Linkage among melanin biosynthetic mutations in Cochliobolus heterostrophus

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Melanin is synthesized by C. heterostrophus from acetate via pentaketide and several dihydroxynaphthalene intermediates (Tanaka et al. 1991 Mycol. Res. 95:49-56), as it is for certain other fungi (Bell and Wheeler 1986 Ann. Rev. Phytopathol. 24:411-451; Kubo et al. 1989 Exp. Mycol 13:77-84; Chumley and Valent 1990 Mol. Plant-Microbe Int. 3:135-143). Previously, five melanin deficient mutants of C. heterostrophus were analyzed by Tanaka et al. (Mycol. Res. 95:49-56), who were unable to establish complete linkage relationships because three of the mutations (alb1, alb3, and brn1) showed no recombination when crossed to each other, and were unlinked to the other two (sal1 and pgr1), which mapped about 12 cM apart. A sixth color mutation, scr1, represented a third linkage group, but there was no evidence of its involvement in melanin biosynthesis. Independently, we have recovered six melanin-deficient mutants, one of which (alb1, Leach et al. 1982 J. Gen. Microbiol. 128:1719-1729) was included in the study of Tanaka et al. and maps to chromosome 1 on the C. heterostrophus RFLP map (Tzeng et al. 1992 Genetics 130:81-96). We report here that our remaining five melanin-deficient mutants [crm1 (light cream), crm2 (dark cream), brn1 (brown), rsy1 (rose), and probably gra3 (gray)] are linked to, but are not allelic with, alb1 (white) and constitute a gene cluster on chromosome 1 as shown in Figure 1. Since alb1 is the only mutant common to both our analysis and that of Tanaka et al., we do not know which (if any) of the remaining mutants in our collection correspond to those in the previous study.

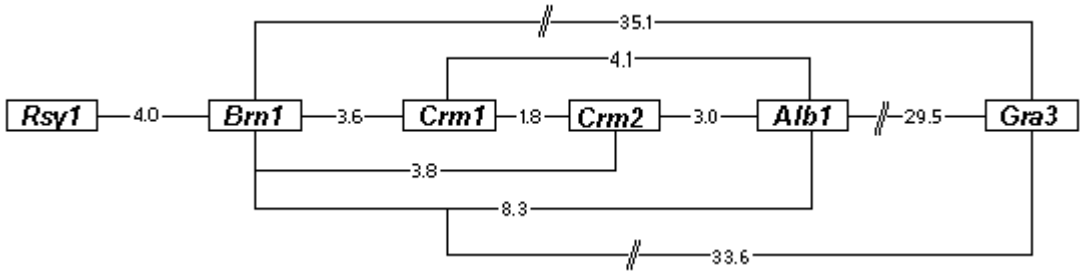

Figure 1. Proposed map of the gene cluster encoding melanin biosynthetic enzymes on chromosome 1 of C. heterostrophus, based on the two point cross data in Table 1. The gene order and the map distances must be considered tentative, since two point data may not accurately reflect recombination frequencies and do not prove gene order. Note that Rsy1 could be on either side of Brn1 and that linkage of Gra3 to the cluster is tenuous because of the distance between them. Numbers indicate cM.

In addition to the randomly isolated ascospores reported in Table 1, a few complete tetrads (four sets of twins/ascus) were recovered from each cross; they were predominantly parental ditypes

with at least one tetratype ascus from each pair of parents (nonparental ditypes were not found in any progeny). Color patterns in tetratype asci indicated the following epistatic relationships [evidence for epistasis of brn1 over rsy1 is based on the relative high abundance of brn1 progeny from the brn1 X rsy1 cross (Table 1), not on tetratype asci]:

alb1 > crm1 > crm2 > brn1 > rsy1 > gra3

Table 1. Progeny tests of two point crosses between C. heterostrophus color mutants.

Cross		Number of progeny			Map distance[a]
Parent 1	Parent 2	Parent 1 type	Parent 2 type	Wild type	(cM)
brn1	alb1	47	45	4	8.3
brn1	crm1	75	86	3	3.6
brn1	crm2	76	80	3	3.8
alb1	crm1	74	70	3	4.1
alb1	crm2	97	97	3	3.0
crm1	crm2	612	513	10	1.8
gra3	alb1	58	92	26	29.5
gra3	brn1	27	34	13	35.1
gra3	crm1	24	55	16	33.6
rsy1	brn1	87	108	4	4.0

[a] Recombinant progeny included wild types (recognizable by their dark brown-green color) and double mutants (which could not be recognized because they expressed the color of the epistatic parent). The number of double mutants in each progeny was assumed to equal that of wild types, and the wild type number was doubled to calculate the recombination frequency.

An attempt was made to test the gene order shown in Figure 1 by scoring progeny from the three point cross alb1;brn1 X crm2. Of 945 progeny isolated, 478 were alb1, 431 were crm2, 24 were brn1, and 12 were wild type. These data are not consistent with the gene order Brn1-Alb1-Crm2, which would require that the Brn phenotype represent double cross overs as the least frequent class, which it is not. However, because epistasis obscured certain recombinant classes of progeny, the data do not distinguish between the other two possible gene orders: Brn1-Crm2-Alb1 (which the two point data in Table 1 suggest) and Crm2-Brn1-Alb1.

Although albino (alb1) strains of C. heterostrophus cannot survive in the field, they cause lesions qualitatively similar to those of wild type on the host plant (corn) in a growth chamber or greenhouse (Fry et al. 1984 Phytopathology 74:175-178). To determine if there are quantitative differences in virulence among color mutants or between color mutants and wild type, lesions on

corn plants (in a greenhouse) caused by strains carrying alb1, crm1, crm2, brn1, or rsy1 were counted and measured, and compared with the sizes and numbers of lesions caused by wild type. No statistically significant differences were detected in any pair-wise comparison, suggesting that melanin plays no significant role in the virulence of C. heterostrophus to its host, in contrast to certain other fungi such as Colletotrichum lagenarium (Kubo et al. 1991 Mol. Plant-Microbe Int. 4:440-445) and Magnaporthe grisea (Chumley and Valent 1990 Mol. Plant-Microbe Int. 3:135-143), which require melanin for penetration of the host epidermis.