmers
		2	AR 25	—	A. Radford
		3	Y254M165	UV	FGSC*
		4	al <sup>c</sup>	X-ray	FGSC
		5	Y246M2	UV	FGSC
		6	G2K30	—	FGSC
Pale rose-white 'albinos'	{	7	15300 ( <i>al-2</i> )	X-ray	FGSC
		3	(Y254M165)	UV	FGSC
		8	car-1	UV	FGSC
		9	Y256M220	UV	FGSC
Cream 'albinos'	{	10	Y254M232	UV	FGSC
		11	ALS-19-S79	UV	D. D. Perkins†
Lemon- yellow 'albinos'	{	12	34508 (aurescent)	UV	FGSC
		13	ALS-22-S82	UV	D. D. Perkins†
		14	ALS-25-S72	UV	D. D. Perkins†
		15	ALS-23-S70	UV	D. D. Perkins†
		16	ALS-4-S48-64	UV	D. D. Perkins†
Very pale yellow 'albinos'	{	17	RES-25-4y	Spon- taneous	S. F. H. Threlkeld
		18	80-96	X-ray	FGSC
Arginineless ( <i>arg-6</i> )	—	2997	UV	FGSC	
					19
Lysineless ( <i>lys-3</i> )	—	4545	X-ray	FGSC	
					20

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† Isolated by Alice L. Schroeder.

The doubly marked strains used in the transposition sets (Fig. 1) were synthesized by crossing the selected albino strains (Table 1) to *arg-6* and *lys-3* strains respectively and recovering the double mutants by manual isolation.

The order of the *al* loci was determined by examining the numbers of prototrophs with wild-type pigmentation from the transposition sets, as outlined in Fig. 1. Ascospores from transposition set crosses were suspended in selective medium and heat shocked for 40 min at 60 °C; the suspensions were then poured into Petri plates. The plates were incubated for 14 days at 25 °C under intense fluorescent illumination before scoring. The optimal plating density was found to be 300–400 spores per plate, providing a prototroph frequency of 15–20 colonies per

plate. Direct identification of the pigmentation of prototrophs on the plates presented no difficulties, except with crosses involving a pair of lemon-yellow strains; in these cases all suspected recombinants were subcultured in small tubes and their pigmentation was compared with parental cultures for reliable identification. Arbitrarily, the number of spores plated per cross was set at 3000 to give a resolution of 0.06 crossover units. In several instances when the transposition sets produced no prototrophs with wild-type pigmentation, an additional 3000 spores were plated.

Transposition set description	Alternative or possible orders	
	Order I	Order II
<p><i>arg-6 al'</i></p> <p>×</p> <p><i>al'' lys-3</i></p> <p>+ + + +</p> <p>(wild-type prototroph recombinant)</p>	<p><i>arg-6 al' + +</i></p> <p>— — — — </p> <p>— — — — </p> <p>+ + <i>al'' lys-3</i></p> <p>— — — — </p> <p>+ +</p> <p>Wild-type (pigmentation) prototroph frequency if Order I is correct &gt;</p>	<p><i>arg-6 + al' +</i></p> <p>— — — — </p> <p>— — — — </p> <p>+ <i>al'' + lys-3</i></p> <p>— — — — </p> <p>+ +</p> <p>Wild-type (pigmentation) prototroph frequency if Order II is correct &gt;</p>
<p><i>al' lys-3</i></p> <p>×</p> <p><i>arg-6 al''</i></p> <p>+ + + +</p> <p>(wild-type prototroph recombinant)</p>	<p>+ <i>al' + lys-3</i></p> <p>— — — — </p> <p>— — — — </p> <p><i>arg-6 + al'' +</i></p> <p>— — — — </p> <p>+ +</p> <p>Wild-type (pigmentation) prototroph frequency if Order I is correct &lt;</p>	<p>+ + <i>al' lys-3</i></p> <p>— — — — </p> <p>— — — — </p> <p><i>arg-6 al'' + +</i></p> <p>— — — — </p> <p>+ +</p> <p>Wild-type (pigmentation) prototroph frequency if Order II is correct &lt;</p>

Fig. 1. Ordering rationale from outside marker transposition set crosses.

An estimate of the total number of spores plated was obtained from counts of prototrophs from crosses between *arg-6* and *lys-3* strains. The parameter so derived is an approximation as it is well known that prototroph frequencies in *Neurospora* are dependent on various background effects (Jessop & Catcheside, 1965). Fluctuations due to background effects would also be reflected in the *al' + al''* distances described in Fig. 2. However, calculations based on the *arg-6* × *lys-3* crosses using six different backgrounds used in this study resulted in a standard deviation of no more than ± 6.5% of the mean prototroph frequency.

Table 2. Prototrophic colonies with wild-type pigmentation as a fraction of the total number of prototrophs scored\*

arg-6 parent	lys-3 parent													
	3	4	5	6	7	8	9	11	12	14	15	17	19	20
1	-	-	-	-	-	-	-	-	-	$\frac{3}{414}$	-	$\frac{13}{503}$	-	-
2	$\frac{1}{185}$	$\frac{2}{104}$	-	-	$\frac{0}{-}$ †	$\frac{0}{96}$	-	$\frac{1}{260}$	$\frac{2}{439}$	-	$\frac{3}{314}$	-	$\frac{5}{141}$	$\frac{3}{115}$
3	$\frac{0}{536}$	- †	-	$\frac{2}{420}$	$\frac{0}{250}$	-	$\frac{3}{1875}$	-	$\frac{8}{196}$	$\frac{0}{175}$	$\frac{1}{-}$	$\frac{17}{796}$	$\frac{14}{151}$	-
4	$\frac{0}{730}$	$\frac{0}{138}$	$\frac{0}{258}$	-	$\frac{5}{611}$	$\frac{0}{104}$	$\frac{265}{265}$	$\frac{4}{392}$	$\frac{5}{598}$	$\frac{0}{61}$	-	$\frac{3}{820}$	$\frac{12}{1335}$	$\frac{1}{203}$
7	$\frac{0}{330}$	$\frac{8}{488}$	$\frac{5}{146}$	$\frac{1}{497}$	$\frac{0}{1146}$	$\frac{0}{144}$	$\frac{0}{774}$	-	$\frac{13}{763}$	$\frac{5}{-}$	$\frac{12}{511}$	$\frac{8}{511}$	$\frac{37}{502}$	$\frac{12}{231}$
8	$\frac{0}{42}$	-	-	$\frac{2}{411}$	$\frac{1}{2626}$	-	$\frac{0}{250}$	-	$\frac{0}{250}$	$\frac{0}{-}$	$\frac{1}{370}$	$\frac{5}{266}$	$\frac{9}{258}$	-
10	$\frac{0}{240}$	$\frac{8}{188}$	-	-	$\frac{0}{466}$	$\frac{0}{241}$	$\frac{0}{592}$	$\frac{4}{121}$	$\frac{5}{428}$	-	-	$\frac{7}{105}$	$\frac{10}{205}$	$\frac{11}{328}$
11	$\frac{0}{182}$	$\frac{2}{562}$	$\frac{5}{419}$	$\frac{2}{168}$	$\frac{1}{660}$	$\frac{0}{241}$	$\frac{1}{750}$	$\frac{0}{24}$	$\frac{8}{3101}$	$\frac{0}{-}$	-	$\frac{1}{279}$	$\frac{3}{992}$	$\frac{2}{271}$
12	$\frac{0}{533}$	$\frac{0}{146}$	-	$\frac{3}{374}$	$\frac{0}{678}$	$\frac{0}{160}$	$\frac{1}{808}$	-	$\frac{0}{811}$	$\frac{0}{-}$	$\frac{1}{210}$	$\frac{14}{1646}$	$\frac{2}{437}$	$\frac{2}{463}$
13	$\frac{0}{-}$	$\frac{0}{42}$	-	-	$\frac{0}{314}$	-	$\frac{0}{770}$	-	$\frac{0}{660}$	$\frac{0}{-}$	-	$\frac{0}{-}$	$\frac{0}{357}$	-
15	$\frac{0}{73}$	$\frac{0}{281}$	$\frac{0}{523}$	-	$\frac{0}{-}$	$\frac{0}{162}$	$\frac{0}{327}$	-	$\frac{0}{523}$	-	-	-	$\frac{2}{274}$	$\frac{2}{432}$
16	$\frac{0}{968}$	$\frac{4}{599}$	$\frac{3}{585}$	-	-	$\frac{0}{253}$	$\frac{0}{319}$	$\frac{0}{116}$	$\frac{0}{1347}$	-	-	-	$\frac{2}{146}$	$\frac{2}{331}$
17	$\frac{0}{1186}$	$\frac{2}{179}$	$\frac{2}{58}$	$\frac{1}{480}$	$\frac{0}{1106}$	$\frac{0}{197}$	$\frac{0}{760}$	$\frac{1}{102}$	$\frac{0}{1499}$	$\frac{0}{-}$	$\frac{0}{-}$	$\frac{1}{626}$	$\frac{6}{1221}$	$\frac{1}{447}$
18	$\frac{0}{215}$	-	-	-	$\frac{0}{-}$	-	$\frac{1}{280}$	-	$\frac{0}{-}$	$\frac{1}{-}$	$\frac{1}{-}$	$\frac{2}{-}$	$\frac{0}{-}$	-
19	$\frac{0}{461}$	$\frac{1}{848}$	$\frac{1}{38}$	$\frac{0}{156}$	$\frac{0}{1123}$	$\frac{0}{190}$	$\frac{0}{370}$	$\frac{0}{76}$	$\frac{0}{754}$	$\frac{0}{-}$	$\frac{0}{180}$	$\frac{2}{274}$	$\frac{0}{721}$	$\frac{0}{610}$
20	-	-	-	-	$\frac{0}{84}$	-	$\frac{0}{201}$	-	$\frac{0}{87}$	$\frac{0}{177}$	$\frac{0}{339}$	-	$\frac{0}{178}$	-

\* Frequencies represent cumulative scores for one or more trials with the same or transposed mating types.

† '-' indicates an infertile cross.

‡ '0/-' indicates a situation where the total number of prototrophic colonies were not discrete enough for an accurate count, e.g. because of 'sorbosc escape' or arg-6 reversion. Map distance approximation based on modal prototroph frequency of 200 per trial.

Conidial isolates were made in order to detect and exclude from the recombinant wild-type prototroph class any complementing heterokaryons formed either from pseudowild types or from the anastomoses of hyphae from adjacent ascospores. Thus conidial suspensions from prototrophic colonies showing wild-type pigmentation were plated on medium supplemented with arginine, and when the subsequent colonies were 24 h old a further conidial suspension was made and plated again on arginine-supplemented medium.

### 3. RESULTS

The recombination results are summarized in Table 2 and Fig. 2. They indicate that the *al* markers span a region 1.5–2.0 map units in length, and that this region appears to incorporate two major subunits. The first subunit consists of a cluster of closely linked mutant sites approximately 0.2 map units long which express a pure white or a pale rose-white ‘albino’ phenotype. The second subunit is separated from the first by an interval of approximately 0.5 units. The phenotypes cream, lemon yellow, pale yellow, and pure white were expressed by mutants in the second subunit, which occupied approximately 0.5 map units. Further evidence concerning the existence of two subunits within a single gene complex is presented elsewhere (Subden & Threlkeld, 1969).

Because of background heterogeneity the primary purpose was only to order the mutant sites. In some exceptional crosses, involving approximately equal map distances, differences in recombination frequencies were markedly high; e.g. for the cross Y254M165 × 34508 the prototroph frequency was  $48.2 \times 10^{-4}$ , while for the cross Y254M165 × ALS-23-S70 the prototroph frequency was  $5.0 \times 10^{-4}$ .

Complementary heterokaryons accounted for 30–35% of prototrophic colonies with wild-type pigmentation obtained from crosses with a parent from each subunit. Furthermore, it was found that successive conidial isolates were necessary to exclude from the complementing heterokaryon class, contamination by conidia from adjacent albino prototrophic colonies on the original plate.

The existence of high negative interference was detected after examining the overall ratio of single to triple crossovers from the transposition sets. From the calculated map distance the expected ratio was 400:1 and the observed was 31:1 ( $\chi^2 = 6.1$ ,  $P < 0.05$ ).

Of the crosses attempted, 22% failed to produce a sufficient number of ascospores (< 1000) to provide sufficient prototrophs. Confidence limits (10%) that had previously been calculated (Dorfman, 1964) for the significance of prototroph frequencies were not attainable even for 3000 spores with the system described. Accordingly a series of duplicate crosses was performed and their results compared by means of Poisson and contingency tests which validated their significance irrespective of the extended confidence limits (Subden, 1969).

The data presented in Fig. 2 cite only the results from the postulated single crossover classes and data are omitted where the presence of prototrophs with wild-type pigmentation shows the occurrence of multiple crossovers. In general, data for

strains carrying the *al<sup>c</sup>* marker tended to be ambiguous, and it is probable that these strains carry some chromosome rearrangement. The map position of *al<sup>c</sup>* was derived by minimizing the number of inconsistencies among the transposition set results. The strain 46-11-68 *arg-6* was sterile in all but three crosses, which, in contrast, were normal in all respects. It is possible that the successful production of a cross, like that of a heterokaryon, requires a specific combination of genes in

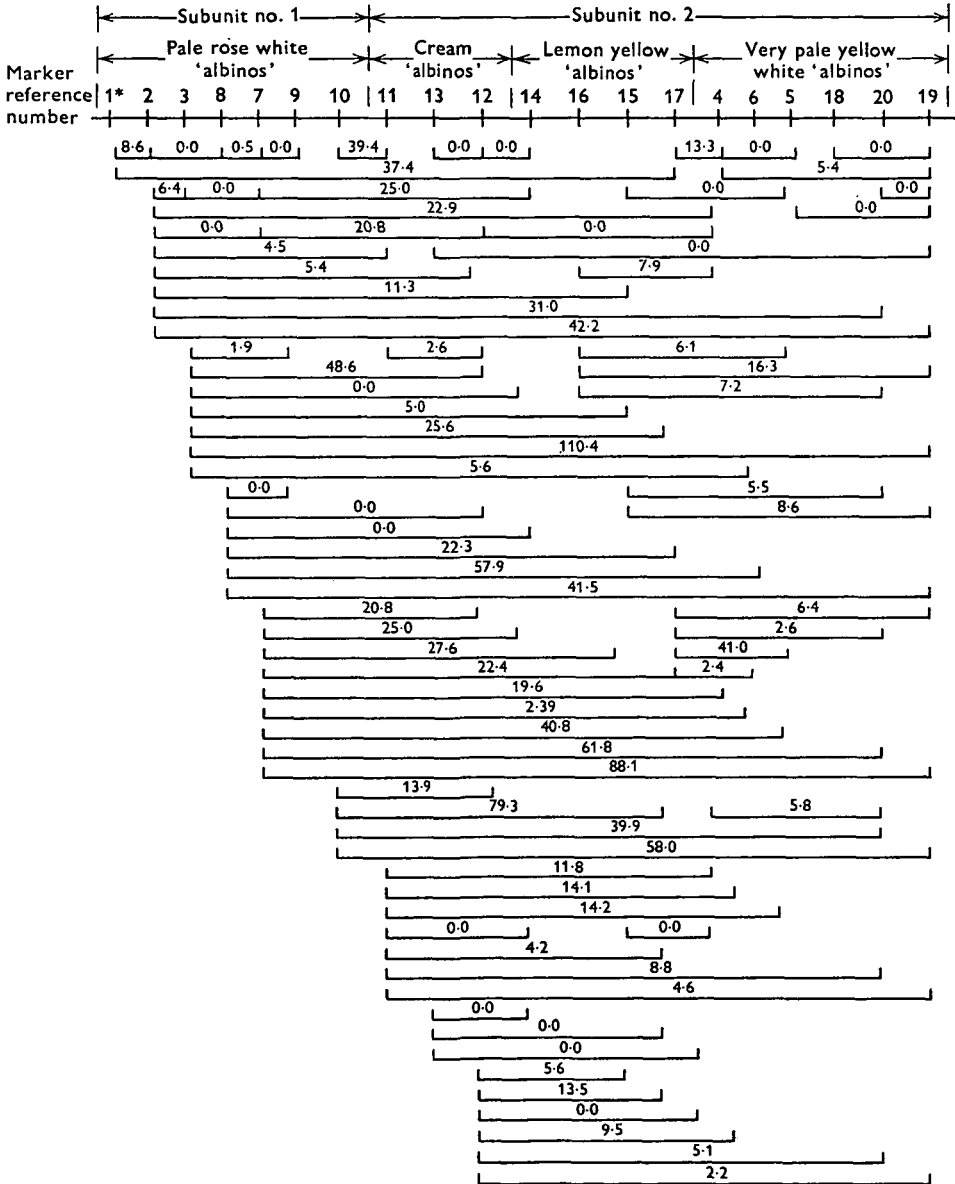


Fig. 2. Map distance approximations ( $\times 10^4$ ) for all *al* regions. Distances cited for more than one trial are expressed as averages. Site spaces are equidistant for graphic purposes only.

addition to the mating type loci. Strain ALS-22-S82 *arg-6* produced no prototrophs with wild-type pigmentation with any cross attempted. The synthesis of the doubly marked strain ALS-22-S82 *arg-6* required the isolation of >3000 spores before the double mutant was recovered. The ALS-22-S82 *lys-3* strain was synthesized but was sterile in all crosses. It is possible that ALS-22-S82 is a deletion mutant for a region distal to ALS-19-S70, resulting in chromatid pairing problems hindering successful crossovers.

#### 4. DISCUSSION

Although the order of markers is in agreement with that published for the four strains (15300, 34508, *al<sup>c</sup>*, JH216) previously studied by Huang (1964), the inconsistencies in the distances are too great to be explained by reference to the approximation limits. They probably reflect the omission by previous workers of the analysis of conidial isolates. As a result complementing heterokaryons would be counted as recombinants and so lead to false increases in map distances. Pseudo-wild types have been reported for this region by Pittenger (1954) presumably with a crossover between the centromere and the mating type locus to obtain homozygosity for the mating type locus.

The existence of identical phenotypes (pure white 'albino') in both complementary subunits suggested that the gene functions are interrelated, and this is consistent with the available biochemical data (Harding, 1968). Few conclusions can be made about the mode of gene action of the loci as the results may be explained equally well by both current hypotheses: (1) There is a polyfunctional enzyme performing successive dehydrogenations on the same substrate molecule. This would suggest that the various phenotypes are the result of impaired enzyme efficiency. The various phenotypes would reflect a graduated decrease in the quantity of carotenoids present from initial substrates to end-products (compared with wild-type concentrations). (2) An enzyme aggregate exists in which each enzyme is responsible for a specific substrate dehydrogenation and the phenotypes represent the diminution of all pigments synthesized subsequent to the lesion. Map distance approximations could accommodate this scheme.

Complementation studies with a large number of *al* strains (Subden & Thelkeld, 1969) although not completely conclusive, do suggest that the gene products of the two loci are interrelated (there are several non-complementing pairs). Furthermore, it would also appear that the unit previously occupied by *aur*-type strains is subdivided into complons or possibly cistrons equivalent to the phenotypic divisions shown in Fig. 2. Preliminary accumulation studies (Subden, 1969; Harding, 1968) with *Neurospora* carotenoid mutants support the contention that the mutant phenotypes are related to specific blocks early in the carotenoid synthetic pathway.

## REFERENCES

- DODGE, B. O. (1930*a*). Breeding albinistic strains of the *Monilia* bread mold. *Mycologia* **22**, 9.
- DODGE, B. O. (1930*b*). Inheritance of the albinistic non-conidial character in interspecific crosses in the genus *Neurospora*. *Proc. Intern. Botan. Congr. 5th Cambridge 1930*, 358.
- DOERMANN, A. H. (1944). A lysineless mutant of *Neurospora* and its inhibition by arginine. *Arch. Biochem.* **5**, 373.
- DORFMAN, B. Z. (1964). Complementation and meiotic recombination at the *ad-5-7* locus in *Saccharomyces cerevisiae*. Ph.D Thesis, Yale University.
- HARDING, R. W. (1968). The carotenoids of *Neurospora crassa*. Ph.D. Thesis, California Institute of Technology.
- HUANG, P. C. (1964). Recombination and complementation of albino mutants in *Neurospora*. *Genetics* **49**, 453.
- HUNGATE, M. V. G. (1945). A genetic study of albino mutants of *Neurospora crassa*. M.A. Thesis, Stanford University.
- JESSOP, A. P. & CATCHESIDE, D. G. (1965). Interallelic recombination at the *his-1* locus in *Neurospora crassa* and its genetic control. *Heredity* **20**, 237.
- OLIVE, L. S. (1956). Genetics of *Sordaria fimicola* I. Ascospore color mutants. *Am. J. Bot.* **43**, 97.
- PITTENGER, T. J. (1954). The general incidence of pseudo-wild types in *Neurospora crassa*. *Genetics* **39**, 326.
- SUBDEN, R. E. (1969). Some studies on loci associated with carotenogenesis in *Neurospora crassa*. Ph.D Thesis, McMaster University.
- SUBDEN, R. E. & THRELKELD, S. F. H. (1968). Genetic and complementation studies of a new carotenoid mutant of *Neurospora crassa*. *Can. J. Genet. Cytol.* **10**, 351.
- SUBDEN, R. E. & THRELKELD, S. F. H. (1969). Some aspects of complementation with carotenogenic *al* loci in *Neurospora crassa*. *Experientia* (in Press).
- WESTERGAARD, M. & MITCHELL, H. K. (1947). *Neurospora*. V. A synthetic medium favouring sexual reproduction. *Am. J. Botany* **34**, 573.