Ascospore Mutants and Genetic Map of *Ascobolus immersus* Stock 28

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Three hundred and seventy mutants of the ascomycete *Ascobolus immersus* stock 28 were isolated. They were assigned to at least 75 loci. Mutations in 33 loci affect ascospore morphology: 19 of these loci affect pigmentation, 5 affect pigment distribution and 9 affect shape. The reliability of various phenotypes and the role of multigenic control on ascospore morphogenesis are discussed. Other mutations affect the mycelial growth rate or morphology and sporulation. A total of 56 loci have been mapped in 13 linkage groups corresponding to at least nine chromosomes. Using an intermediary strain, successful crosses between two intersterile stocks of *Ascobolus* (28 and 50) demonstrated that 12 pairs of mutants isolated in each stock correspond to 12 homologous loci.

INTRODUCTION

Ascobolus immersus has been widely used in the study of meiotic recombination (Lissouba *et al.*, 1962; Decaris *et al.*, 1974; Rossignol *et al.*, 1979) and also more recently in the study of genetic instability (Decaris *et al.*, 1981). The most important attribute which makes this ascomycete useful for genetic studies is the ease with which mutations affecting shape or spore pigmentation can be detected, isolated and analysed. Moreover, these correspond to autonomously expressed genes which alter the phenotype of a single cell. Thus, the genotype of an ascospore may be inferred directly from its shape or colour. Tetrad analysis involving samples of 10^3 to 10^4 asci provides a system with an unusually high resolving power. However, despite earlier attempts (Yu Sun, 1966; Paszewski *et al.*, 1966; Paquette, 1973), only limited genetic maps were available at the beginning of this study. Apart from its intrinsic value, formulating an extensive genetic map seemed important to us for at least three reasons.

(1) Highly detailed recombination studies have been performed on loci controlling ascospore pigmentation. Ascospore mutants readily permit the routine detection of asci showing aberrant segregations by using crosses between mutant and wild type. The isolation of spore colour mutants in numerous different genes, of known location, was necessary for a study of the distribution of conversion frequencies and intragenic recombination frequencies throughout the genome (Nicolas, 1979).

(2) Numerous ascospore mutants showing distinctive patterns of aberrant segregations are known in some loci (Leblon, 1972). However, the relationship between the various types of aberrant segregations and reciprocal recombination or crossing-over could not be studied because flanking markers were not available. Genetic mapping should identify outside markers that flank spore pigmentation genes.

(3) The genetic studies in *Ascobolus* conducted in different laboratories utilize five intersterile strains. It seemed to us that the extent to which the strains share genetic homology could be determined by comparative phenotype analysis and the construction of a detailed genetic map.

Many different mutants were isolated in *Ascobolus immersus* stock 28. Their analysis resulted in the mapping of 56 loci on 13 linkage groups. These groups corresponded to at least nine distinct chromosomes. Successful allelism tests between stock 28 and stock 50 were also carried out.

METHODS

Stocks. All the mutants of *Ascobolus immersus* were isolated in stock 28 (Rizet *et al.*, 1969) used in our laboratory for the study of meiotic recombination. Some mutants belonging to stock 50, used in the study of genetic instability by Decaris *et al.* (1978), and some strains derived from stock 51 were also used in order to test the allelism between the loci of stocks 28 and 50. Most crosses between stocks 28 and 50 are sterile. Crosses between stock 51 and stock 28 or 50 are fertile. Stocks 28, 50 and 51 were collected in geographically distinct areas.

Stocks *S* (Paszewski *et al.*, 1966), *S2* (Paquette, 1973) and *Pasadena* (Yu Sun, 1966) are mentioned in the text. These also have different geographical origins.

Media. Details of culture media and crossing techniques have been previously described (Lissouba *et al.*, 1962; Yu Sun, 1964). All crosses were done in Petri dishes, on sterile horse dung medium.

Isolation of mutants. Ascospore mutants were isolated from asci having 4 wild-type : 4 mutant spores formed in crosses between two wild-type strains. Leaky mutants leading to aborted ascospores were isolated from asci having 3 aborted : 5 wild-type spores. One of the five 'wild-type' spores was subsequently shown to give aborted ascospores in its progeny. Thus, in a cross with wild-type, most of the resultant asci showed 4 wild-type : 4 aborted ascospores. Mycelial growth mutants were isolated according to three procedures. In procedures 1 and 2, ascospores suspended in distilled water were spread on germination medium and germination was induced by heat-shock (60 min at 54 °C). In procedure 1, every germinated spore was transferred individually to growth medium. In procedure 2, ascospores with a mutant or delayed germination phenotype were selected before transfer. In these two procedures strains with a slower growth rate or a different growth pattern were retained after 3 d incubation at 25 °C or 36 °C. In procedure 3, germinated half-asci (four ascospores corresponding to two pairs of sister ascospores) from a cross between a mutagen-treated wild-type strain and an 'aborted ascospore' mutant strain (*abo1*) were collected directly on germination medium, germinated and transferred to fructification medium. Fertile cultures having apothecia restricted to a small region of the surface plate were retained. They corresponded to a wild-type × slow growth strain cross, the fructifications of which were produced on the area covered by both the wild-type and the slower growth strain. In a second step pure mycelial mutant strains were derived from projected ascospores. This procedure selected normal fertile slow-growth mutants.

Mutagen treatment. Induced mutants were isolated from the progeny of crosses in which one of the wild-type parental strains had been treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, ethyl methanesulphonate or ICR170.

Segregation. The segregation of each mutant phenotype was tested by crossing the presumptive mutant to a reference wild-type strain. Segregations were scored in the projected asci or in mycelial cultures derived from single ascospores.

Locus assignment. Cis-trans tests of functional allelism are difficult to achieve in *A. immersus*, both at the mycelial and at the ascospore level. Heterokaryons are obtained only with great difficulty and ascospores typically possess only one haploid nucleus. Most of the mycelial growth mutants could be assigned to distinct genetic loci and mapping them posed no special problems. In contrast, independently isolated ascospore colour mutants exhibiting the same or similar phenotypes often showed relatively close or extremely close linkage. A set of mutants was classified as belonging to the same locus if, when intercrossed, the recombination frequencies showed a continuous variation within the set. The observed range of variation was quite different from one locus to another: among the extreme ranges were *b9* (0 to 2.5×10^{-4} wild-type recombinants) and *b2* (0 to 8×10^{-2}) (Nicolas, 1979).

Genetic mapping. All genetic data were obtained by the analysis of unordered octads.

To estimate the second division segregation (SDS) frequencies, the mutants were crossed with a *b10* spore colour mutant which was the most closely centromere-linked spore colour gene. For mycelial mutants, the SDS frequency was also investigated in crosses with double-mutant strains (*b7.b10*, *b7.b8* or *b9.b10*) in which the single mutants expressed distinct phenotypes: *b7*, pink; *b8*, white; *b9*, white; *b10*, granular. In such crosses, the parental ditypes (PD) were easily distinguished from non-parental ditypes (NPD) and tetratype (T) asci. In each double-mutant strain the two reference markers are linked to different centromeres. In crosses involving a third locus, PD asci for the two reference markers were selected and the frequency of T asci for the third locus could then be considered as its actual frequency of SDS when it was independent of the two reference markers. Incidental linkage was also easily detected.

Mapping loci into linkage groups was achieved by the following successive steps: (1) cross to the centromere markers of each characterized chromosome; (2) cross to independent markers unlinked to their centromere; (3) two-point, and eventually multi-point, crosses in order to assign a precise locus to mutants mapping in the same linkage group. The criterion for linkage was that the ratio of PD to NPD significantly exceeded unity. Gene distance in centimorgans (cM) was estimated by the equation proposed by Perkins (1949): $[(T + 6NPD)/(PD + T + NPD)]50$.

RESULTS

Mutant phenotypes

Ascospore mutants. These fell into three classes according to their primary phenotype: (i) ascospore colour, (ii) distribution of spore pigment, (iii) ascospore shape.

(i) *Mutants with altered ascospore colour:* the description and origin of the 268 spore colour mutants so far isolated are given in Table 1. These mutants were distributed in 19 loci designated *b1* to *b19*. Loci *b1*, *b2* and *b3* were first reported by Leblon (1972, 1974), and *b4* and *b6* by Girard & Rossignol (1974). This number is close to the 20 'series' of white spore mutants reported by Lissouba *et al.* (1962) in stock *S*. Two mutant phenotypes are known in nine of the loci. In the locus *b2*, five mutant phenotypes have been reported previously (Mekki-Berrada *et al.*, 1976; Leblon & Paquette, 1978).

(ii) *Mutants with an altered distribution of spore pigment:* the description and origin of these mutants are given in Table 1. Sixteen mutants showed a partitioning of pigment into granules within the spore. The mutants could be assigned to four loci designated *grn1* to *grn4*. Locus *grn1* was previously reported by Paquette (1978). Two belted mutants fell within the *cin* locus. Although the distribution pattern of pigmentation may differ from one mutant to another, this pattern was clearly locus specific.

(iii) *Mutants with altered ascospore shape:* the description and origin of these mutants are given in Table 2. The 15 round spore mutants could be assigned to six loci designated *rnd1* to *rnd6*. Locus *rbd1* was previously reported by Paquette (1978). Three large size spore mutants fell into three loci called *gov* (large size and oval shape), *gom* (large size, oval shape and sparse mycelial growth) and *glo* (large size and thin shape).

Although these mutants were selected phenotypically on the basis of their ascospore shape, most exhibited mutant-associated pleiotropic characteristics affecting spore pigmentation, spore germination, mycelial growth, cross-fertility or sporulation. No mutant-associated character was detected for the mutants affected in ascospore pigmentation or mycelial growth, except for the five *b3* mutants (which showed reduced fertility) and *per4* (which had pink ascospores). The associated mutant characters for mutants involved in ascospore shape were observed in eight out of the nine loci, the single exception being *rnd3*. Pleiotropy was tested by looking for a separation between the associated characters in the progeny of crosses to wild-type, but no separation was ever observed.

Mycelial growth mutants. Twenty-eight mutants affecting mycelial growth were isolated (Table 3). Some of them showed a low fertility when crossed to other mycelial mutants. Hence only map position could be used to determine whether intersterile mutants corresponded to distinct loci. Of the 25 loci studied, all but two were defined by one allele. Mutants were classified into three groups according to their phenotypes: wave (*vag1* to *vag10*), clock (*per1* to *per4*) and other slow-growing or abnormal mycelial pattern mutants (*myc* loci). Wave and clock mutants have been described previously (Chevaugnon, 1959; Yu Sun, 1964; Paquette, 1973).

Miscellaneous mutants. Table 3 also shows the descriptions and origins of various other mutants occupying 17 additional loci. These mutants exhibited diverse phenotypes such as sensitivity to low temperature (*cry1*), aborted ascospores (*abo1*, *abo2*), allele-specific locus non-specific suppressor (*su2* to *su4*), locus-specific suppressor (*sup12*, which suppresses pink-spored mutants in *b12*), brownish or yellow mycelial coloration (*brn1*, *brn2*, *jau1*) and

Table 1. *Phenotype and origin of ascospore pigmentation mutants*

Locus	SDS (%)	Linkage group	Mutagenic origin†	No. of alleles		Distribution of ascospore pigment
				White	Pink	
* <i>b1</i>	43	II	SP NG EMS ICR	51	4	
* <i>b2</i>	66	13	SP NG EMS ICR	76	0	belted ascospore (1), double belt (1), eclatée (1), tachtée (1)
<i>b3</i>	66	IX	NG EMS	5	0	
* <i>b4</i>	66	II	SP NG EMS	10	1	
* <i>b5</i>	63	I	NG EMS	4	0	
<i>b6</i>	64	II	NG EMS ICR	6	1	
* <i>b7</i>	16	II	SP NG ICR	16	2	
* <i>b8</i>	14	III	SP NG EMS ICR	11	0	granular (1)
<i>b9</i>	3	IV	NG EMS ICR	15	0	
* <i>b10</i>	1	V	EMS ICR	5	0	granular (1)
<i>b11</i>	65	—	NG EMS	25	3	
* <i>b12</i>	57	10	EMS	0	6	
* <i>b13</i>	53	VII	SP NG EMS ICR	12	0	
<i>b14</i>	67	IX	SP NG	2	1	
<i>b15</i>	64	—	SP EMS	2	0	
* <i>b16</i>	62	13	EMS	2	0	
<i>b17</i>	—	V	EMS	1	0	
* <i>b18</i>	48	IX	SP EMS	4	0	
<i>b19</i>	—	I	NG ICR	1	2	
* <i>grn1</i>	35	II	SP NG EMS			white ascospore, numerous small pigmented granules (7)
<i>grn2</i>	55	I	NG			pink ascospore, few granules (2)
<i>grn3</i>	69	11	NG EMS			pink granular ascospore (1), white ascospore with two granules (1)
<i>grn4</i>	53	VII	SP EMS			white ascospore, granular (5)
<i>cin1</i>	21	VIII	EMS			belted ascospore (2)

* Homologous loci known in stock 50.

† SP, Spontaneous; NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; EMS, ethyl methanesulphonate; ICR, ICR170.

Table 2. *Phenotype and origin of ascospore shape mutants*

Locus	SDS (%)	Linkage group	No. of alleles	Mutagenic origin*	Phenotype†			
					Ascospore morphology	Ascospore pigmentation	Ascospore germination	Mycelium growth
<i>rnd1</i>	55	I	7	SP NG EMS	round	dark violet(+)	+	+
<i>rnd2</i>	25	I	1	EMS	round	granular	poor	clock
<i>rnd3</i>	65	10	4	NG EMS	round	+	+	+
<i>rnd4</i>	42	III	1	EMS	round	+	poor	clock
<i>rnd5</i>	64	—	1	SP	round	white	spontaneous	sparse, few branches
<i>rnd6</i>	63	12	1	NG	round	+	+	sparse, few branches
<i>gov</i>	59	VII	1	EMS	large	white	+	+
<i>gom</i>	—	II	1	NG	large	white	+	sparse, few branches
<i>glo</i>	57	IX	1	SP	large	+	poor	sparse, few branches

* Symbols as in Table 1.

† +, Wild-type phenotype. Wild-type ascospores are ovals, dark violet.

sporulation deficiency (*spo1* to *spo6*). The mating type genotypes (*mt*⁺ and *mt*⁻) represent naturally occurring differences and mutations at this locus were not detected.

Genetic map

Loci were mapped according to classical methods (Perkins, 1949; Mortimer & Hawthorne, 1975) using the following steps: determination of SDS frequencies; determination of the

Table 3. *Phenotype and origin of mycelial growth mutants and miscellaneous mutants*

Locus	SDS (%)	Linkage group	Mutagenic origin*	No. of alleles	Phenotype
<i>vag1</i>	—	—	NG	1	wave
<i>vag2</i>	24	VI	NG	1	thick wave
<i>vag3</i>	—	II	NG	1	wave
<i>vag4</i>	75	11	NG	2	thick wave
<i>vag5</i>	—	11	NG	1	wave
<i>vag6</i>	—	I	SP	1	wave
<i>vag7</i>	—	IV	NG	1	wave
<i>vag8</i>	—	III	NG	1	wave
<i>vag9</i>	28	II	NG	3	wave (one allele is thermosensitive)
<i>vag10</i>	40	VI	SP	1	wave
<i>per1</i>	0	IV	NG	1	clock, numerous periodic rings, germination at 54 °C but not at 39 °C
<i>per2</i>	—	—	NG	1	clock, few rhythmic rings
<i>per3</i>	0	I	NG	1	clock, numerous rhythmic rings
<i>per4</i>	—	—	NG	1	clock, numerous rhythmic rings, pink ascospores
<i>myc1</i>	42	VII	NG	1	thin
<i>myc2</i>	—	—	NG	1	thin, poor ascospore germination
<i>myc3</i>	34	VII	NG	1	thick slow
<i>myc6</i>	57	IX	NG	1	colonial
<i>myc11</i>	40	V	NG	1	slow
<i>myc21</i>	—	10	NG	1	colonial
<i>myc26</i>	—	—	NG	1	thin
<i>myc27</i>	—	II	NG	1	slow
<i>myc28</i>	—	II	NG	1	wave, poor ascospore germination
<i>myc29</i>	—	—	NG	1	wave
<i>myc30</i>	—	II	NG	1	wave
<i>cry1</i>	—	III	NG	1	cold temperature sensitive
<i>abo1</i>	—	II	EMS	1	aborted ascospore
<i>abo2</i>	—	—	EMS	1	aborted ascospore
<i>su2</i>	—	VI	NG	17	allele-specific suppressor (Leblon, 1972)
<i>su3</i>	—	—	NG	2	allele-specific suppressor
<i>su4</i>	—	—	NG	1	allele-specific suppressor
<i>sup12</i>	—	12	SP	1	specific suppressor of locus <i>b12</i>
<i>brn1</i>	—	13	SP	1	brown mycelium (Paquette, 1978)
<i>brn2</i>	—	—	NG	1	brown mycelium
<i>jaul</i>	—	—	NG	1	yellow mycelium
<i>spo1</i>	60	IX	SP	1	sporulation deficient
<i>spo2</i>	—	—	NG	1	sporulation deficient
<i>spo3</i>	—	—	NG	4	sporulation deficient
<i>spo4</i>	—	—	NG	1	sporulation deficient
<i>spo5</i>	—	—	NG	1	sporulation deficient
<i>spo6</i>	—	—	NG EMS	2	sporulation deficient
<i>mt</i>	0	I	SP	1	mating type

* Symbols as in Table 1.

number of independent centromere-linked mutant groups; determination of the number of linkage groups whether or not they were centromere-linked; mapping of linkage groups.

Second division segregation frequencies. The SDS frequency of *b10* was estimated in a cross including four independent centromere markers: two ascospore colour loci (*b7*, pink; *b10*, granular), one clock-growing mycelial locus (*per1*) and the mating-type locus (*mt*) (Table 4). From this cross, SDS of *b10* was estimated at 1.3% of total meiosis and that of *b7* at 15.8%. Therefore the *b10* locus was chosen as a reference centromere marker to determine SDS frequencies of other loci in two-point crosses.

Second division segregation frequencies were calculated from data of crosses with *b10* or with *b7.b10* (or other double-mutant strains, see Methods). The values obtained are shown in

Table 4. Analysis of the cross $\frac{b7}{+} \frac{+}{b10} \frac{+}{per1} \frac{mt^-}{mt^+}$

(a) Segregation of *b7* (denoted by 7) and *b10* (denoted by 10) in a sample of 2000 asci (7 + = pink; + 10 = granular; + + = dark violet; 7 10 = white)

	Genotype	No.
PD	+ 10, + 10, 7 +, 7 +	1001
NPD	+ +, + +, 7 10, 7 10	683
T	+ 10, + +, 7 10, 7 +	316

(b) Segregation of *b7*, *b10*, *per1* (denoted by P) and eventually *mt* [denoted by (+), (-)] in a sample of 184 tetratype asci for *b7* and *b10*

	Genotype	No.
SDS <i>b7</i>	+ + +, 7 + +, + 10 P, 7 10 P	103
	+ + P, 7 + P, + 10 +, 7 10 +	71
SDS <i>b10</i>	7 + + (+), 7 10 + (+), + + P (-), + 10 P (-)	3
	7 + P (-), 7 10 P (-), + + + (+), + 10 + (+)	2
	+ + P (-), 7 10 P (-), 7 + + (+), + 10 + (+)	2
SDS <i>b7</i> and <i>b10</i>	+ + P (+), 7 10 P (+), 7 + + (-), + 10 + (-)	1
	+ + + (+), 7 10 + (+), 7 + P (-), + 10 P (-)	2

From these results the SDS frequencies for *b7* and *b10* were estimated to be 15.8% and 1.3%, respectively.

Table 5. Mapping of centromeres

For each entry, the three values represent PD, NPD and T. The values in small squares are calculated SDS frequencies (%).

	II <i>b7</i>	III <i>b8</i>	IV <i>b9</i> or <i>per1</i> *	V <i>b10</i>	VI <i>vag2</i>	VII <i>grn4</i>	VIII <i>cin1</i>	IX <i>b18</i>	Linkage groups
<i>mt</i>	25 15 3	14 9 1	15* 28 0	12 31 0 0	30 25 10	4 5 5	6 10 2	2 12 14	I
<i>b7</i>		216 207 94	17* 14 1	736 811 453 23	75 92 52	66 52 105	42 48 42	46 37 137	II
<i>b8</i>			4* 5 6	805 909 286 14	75 92 52	63 65 167	56 43 47	38 40 122	III
<i>b9</i> or <i>per1</i> *				1007 926 67 3	15 11 5	24 58 59	52 47 41	158 210 532	IV
<i>b10</i>					13 13 4 13	485 463 1052 53	825 746 429 21	508 532 960 48	V
<i>vag2</i>						5 5 18	13 9 19	3 10 12	VI
<i>grn4</i>							20 22 58	34 46 120	VII
<i>cin1</i>								40 36 124	VIII

Fig. 1. Genetic map of *Ascobolus immersus* stock 28. Where map positions are shown in parentheses, the order of the loci is unknown.

Tables 1, 2 and 3 for corresponding loci, wherever they were based on sufficiently large samples.

Centromere mapping. The results of the 36 possible combinations of two-point crosses between nine centromere markers are shown in Table 5. In every cross, the distribution of PD, NPD and T asci indicates that both loci are linked to their centromere. The results indicate that the nine loci are located on nine different chromosomes. The gene-centromere distance of seven of these loci is less than 12 cM, when calculated as the percentage of T/2 asci in crosses involving the *b10* locus. Three loci are closely linked to their centromere. As shown in Table 4, *b10* gave 1.3% SDS and no SDS were detected for *per1* in a sample of 184

Table 6. *Linkage groups of Ascobolus immersus stock 28*

Data shown are from octad analyses indicating linkage of markers in linkage groups I to IX, 10 to 13. For each linkage group a minimum sample of crosses necessary to establish the genetic map is presented. These correspond to the following five types of crosses.

(A) Cross with a reference centromere-linked locus (*b10*, *b7* or *mt*) to estimate SDS frequencies.

(B) Two-point cross between linked markers.

(C) Three-point cross involving two linked genes close to their centromere and a third independent centromere-linked marker (symbol C, corresponding to either *b7*, *b8*, *b9*, *b10* or double mutants *b7.b8* or *b9.b10*, see Methods). To enrich the fraction of asci giving information relating to gene order, asci showing FDS or SDS for the spore pigmentation marker were selected for analysis.

(D) Multi-point cross between linked genes. Selected asci were analysed; this enriched the fraction of informative asci leading to the order proposed.

(E) Multi-point cross between linked genes. Unselected octad analysis. The order of markers designated in the cross defines the successive intervals I and II. For example, in linkage group I cross *rnd2.rnd1. + x + +.b5*, ICO(I) means one crossover between *rnd2* and *rnd1* and ICO(II) means one crossover between *rnd1* and *b5*.

Linkage group I

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>rnd2</i> × <i>b7</i> <i>grn2</i> × <i>b10</i> <i>rnd1</i> × <i>b10</i> <i>b5</i> × <i>b10</i>			492 471 475 388	266 1098 1094 1254	288 431 431 355	25 55 55 55
(B) <i>mt</i> × <i>per3</i> <i>mt</i> × <i>rnd2</i> <i>mt</i> × <i>rnd1</i> <i>rnd2</i> × <i>rnd1</i>			22 21 22 739	0 5 33 450	0 0 2 38	0 0 40 27
(D) <i>rnd1.b19. + x + +.vag6</i>	PD <i>rnd1-b19</i> T <i>rnd1-b19</i>	<i>rnd1-b19</i> <i>rnd1</i> and <i>b19-vag6</i> <i>b19-vag6</i> <i>rnd1-vag6</i> <i>rnd1-mt</i> <i>b19</i> and <i>vag6-mt</i>	8270 89 15 0 6 1	18 2 0 15 5 11	0 0 0 0 1 0	0.1 1
(E) <i>rnd2.rnd1. + x + +.b5</i> <i>grn2.rnd1. + x + +.b5</i>	OCO 29 672	ICO(I) 15 81 ICO(II) 20 446 2CO(I) 0 7 2CO(II) 42 77 3CO(I+II+II) 0 8				

Linkage group II

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>b1</i> × <i>b10</i> <i>b6</i> × <i>b10</i> <i>grn1</i> × <i>b10</i>			533 301 630	854 1278 700	613 421 670	43 64 35

Linkage group III

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>rnd4</i> × <i>b10</i>			287	424	289	42
(B) <i>b8</i> × <i>rnd4</i> <i>cry1</i> × <i>rnd4</i>			358 16	140 5	7 0	18 4
(C) (C). <i>b8</i> . + × (C).+. <i>rnd4</i>	FDS <i>b8</i>	<i>b8-rnd4</i>	108	13	0	
(E) <i>vag8.b8.</i> + × +.+. <i>cry1</i>	OCO 74	ICO(I) 14 ICO(II) 10				

Linkage group IV

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(B) <i>per1</i> × <i>b9</i>			24	1	0	
(C) (C).+. <i>b9</i> × (C). <i>per1</i> . +	FDS <i>b9</i> SDS <i>b9</i>	<i>per1-b9</i> <i>per1-b9</i>	36 4	0 31	0 0	
(C).+. <i>b9</i> × (C). <i>vag7</i> . +	FDS <i>b9</i> SDS <i>b9</i>	<i>vag7-b9</i> <i>vag7-b9</i>	22 24	0 1	0 0	
				(FDS <i>vag7</i>)		

Linkage group V

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>myc11</i> × <i>b7</i>			10	12	8	40
(B) <i>myc11</i> × <i>b10</i> <i>myc11</i> × <i>b17</i>			14 14	14 4	1 0	29

Linkage group VI

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>vag10</i> × <i>b7</i>			4	5	6	
(B) <i>vag2</i> × <i>vag10</i> <i>vag2</i> × <i>su2</i>			23 27	7 12	0 0	12 15
(C) <i>(C).vag2</i> .+ × <i>(C).+ .vag10</i> <i>su2</i> . <i>(C).</i> + × <i>(C).vag2</i>	T <i>su2-vag2</i>	<i>su2-vag2</i>	OCO 11 —	ICO(I) 4 7	ICO(II) 5 10	

Linkage group VII

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>b13</i> × <i>b10</i> <i>myc3</i> × <i>b10</i> <i>myc1</i> × <i>b10</i>			430 27 12	1061 20 18	509 11 13	53 34 42
(B) <i>grn4</i> × <i>b13</i> <i>grn4</i> × <i>gov</i> <i>b13</i> × <i>gov</i> <i>myc3</i> × <i>b13</i> <i>myc3</i> × <i>myc1</i> <i>myc1</i> × <i>grn4</i>			110 159 152 4 20 8	88 169 91 18 11 11	8 23 3 4 0 0	33 44 22 18
(C) <i>myc3</i> . <i>(C).</i> + × <i>(C).grn4</i>	FDS <i>grn4</i>	<i>myc3-grn4</i>	12	3	2	
(D) <i>myc3</i> .+ × <i>gov</i> . <i>grn4</i>	PD <i>gov-grn4</i>	<i>gov-myc3</i>	14	30	4	
(E) <i>gov</i> .+ × <i>grn4</i> × <i>b13</i> .+	OCO 562	1CO(I) 135	2CO(I) 3	2CO(I+II) 75	3CO(I+II+II) 3	

Linkage group IX

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>myc6</i> × <i>b10</i> <i>spo1</i> × <i>mt</i> <i>b14</i> × <i>b10</i> <i>glo</i> × <i>b10</i> <i>b3</i> × <i>b10</i>			8 31 222 161 286	17 102 1346 395 1336	5 37 436 128 378	57 60 67 57 66
(B) <i>myc6</i> × <i>b14</i> <i>spo1</i> × <i>b14</i> <i>b18</i> × <i>b14</i> <i>b14</i> × <i>b3</i> <i>glo</i> × <i>b3</i>			15 10 504 178 100	10 3 198 300 91	1 0 2 22 4	1 0 15 43 29
(D) <i>myc6</i> .+. <i>b14</i> × +. <i>b18</i> .+ <i>b18</i> . <i>b14</i> .+ × +.+. <i>glo</i>	T <i>b14-b18</i> PD <i>b18-b14</i>	<i>myc6-b14</i> <i>b14-glo</i>	0 338	9 1	0 0	
(E) <i>myc6</i> .+. <i>b18</i> . <i>b14</i> × +. <i>spo1</i> .+. +.	PD <i>b14-b18</i>	OCO 1CO(I) 15 1	1CO(II) 3			

Linkage group 10

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>b12</i> × <i>b10</i> <i>rnd3</i> × <i>b10</i>			399 70	1145 231	456 56	57 65
(B) <i>b12</i> × <i>myc21</i>			30	9	1	19
(D) <i>b12</i> . <i>myc21</i> .+ × +.+. <i>rnd3</i>	PD <i>b12-rnd3</i> T <i>b12-rnd3</i>	<i>b12-rnd3</i> <i>b12-myc21</i> <i>b12-myc21</i> <i>rnd3-myc21</i>	262 22 19 19	180 1 20 20	21 0 0 0	33

Linkage group 11

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>grn3</i> × <i>b7</i> <i>vag5</i> × <i>b7.b8</i> <i>vag4</i> × <i>mt</i>			72 2 9	288 16 48	56 2 7	69 64
(B) <i>grn3</i> × <i>vag4</i> <i>vag5</i> × <i>vag4</i>			17 19	22 15	1 1	35 30
(E) <i>grn3.vag5</i> .+ × +.+. <i>vag4</i>	OCO 18	1CO(I) 6 1CO(II) 6 2CO(I) 1	2CO(I+II) 12	3CO(I+I+II) 4	3CO(I+II+II) 1	

Linkage group 12

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>rnd6</i> × <i>b7</i>			41	131	35	63
(B) <i>rnd6</i> × <i>sup12</i>			482	18	0	2

Linkage group 13

Cross	Selection	Pair considered	PD	T	NPD	SDS (%) or distance (map units)*
(A) <i>b16</i> × <i>b10</i> <i>b2</i> × <i>b10</i>			386 344	1246 1268	368 388	62 63
(B) <i>b16</i> × <i>brn1</i> <i>b16</i> × <i>b2</i>			16 516	2 1279	0 193	61

* SDS (%) for crosses of type A; genetic distances (map units) for crosses of types B, C and D. Distances were calculated for samples of more than 30 octads.
† Phenotypes of PD, NPD, T asci in *b1.b6* × *b4* and *b1.b4* × *b6* crosses were, respectively, 8 white spores, 4 white : 4 wild-type spores and 6 white : 2 wild-type spores.

‡ In the cross *myc28.myc27*.+ × +.+.*b1*, the marker order was partially suggested by the analysis of asci showing a normal segregation for *b1* (4+ : 4*b1*). Subsequently, it was established from analysis of 5 : 3 and 3 : 5 segregations of *b1* taking the advantage of the frequent association between such aberrant segregations and adjacent crossovers.

asci. Furthermore, in four crosses involving three independent centromere-linked markers, no SDS were observed for the mating-type locus in a sample of 132 asci showing ditype segregation for the two other markers.

Mapping of linkage groups. The genetic map of the nine centromere-linked groups (indicated by roman numerals I to IX) and the four centromere-independent linkage groups (indicated by arabic numerals 10 to 13) is shown in Fig. 1 and the detailed results are given in Table 6. Within each group all the loci exhibited linkage with at least one other locus. When two genes were found loosely linked in a trans-cross, linkage was verified in the corresponding cis-cross. Distances obtained in two-point crosses are given in cM. These distances may show considerable variation from cross to cross, depending on the strains used (Girard & Rossignol, 1974). Such fluctuations account for the non-additivity observed in, for example, linkage groups II and VII. The relative order of genes was most often determined in three-point crosses involving either three linked genes, leading to an estimation of their relative order, or two linked genes close to their centromere and a third independent centromere-linked marker which gave an estimation of the relative order of the centromere and its two linked genes; see legend to Table 6. In two three-point crosses, interference could be checked (cross *b13* × *grn4.gov* on linkage group VII, and *vag4* × *grn3.vag5* on linkage group 11) but no chromosome or chromatid interference was observed. A similar result was found in the *Pasadena* stock (Stadler *et al.*, 1970). This is consistent with the absence of SDS values significantly higher than 66% for the 42 mutants studied.

Two loci *b11* and *b15* are unlinked to their centromere and are independent of all linkage groups (Nicolas, 1978). Fifty-six loci out of the 75 loci defined above are located in 13 linkage groups (I to IX, 10 to 13).

Inter-stock crosses

Direct crosses between stock 28 and stock 50 strains were first performed to test for the allelism of mutants. Crosses were usually intersterile and formed no apothecia. A few crosses did produce apothecia but ascospores were absent or showed abnormal morphologies.

Successful crosses were obtained by using stock *SI*, which was compatible with both stocks 28 and 50. Two intermediary stocks were constructed by crossing 28 × *SI* and 50 × *SI*. Tests for allelism could then be performed by crossing 28–*SI* intermediary stock with 50 and by crossing 50–*SI* intermediary stock with 28. Reasonable fertility and normal morphology of asci were frequently obtained in these crosses.

Recombination frequencies between reference mutants of ascospore colour loci of stocks 28 and 50 were studied. Most asci were PD in the progeny of 12 crosses: *b2* × 179, *b4* × 230, *b5* × 301, *b12* × 200, *b13* × 149, *b16* × 533, *b18* × 197, *grn1* × 441 and *b1* × 141, *b7* × 138, *b8* × 184, *b10* × *B* (Rizet *et al.*, 1979), showing that these 12 pairs of mutants correspond to 12 homologous loci in stocks 28 and 50.

DISCUSSION

Among the 75 loci defined in stock 28 of *Ascobolus immersus*, 33 involved ascospore phenotype. Of these loci, 24 controlled pigment production or pigment distribution. In addition, a locus-specific suppressor of *b12* mutants was isolated. The involvement of so many loci emphasizes the complexity of the mechanisms involved in the regulation of spore pigmentation. Obviously a lack of pigmentation may be due to different metabolic alterations such as pigment synthesis blocked at different stages, non-attachment of the pigment to the cell wall because the pigment or its precursors are altered, or because structural abnormalities of the spore wall prevent pigment fixation.

The existence of several *b2* alleles with no pigmentation, weak pigmentation or modified partitioning of pigment strongly suggests that this gene product is a structural protein bound

to the spore wall and interacting with pigment expression. The absence of pleiotropy and the autonomous expression of the spore pigmentation mutants indicates that many genes are expressed during a limited part of the life cycle, such as sporulation which occurs after the first post-meiotic division.

Nine loci controlled ascospore shape. Mutants in these loci also displayed an autonomous expression, but most exhibited a pleiotropic effect. The most conspicuous pleiotropies concerning spore pigmentation, mycelial growth and sporulation for spore shape mutants may indicate that the gene product plays a role during different parts of the life cycle.

The genetic map of stock 28 shows at least nine chromosomes. Two chromosomes carry markers on both arms. Cytological observations, with an optical microscope, by Zickler (1967) suggested that the haploid number of chromosomes was 12. More recent observations using serial sections and electron microscopy indicate that this number is probably 11 (D. Zickler, personal communication). Hence, in this study only two chromosomes might not have been marked genetically. The six independent genetic regions not linked to a centromere (linkage groups 10 to 13, *b11*, *b15*) may define the two remaining chromosomes or they may represent distal segments of the nine genetically defined chromosomes. The minimal total length of the linkage map is about 980 map units. This value was calculated as the sum of the genetic distances observed in the successive intervals in two-point crosses (linkage groups I to 13), with the addition of 300 units (50 units each) for the four linkage groups 10 to 13 and each locus *b11* and *b15*, since they correspond to regions genetically independent of all linkage groups and independent of each other. Partial genetic maps were previously based on data from different stocks of *Ascobolus immersus*: *Pasadena* stock (Yu Sun, 1966), European *S* stock (Paszewski *et al.*, 1966), stock *S2* (Paquette, 1973). The search for molecular homology between loci in distinct stocks by using identical mutant phenotypes can only be tentative, since mutants previously mapped showed mostly the same white-spored phenotype. Taken collectively the results of tests for allelism between stocks 28 and 50 reported here and genetic mapping in stock 50 (B. Decaris, personal communication) point to the following similarities among the five *Ascobolus* stocks. The mating-type locus is always very closely linked to a centromere. Allelism between loci 75 (stock *S*) and *b2* (stock 28) was previously reported (Decaris *et al.*, 1974) and can now be extended to locus 179 (stock 50). Loci *b2* and *b16* are loosely linked in both stocks 28 and 50. Chromosomes I of stocks 28 and 50 share five loci in the same order: *mt*, *grn2*, *rnd1*, *b19* (white and pink alleles were isolated in stocks 28 and 50) and *b5*. Linkage groups II (stock 28), I (stock *S*) and II (stock *S2*) share at least two white ascospore loci and one granular ascospore locus linked to a centromere. Linkage groups II of stocks 28 and 50 share four loci in the same order: *grn1* on the left arm, *b7*, *b1* and *b4* on the right arm.

Allelism tests between stocks 28 and 50 are in progress. They should be helpful in the comparison of genetic recombination parameters in homologous loci and homologous inter-genic regions.

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