BIOCHEMICAL AND MORPHOLOGICAL MUTANTS OF *ASCOBOLUS IMMERSUS*¹

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Ascobolus immersus is a valuable organism for genetic studies, because (1) the percentage of ascospore germination is high (95 percent), (2) the time required for one generation is short, and (3) certain spore color markers may be screened with ease (RIZET, LISSOUBA and MOUSSEAU 1960; LISSOUBA 1960; LISSOUBA, MOUSSEAU, RIZET and ROSSIGNOL 1962). Polarization in nonreciprocal recombination between spore color mutants, observed by the above authors, is the most important genetic result obtained with this species, though, as pointed out by STADLER (1963), interpretation could be facilitated by the use of closely linked outside genetic markers. One obstacle to the accumulation of such genetic markers has been the lack of a minimal medium essential to the selection of biochemical mutants. Recently YU-SUN (1964) formulated a synthetic medium of known chemical composition for the vegetative growth and fruiting of *A. immersus*. This paper reports mutant strains of *A. immersus* with nutritional deficiencies, and also morphological mutants. Linkage relationships of some of the mutants are discussed.

MATERIALS AND METHODS

Strains: Two locally collected wild-type strains, P5 and K5 (YU-SUN 1964), were used both as sources of mutants and in crosses.

Media: The basal, or minimal medium used consists of 5 g asparagine, 0.5 g KH_2PO_4 , 0.6 g K_2HPO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 ml of VOGEL's (1956) micronutrient solution, 5 μg biotin, and 100 μg thiamine, in 1 liter of distilled water. One percent glucose is standardly used as carbon source, and the medium is solidified with 2 percent agar (Difco). The crossing medium, in addition to the salt components of basal medium, contains 0.3 percent yeast extra (Difco), 1.0 percent glucose and 0.2 percent cellulose powder (Whatman) as carbon sources, is solidified and, when necessary, appropriately supplemented to satisfy growth requirements of the strains being crossed. Hereafter this crossing medium, without the supplement, will be referred to as "yeast extract" agar medium.

Dry weight determinations: Cultures in 125 ml Erlenmeyer flasks containing 25 ml liquid basal medium plus various amounts of a supplement were incubated at 25°C for 7 days. The inoculum was a disc, 2 mm in diameter, taken from an actively growing colony on the "yeast extract" agar. At the end of the growth period the mycelium mat was squeezed between filter paper, dried in an oven at 90°C for several hours, and weighed.

Mutagens: Mutations were produced by irradiation with ultraviolet light (UV) and X rays.

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Ascospores plated on 4 percent agar in distilled water were exposed to UV for varied lengths of time. Ascospores suspended in distilled water received 10,000 to 40,000r X rays at the rate of 4,000r per minute. The spores were then heat treated, placed on 4 percent Bacto-agar, and incubated at 35°C for 8–12 hours or longer. Spores which showed a slower growth rate or a different growth pattern were transferred to tubes of yeast extract agar medium supplemented with vitamins, casein hydrolysate and yeast nucleic acid. Ascospore mutants were detected by examination of Petri dish covers with discharged spores. The procedure used for detection of biochemical mutants was essentially that described by RYAN (1950). Since *A. immersus* produces no asexual spores the auxanographic method was carried out by using macerated mycelium.

Crossing techniques: Crosses were made in Petri dishes by placing mycelium of the two opposite mating types about 5 cm apart on the agar surface. When crosses involved one colonial or clock mutant, this mutant was introduced two or three days earlier than the noncolonial type, because its rate of growth is slower. These cultures were incubated at laboratory temperatures.

Ascospores were collected by inverting Petri dishes containing 4 percent Bacto-agar over a culture. Numerous groups of eight ascospores were obtained. The eight spores of each tetrad were separated from each other, subjected to heat treatment and isolated when necessary. Unless otherwise stated, at least 95 percent germination was obtained after heat treatment at 50°C for 60 to 75 minutes, followed by incubation at 35°C for 8 to 12 hours or longer. Granular-mutant ascospores germinate spontaneously at room temperature or at 35°C, but they will not germinate after heat treatment at 50°C.

RESULTS

Production, isolation and characterization of mutants: A total of 24 mutants induced by UV or by X rays, are listed in Table 1. Seven biochemical mutants are designated by the various compounds that they required for growth. When allelism has not yet been established either by direct test or by map position, different mutants with the same requirement or name are distinguished by their isolation numbers in parentheses following the name or gene symbol. Except for the adenine requiring mutant which produces a reddish pigment on agar medium, all biochemical mutants are morphologically similar to wild type when they are grown on yeast extract agar, or on minimal medium supplemented with appropriate growth factor. Strain 2 reverted to leucine independence after seven months. The other six biochemical mutants have remained stable since they were isolated four to nine months ago.

Sixteen morphological mutants are classified into three groups according to their ascospore color, mycelium color, and colony morphology. The first group includes mutants that differ from wild type in ascospore color or distribution of pigment. The white mutants produce ascospores which remain colorless even at maturity. Figure 1 shows 4 red (wild type) and 4 white ascospores from one ascus. Ascospore pigmentation is restricted to loosely attached particles in the granular mutant, as shown in Figure 2, whereas it is evenly distributed in wild type. Both granular and white mutants have been reported by LISSOUBA, *et al.* (1962). The second group consists of one mutant which produces brown mycelium (Figure 3) on yeast extract agar. This brown color was not observed on minimal medium. The third group comprises 11 colonial mutants. These mutants are characterized by the formation of dense, restricted colonies unlike those of the wild type which has a spreading growth habit (Figure 4). They grow 1.5 to 4 mm per day at 25°C on an agar surface, whereas the wild type grows about

TABLE 1

Biochemical and morphological mutants of Ascobolus immersus

Name and isolation number*	Symbol*	Mutagenic agent	Characteristics
<i>Biochemical</i>			
leucine(1)	<i>leu</i> (1)	X rays	leucine requiring
leucine(2)	<i>leu</i> (2)	X rays	leucine requiring
cysteine(3)	<i>cys</i> (3)	X rays	cysteine requiring
cysteine(6)	<i>cys</i> (6)	X rays	cysteine requiring
cysteine(8)	<i>cys</i> (8)	X rays	cysteine requiring
adenine(5)	<i>ad</i>	X rays	{ adenine requiring; responds to hypo-xanthine, not to guanine; produces red pigment
lysine(7)	<i>lys</i>	X rays	
unknown(9)	<i>un</i>	UV	nutritional requirement unknown
<i>Morphological</i>			
white-1(10)	<i>w-1</i>	UV	{ white ascospores (Figure 1)
white-2(11)	<i>w-2</i>	X rays	
white-3(12)	<i>w-3</i>	X rays	
granular(13)	<i>gr</i>	X rays	{ pigment of ascospore in form of granules (Figure 2)
brown(14)	<i>br</i>	UV	
clock-1(15)	<i>clo-1</i>	UV	{ brown mycelium (Figure 3)
clock-2(16)	<i>clo-2</i>	UV	
clock-3(19)	<i>clo-3</i>	X rays	
clock(17)	<i>clo</i> (17)	X rays	
clock(18)	<i>clo</i> (18)	X rays	
clock(20)	<i>clo</i> (20)	X rays	{ Growth in concentric rings, about one ring a days at 25°C (Figures 5 to 8)
wave-1(21)	<i>wa-1</i>	X rays	
wave(22)	<i>wa</i> (22)	X rays	{ wave-like growth (Figure 10)
colonial(23)	<i>co</i> (23)	X rays	
colonial(24)	<i>co</i> (24)	X rays	{ slow and compact growth
colonial(25)	<i>co</i> (25)	X rays	

* Isolation numbers are shown in parentheses. Following gene names or symbols, they identify strains not yet sufficiently tested for allelism.

20 mm per day. Six of the 11 colonial mutants have been designated "clock" because they resemble the clock mutant of *Neurospora* described by SUSSMAN, LOWRY, and DURKEE (1964). These mutants form concentric rings, the first one appearing approximately 24 hours after inoculation with germinating ascospores or mycelial fragments. The rings are composed of highly branched dense hyphae alternating with thinner and faster growing hyphae. Mutants *clo-1* and *clo-2* form more conspicuous rings on yeast extract agar than on minimal agar but the reverse is true of *clo-3* (Figure 5). The rings of *clo*(17), *clo*(18), and *clo*(20) are equally conspicuous on both media. All clock mutants form about one ring of growth per day at 25°C (Figures 5 and 8). They grow slower and form fewer rings at lower temperatures and faster and more rings at higher temperatures. Figure 6 shows that *clo*(18) forms about 0.6 rings per day at 17°C and Figure 7 shows that *clo-1* forms about 1.4 rings per day at 35°C. The clock characteristic

persists either in the dark or in light of ordinary intensities, but under strong light (about 1,000 foot-candles or 10,760 meter-candles) the mutants tend to change to the wild phenotype, as shown in Figure 9. Such phenotypically wild-type hyphae again formed the characteristic clock colony after being transferred to yeast extract agar and low light intensity, indicating that there was no persistent change. With the exception of *clo-1* and *clo-2*, the various clock mutants differ from each other by their growth rate, colony characteristics, and sometimes by pigment formation on certain medium. The mycelia of *clo*(17) and *clo*(18) turn to a brown color with age on yeast extract agar, but not on minimal medium, as shown in Figures 6 and 8. *clo*(20) produces a yellow pigment on yeast extract agar. *clo-1* and *clo-2* are similar in growth rate and colony morphology; however, *clo-2* sometimes expresses a nearly wild phenotype on minimal agar medium, under light of low intensity or in the dark, whereas *clo-1* has not been observed to change its growth habit under the same condition. It will be shown below that *clo-1* and *clo-2* are not linked.

The two wave mutants form compact mycelium in the first four or five days after inoculation, and then form alternating thin and thick mycelium in the form of wave-like progression (Figure 10). The term *wave* was used by CHEVAUGEON (1959) to describe a periodic mutant of *Ascobolus*.

The three nonperiodic colonial mutants form compact colonies of different rates of growth on yeast extract agar medium. All 11 colonial mutants are very fertile.

Nutritional requirements: (1) Lysine-requiring strain 7. Growth of strain 7 is almost linearly proportional to lysine concentration, approximately doubling in dry weight at each doubling in concentration over an eightfold range (Figure 12B). An equivalent amount of growth requires twice the concentration of the racemic mixture as of the L-isomer, indicating that the unnatural D-isomer is not used. In this respect the lysine deficient strain of *Ascobolus* resembles strain 4545 of *Neurospora* (DOERMANN 1944) which also requires L-lysine even though *Neurospora* has a D-amino acid oxidase attacking D-lysine (HOROWITZ 1944). DOERMANN (1944) also found that a lysineless mutant of *Neurospora* was inhibited by arginine, growth being reduced to one half by an equimolar ratio of arginine and lysine. As shown in Table 2, arginine is an even stronger competitive inhibitor of lysine in strain 7 of *Ascobolus*, growth being almost completely

FIGURES 1-11.—Photographs of mutant strains. FIGURE 1. Four red (wild) and four white ascospores from one ascus ($\times 120$). FIGURE 2. Four red and four granular ascospores from one ascus ($\times 164$). FIGURE 3. Eight single ascospore cultures, four white mycelium (wild) and four brown, from one ascus. FIGURE 4. Wild strain showing spreading growth habit (14 hours). FIGURES 5-10. Appearance of various colonial mutants on Petri plates; 9-day cultures, *ca.* $\times 0.5$: FIGURE 5. clock-3 on minimal agar at 25°C; FIGURE 6. clock(18) on minimal agar at 17°C (it formed about 0.6 ring per day); FIGURE 7. clock-1 on yeast extract agar at 35°C (it formed about 1.4 rings per day); FIGURE 8. clock(18) on yeast extract agar at 25°C; FIGURE 9. clock-2 on yeast extract agar under intense light (it changed to wild-type growth); FIGURE 10. wave(22) on yeast extract agar at room temperature; FIGURE 11. Four wave-1 from white spores, and four wild type from red spores, from one ascus ($\times 20$).

FIGURE 12.—The growth of six biochemical mutants on various concentrations of required substances. Each point represents the average dry weight of mycelium from eight flasks in two experiments, four replicates in each experiment. A. Strain 5 in adenine. B. Strain 7 in L-lysine, ●, and in DL-lysine, ○. C. Strains 3, ○ and 8, ●, in L-cysteine, and strain 6 in L-methionine, △. D. Strain 1 in L-leucine.

TABLE 2

Inhibition of strain 7 by arginine at various molar ratios of lysine and arginine in basal medium

L-lysine, millimole per 25 ml	L-arginine, millimole per 25 ml				
	0	0.005	0.01	0.02	0.04
0	0	0	0	0	0
0.005	43	2	1	0	0
0.01	79	16	1	1	0
0.02	140	119	13	1	0
0.05	141	143	145	151	7

Dry weights from 7-day cultures are given in mg per flask. Each dry weight value in the table is the average of 8 flasks from 2 experiments, 4 replicates in each experiment.

inhibited by equimolar concentrations. Alpha-aminoadipic acid, which is utilized by lysineless strain 33933 of *Neurospora* (MITCHELL and HOULAHAN 1948), did not support growth of strain 7 of *Ascobolus* at concentrations of from 0.005 to 0.4 moles per 25 ml. Strain 7 formed abundant apothecia on yeast extract agar supplemented with either 10 mg of L-lysine or 0.5 ml casein hydrolysate (Nutritional Biochemical Corp.) per 100 ml of medium. Apothecial formation on unsupplemented yeast extract agar was very poor.

(2) Adenine-requiring strain 5. The response to adenine is shown in Figure 12A. This mutant has been tested on the basal medium supplemented with adenine, hypoxanthine, adenosine or guanine. It responds to hypoxanthine, weakly to adenosine but not at all to guanine.

(3) Cysteine-requiring strains 3, 6 and 8. Figure 12C shows the growth responses to cysteine or methionine. HOROWITZ (1950) also indicated that cysteine synthesis involved stepwise reduction of sulfate to sulfite, thiosulfate and then to cysteine. All three cysteine mutants of *Ascobolus* grew on thiosulfate, but not on sulfite or sulfate. The response of *cys*(8) to thiosulfate is stronger than that of either *cys*(3) or *cys*(6).

(4) Leucine-requiring strain 1. The growth response to L-leucine is shown in Figure 12D.

Genetic studies: All genetic data presented in Table 3 were derived from unordered tetrad analysis, because the ascospores of *A. immersus* are not arranged in linear order in the ascus. The format of this table was adapted from EL-ANI, OLIVE and KITANI (1961). All loci are included for which recombination data from 17 or more tetrads have been accumulated. Tetrads from which less than seven spores germinated are not included except in instances in which segregation patterns indicated that at least one member of each of the four spore pairs had germinated. The criterion of linkage used is a ratio of PD (parental ditype) to NPD (nonparental ditype) which significantly exceeds 1:1 (PERKINS 1953).

In crosses involving biochemical mutant times wild type or morphological mutant, the biochemical mutant spores can be scored on the agar plate, because they germinate more slowly than either the wild type or the morphological mutants. All colonial mutants are distinguishable from the wild type as soon as their spores germinate by the characteristic production of short hyphae which branch out rapidly but elongate rather slowly. Figure 11 shows 4 white wave-1 (*wa-1*) and 4 red wild-type spores from one ascus (cross 18). A diagram of three linkage groups in which 2 or 4 loci have been located is presented in Figure 13. The map distance between gene and centromere is one half the frequency of second-division segregation. The gene-to-gene interval is measured by the recombination frequency (BARRATT, NEWMAYER, PERKINS and GARNJOBST 1954). The linkage of *mt*, *w-1* and *wa-1* with their respective centromeres was calculated according to the method of WHITEHOUSE (1950; EMERSON 1963). All values are uncorrected map distances.

In linkage group III, the sequence of *w-2-lys-wa-1* could be reversed, since the centromere distance of *w-2* and of *lys* was not determined. It is not known

TABLE 3

Segregation data from crosses involving two or three loci

Cross No.	Cross	PD	T	NPD	Total	Percent T
1.	$mt^+ \times w-1 \times mt^- clo-2 +$					
	mt and $clo-2$	25	18	25	68	26.5
	mt and $w-1$	36	1	31	68	1.5
	$clo-2$ and $w-1$	27	19	22	68	27.9
2.	$++ wa-1 \times w-1 clo-2 +$					
	$w-1$ and $clo-2$	10	16	7	33	48.5
	$w-1$ and $wa-1$	13	12	8	33	36.4
	$clo-2$ and $wa-1$	8	19	6	33	57.6
3.	$wa-1 ++ \times ++ w-2 lys$					
	$wa-1$ and $w-2$	33	10	0	43	23.2
	$wa-1$ and lys	37	6	0	43	14.0
	$w-2$ and lys	38	5	0	43	11.6
4.	$cys(3) gr + \times ++ w-1$					
	$cys(3)$ and gr	5	18	5	28	64.3
	$cys(3)$ and $w-1$	12	5	11	28	17.9
	gr and $w-1$	4	17	7	28	60.7
5.	$w-1 cys(3) gr \times ++ +$					
	$w-1$ and $cys(3)$	9	10	6	25	40.0
	$w-1$ and gr	6	11	8	25	44.0
	$cys(3)$ and gr	3	20	2	25	80.0
6.	$++ un \times clo-2 w-1 +$					
	$clo-2$ and $w-1$	11	13	18	42	31.0
	$clo-2$ and un	13	13	16	42	31.0
	$w-1$ and un	27	4	11	42	9.5
7.	$+ clo-1 \times clo-2 +$	41	...	40	...	...
8.	$wa-1 + \times ++ w-1$	26	17	26	69	24.6
9.	$br + \times ++ w-1$	6	10	6	22	45.5
10.	$w-1 gr \times ++$	82	297	76	455	65.3
11.	$w-1 cys(3) \times ++$	10	20	6	36	55.6
12.	$gr + \times ++ w-1$	61	329	69	459	71.7
13.	$w-1 + \times ++ w-2$	57	94	58	209	45.0
14.	$clo-2 mt^- \times ++ mt^+$	18	6	17	41	14.6
15.	$lys + \times ++ w-1$	19	18	19	56	32.1
16.	$br mt^+ \times ++ mt^-$	5	14	4	23	60.9
17.	$cys(3) + \times ++ w-2$	13	33	10	56	58.9
18.	$wa-1 + \times ++ w-2$	96	25	0	121	20.7
19.	$lys + \times ++ w-2$	81	9	0	90	10.0
20.	$ad w-1 \times ++$	72	40	25	137	29.2
21.	$clo-1 + \times ++ w-2$	28	47	25	100	47.0
22.	$clo-2 + \times ++ w-2$	22	52	28	102	51.0
23.	$+ gr \times w-2 +$	189	770	177	1136	67.8
24.	$w-1 cys(8) \times ++$	17	1	13	31	3.2
25.	$gr + \times ++ wa-1$	11	37	8	56	66.1
26.	$w-1 wa(22) \times ++$	6	13	5	24	54.2
27.	$co(24) w-2 \times ++$	6	15	7	28	53.6
28.	$wa-1 mt^+ \times ++ mt^-$	7	11	8	26	42.3
29.	$un mt^+ \times ++ mt^-$	19	0	0	19	0

TABLE 3 (cont.)

Cross No.	Cross	PD	T	NPD	Total	Percent T
30.	<i>clo</i> (17) + × + <i>w</i> -1	14	0	13	27	0
31.	<i>w</i> -3 + × + <i>w</i> -2	132	197	134	463	42.5
32.	<i>clo</i> (18) + × + <i>w</i> -1	11	13	15	39	33.3
33.	<i>clo</i> -3 + × + <i>w</i> -2	44	1	0	45	2.2
34.	<i>clo</i> -3 + × + <i>w</i> -1	4	5	8	17	29.4
35.	<i>cys</i> (6) + × + <i>w</i> -1	5	15	5	25	60.0
36.	<i>wa</i> (22) + × + <i>clo</i> -1	5	16	7	28	57.1
37.	<i>w</i> -3 + × + <i>w</i> -1	211	171	153	535	32.0

PD: parental ditype tetrad; T: tetratype tetrad; NPD: nonparental ditype tetrad.

whether *clo*-3 is left or right of *w*-2. In linkage group II, the arrangement could be *w*-1-centromere-*ad*. The spore sample in cross 20 was not homogeneous; more data will be obtained to determine the linkage relationship of *w*-1 and *ad*. There is little information about *clo*-1, *clo*-2, *gr* and *br*, except that they do not show linkage with any markers tested, as shown in Table 3. In crosses 1 and 2, *clo*-2 has shown different frequencies of second-division segregation, 13 percent and 23 percent. Variation in crossover frequency in fungi has been reported by various workers, e.g. BARRATT *et al.* (1954), STADLER (1956), PERKINS (1959), in *Neurospora* and OLIVE (1956) in *Sordaria*. It has been indicated that differences in temperature and genetic background influence the crossover frequency.

The leucine (1) mutant produced 4 white and 4 red apparently normal ascospores in each ascus when crossed with a white ascospore mutant, but only four spores per ascus germinated and none were leucine-requiring. Strain 1 could contain a chromosomal aberration. When either *cys*(8) or *cys*(6) was crossed with *w*-1, spore germination was 60 percent and cultures from only a few spores were cysteine requiring. However, when one of the cysteine-requiring descendants, *w*-1 *cys*(8) or + *cys*(6), from each of these crosses was crossed with wild type (++) and with *w*-1 +, respectively, good germination was obtained (95 percent); the results are shown in Table 3 (crosses 24 and 35).

There have been no data available to indicate the relation between *cys*(3), *cys*(8) and *cys*(6). Crosses between any two of these three mutants or their cysteine-requiring progeny usually produced only 2 or 3 viable spores per ascus,

FIGURE 13.—Maps of linkage groups I, II and III based on data from Table 3.

and no wild progeny were recovered. In a cross of *cys*(8) + × + *cys*(6), six cysteine-requiring spores were obtained from each of two asci. Data in Table 3 indicate that all three cysteine mutants are unlinked to *w*-1 and *w*-2 (crosses 4, 24 and 35).

Loss of fertility in vegetative cultures: The original cultures of *A. immersus* collected in Pasadena in March 1963 are still forming abundant apothecia on agar medium after being subcultured vegetatively for more than a year. On the other hand, several white-ascospore mutant strains of *A. immersus* (137, 1604, and 1472), which were isolated by PROFESSOR RIZET and made available to us by DR. MOGENS WESTERGAARD, formed abundant apothecia on yeast extract agar medium at first, but after a period of 4 months they ceased to form apothecia either on yeast extract agar or on sterilized horse dung. When ascospores of strains 137, 1604, and 1472 were isolated from the Petri plate covers of old apothecial cultures, and then crossed, abundant apothecia were again formed. It seems that reisolation of spores from cultures periodically, or preservation of the strains in the form of ascospores, may prevent or delay the loss of fertility in these strains.

Interstrain infertility: Successful crosses have not so far been obtained between the strains of *Ascobolus immersus* used in the experiments reported in this paper and strains obtained from PROFESSOR RIZET. Such crosses do produce apothecia in compatible matings, permitting mating-type identification, but spore production has not followed.

DISCUSSION

The results show that the nutritionally deficient *Ascobolus* mutants, strains 1, 3, 5, 6, 7, and 8 are able to grow when a suitable supplement is added to the basal medium. Within certain limits, the amount of growth in each case is dependent on the amount of supplement present. It appears, therefore, that these strains are true nutritional mutants. The failure of guanine to provide growth of strain 5 shows that guanine is not readily converted to adenine in that strain. Similarly, the failure of L- α -aminoadipic acid to provide growth of strain 7 shows that L- α -aminoadipic acid is not converted to lysine in strain 7.

The requirement of the unknown biochemical mutant (*un*) has not been determined. It grows slowly on minimal medium supplemented with casein hydrolysate. Growth is better on medium containing yeast extract plus casein.

HOROWITZ (1950) has reported that cysteineless and methionineless mutants were the most frequently obtained following irradiation of *Neurospora*. This may also be the case with *Ascobolus*, as three of the eight biochemical mutants require cysteine or methionine for growth.

Because of the preponderance of colonial mutants, one tends to think that the colonial habit of growth offers certain advantage to this fungus under laboratory conditions. So far, it is not known what biochemical changes are responsible for the formation of colonial mutants of this fungus. One possibility is suggested by the four sorbose-type colonial mutants of *Neurospora* which have less glucose and more glucosamine per unit weight of cell wall than wild type (DE TERRA and TATUM 1963).

The *mt* and *w-1* genes will provide valuable markers for future studies of linkage in this fungus because they give low frequency of second division segregation, indicating little crossing over between these genes and their centromeres. Genetic data show that *A. immersus* has a minimum of three chromosomes. Cytological observations have not been made to determine the haploid number of chromosomes.

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SUMMARY

Eight biochemical mutants and 16 morphological mutants have been obtained in the heterothallic ascomycete *Ascobolus immersus* after X-ray or ultraviolet irradiation of ascospores from wild strains. Six of the biochemical mutants require amino acids and one requires a nucleic acid component. There was a preponderance of colonial mutants among the morphologicals. Both the biochemical and morphological mutants are due to mutations of single genes. Genetic data indicate three linkage groups, with two loci in each of groups I and II and four loci in group III. Mating type and *w-1* are closely linked to their respective centromeres.

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