Proc. Nat. Acad. Sci. USA
Vol. 72, No. 10, pp. 4076–4080, October 1975
Genetics

Meiosis in *Phycomyces*

(linkage/map units/centromere)

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Contributed by M. Delbrück, July 28, 1975

ABSTRACT **A four-factor cross between two strains of *Phycomyces* involving two auxotrophic, one color, and the mating type marker is described. Samples of 40 germspores from 84 individual fertile germsporangia were characterized. The results show: (*i*) The germspores of a germsporangium are derived from one meiosis in approximately 78% of the cases. (*ii*) The four markers are on separate chromosomes. They are nonselective. (*iii*) Analysis of a large sample of germspores from 106 pooled germsporangia confirms that the four markers are unlinked. (*iv*) From the ditype/tetratype ratios it is inferred that each marker is located about 15 map units from its centromere.**

Phycomyces, like other mucoraceous fungi, is heterothallic (1). The two mating types, (+) and (−), are indistinguishable morphologically; when they grow near each other a series of mutually induced biochemical and morphological changes takes place (2–5) culminating in the formation of a highly multinucleate zygospore (6). The zygospore, after a long dormancy (2–6 months, depending on the strains), produces a germsporangium containing germspores containing one to six haploid nuclei each, like those in the vegetative sporangium.

The great majority of the germspores are homokaryotic, possibly because they are formed from protospores containing only one nucleus which undergoes mitotic divisions to bring the number of nuclei up to the known multinucleate state (7).

A feature affecting all previous work on sexual genetics was the lack of regularity of the genotypes in the progeny; often the germspores from a single germsporangium were all infertile, usually several of the expected genotypes were missing, and the ones found varied greatly in number. These irregularities made the analysis of the sexual crosses a difficult task. Burgeff in 1928, based on very limited data from crosses involving morphological markers, found that recombinants are formed and suggested that although many hundreds of nuclei of both mating types enter into the zygospore, in general only one diploid nucleus undergoes meiosis followed by a number of postmeiotic mitoses yielding, in the germsporangium, 7,000 to 15,000 germspores. The rest of the nuclei entering into the zygospore were presumed to abort, either after fusing to form diploids or in the initial haploid state. The cytological studies to establish the karyological events have been inconclusive and the genetic data have been too limited to clarify the sexual genetics of *Phycomyces* and, in general, of the Mucorales. Both aspects have been reviewed recently (6, 8).

With the use of new auxotrophic and color markers and parental strains yielding a shorter dormancy of the zygospores, conditions for high and reproducible germination of the zygospores have been established (9). Clear evidence was found that apogamic nuclei do not contribute to the progeny. In addition, the data suggested that a standard meiotic process is operating in the generation of recombinants (9, 10).

To investigate more precisely the nature of the recombinational process in *Phycomyces*, a four-factor cross involving two auxotrophic, a color, and the mating type markers has been analyzed.

MATERIALS AND METHODS

Strains. The parental strains used in the four-factor cross and their pedigree are displayed in Fig. 1.

Media. SIV was used as a minimal medium. It is similar to SI (4) except that SIV contains 2 g/liter of asparagine·H_2O as a source of nitrogen instead of monosodium glutamate. Various complete media and supplements were used as required (9).

Culture Methods and Analysis. The procedure for the sexual cross and the analysis of the genotypes of the progeny has been described (9).

EXPERIMENTAL

The cross: germination data

Fig. 1 shows the pedigree of the parental strains used in the four-factor cross here reported. The two wild types, UBC21 (+) and NRRL1555 (−) from which the parentals were derived, presumably differ in genetic background, since they were isolated in different places at different times. The (+) parental strain of our cross (C242) resulted from a backcross designed to make the genetic background more isogenic.

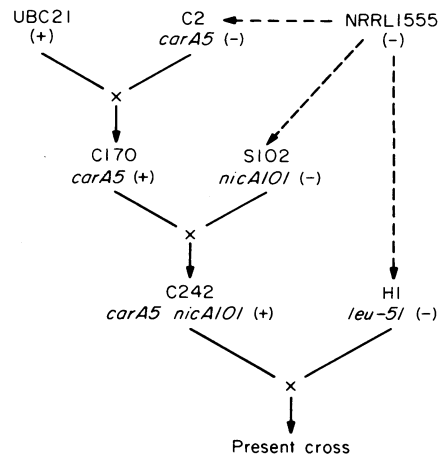

FIG. 1. Pedigree of the strains used. UCB21 and NRRL1555 are the wild types. The genotype is shown below each strain. Continuous lines indicate sexual crosses. Broken lines indicate nitrosoguanidine mutagenesis. *car* designates genes involved in β-carotene synthesis.

Table 1. Progeny samples from 11 germsporangia

Class of germsporangium[b]	Minimal no. of meioses[c]	Viable spores (average)	Genotypes[a]																				Mt[e] het
			I sex	[d]	[d]																		
			II *leu*	+	−	+	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	
			III *nic*	+	+	−	+	+	−	−	−	+	−	+	+	−	−	+	−	+	−	−	+	
			IV *car*	−	+	+	−	+	−	+	−	+	+	−	+	−	+	−	+	−	+	−	+	
1T	1	3000												17									20
1T(6)	1	8000					40																
$2T_{nr}$[f](7)	1	1300			8		31																
$2T_{r}$[g](10)	1	8000																17	22				
3T(14)	1	6000		8						19	3												
4T(28)	1	7000		9		10			11		10												
3T(2)	2	4000				19									17				4				
4T(7)	2	2700		10			3		20								7						
5T(5)	2	2500			14				1					8	14				2				1
6T(3)	2	3500				2	2			6				9						2	18		1
7T(1)	3	3000			1			2	16	8				4					1		8		

[a] The 16 possible genotypes are arranged in pairs of reciprocals. The wild-type allele is represented as +, the mutant allele as −. In the sex marker + represents mating type (+) and − mating type (−).

[b] These class designations indicate the number of genotypes found in the sample taken. In parentheses the number of germsporangia in this class. The first germsporangium listed is unusual in yielding about 50% mating type heterokaryons. One germsporangium (not listed) yielded 100% mating type heterokaryons.

[c] A single meiosis produces at most two genotypes with the same allele of any one gene. By this criterion some samples require at least two, and one requires at least three meioses.

[d] Parental genotypes.

[e] Mating type heterokaryons.

[f] $2T_{nonreciprocal}$.

[g] $2T_{reciprocal}$.

A total of 128 zygospores were set out on water agar. One hundred twelve (88%) germinated and produced germsporangia. Among the germinated zygospores, 84 (75%) yielded germsporangia with viable spores. The remainder yielded no viable spores. Microscopic inspection showed that these sterile sporangia did contain a large number of spores. An additional set of 109 germsporangia were pooled, and the germspore count gave an average of 15,000 per germsporangium. The viability of these pooled germspores on rich acid medium (9) was found to be 40%.

The shortest dormancy, defined as the time elapsed from the day at which mating plates were inoculated to the germination of the first zygospore, was 100 days, about 40 days longer than the shortest dormancy in the cross between the two progenitor wild types, UBC21 and NRRL1555. The reason for this lengthening of the dormancy is probably that C242 was selected, in the backcross of its origin, for a particular combination of markers and not for shortest dormancy. It has been shown that dormancy is determined polygenically (9).

Number of meioses per germsporangium

The germsporangia are classified according to the number of different genotypes found in the samples taken (1T, 2T, . . .). In the case of a germsporangium yielding two genotypes, the two genotypes may be either a reciprocal pair (class $2T_{reciprocal}$) or they may not be reciprocal (class $2T_{nonreciprocal}$). This classification scheme ignores the occurrence of mating type heterokaryons. Table 1 gives, as an example, the analysis of 11 germsporangia according to this classification. For each germsporangium the number of segregants in each genotypic class is given.

In a single meiosis the two alleles of each gene segregate 2:2 (except for conversions). Therefore, each allele of any gene is represented in not more than two of the meiotic products. Germsporangia are classified as resulting from one meiosis if they are compatible with this rule. Otherwise two or more meioses must be assumed. Table 1 shows (first column) the numbers of germsporangia in each class. The majority of them, 65 of 83, are compatible with a single meiosis; 17 require at least two meioses, and only one at least three. These numbers are lower limits, since more than the indicated number of meioses may have occurred in any one germsporangium, with none of the products of the extra meioses being found in the sample taken. However, this source of error is certainly small in view of the fact that the largest class found is that exhibiting four genotypes compatible with a single meiosis. On a random basis this would be a very unlikely event in a four-factor cross with 16 possible genotypes (one out of 35 cases).

Linkage tests

Linkage Between Markers. The average aspects of the recombination mechanisms in *Phycomyces* can be studied either by analyzing a large sample of germspores from a pool of germsporangia or by analyzing samples of germspores from individual germsporangia. The first procedure is simpler but it gives information only about the linkage of the markers. The second procedure determines the frequencies of the different types of germsporangia (1T, 2T, . . ., etc.), giving, therefore, more information about the meiotic process. Only the presence or absence of the various genotypes in the samples tested are counted in order to minimize the effects of secondary mechanisms, such as asynchrony in the postmeiotic divisions of the four haploid products (9).

Table 2 shows the distribution of the parental alleles in the progeny using the two procedures. Both procedures show

Table 2. Parental alleles in the genotypes of the progeny

	carA	*carA*$^+$	*nic*	*nic*$^+$	*leu*	*leu*$^+$	(+)	(−)
No. of genotypes containing the allele in 84 individual germsporangia[a]	136	142	130	148	128	150	135	143
Allele ratio	0.96		0.88		0.85		0.94	
A sample of 500 germspores from 106 pooled germsporangia[b]	242	257	254	256	247	252	248	251
Allele ratio	0.94		0.95		0.98		0.99	

[a] For each allele and each germsporangial sample the number of genotypes containing that allele is counted, irrespective of the number of representatives of that genotype in the sample.
[b] For each allele the number of germspores is counted whose genotype contains that allele.

that the two alleles of each marker gene appear in the progeny in a ratio not differing significantly from 1:1, i.e., they show the absence of allele specific selection.

In Table 3 linkage tests for each of the six possible pairs of the four markers are shown. In no case is linkage clearly indicated, as seen by the approximate 1:1 ratio between parentals and recombinants. The statistical significance of the ratios has been tested by the χ^2 method. Here again, both procedures of analysis give the same results.

Centromere Distances. For a cross involving a pair of unlinked factors, i and k, there exists the fundamental relation (11)

$$t_{ik} = s_i + s_k - \frac{3}{2}s_i s_k,$$

where s_i and s_k are the frequencies of second division segregation of factors i and k (a mapping function for the centromere distances of the factors), and t_{ik} is the frequency of tetratype meioses with respect to the pair i,k.

In organisms permitting observation of all four products of individual meioses the t_{ik} can be determined directly. To obtain the s values from them two factors are not sufficient (two unknowns and one equation). Three factors yield three equations with three unknowns. These can be solved algebraically to determine the three unknowns except, in some cases, for square root ambiguities (12). In a cross with four unlinked factors (our case) one has six equations (six pairs of factors) for four unknowns. The equations can be solved by a computer program designed for nonlinear least square fitting. The errors indicated by the program give an indication of the consistency of the data with the assumptions of regular meioses and nonlinkage of the markers.

To obtain the input values t_{ik} we classified each germsporangial sample compatible with one meiosis as to whether the tetrad was ditype or tetratype with respect to each marker pair. Two calculations are given. Case (A): omitting cases requiring assumptions about the genotypes lost; Case (B): for the samples showing only one genotype, or two nonreciprocal ones, or three, we assumed that genotypes had been lost as explained in the next section. In the very few cases where the classification was ambiguous we assigned equal probability to ditypes and tetratypes.

Table 3. Tests for linkage between factor pairs

	Occurrence in the 84 individual germsporangia[b]			Occurrence in a sample of 500 germspores from a pool of 106 germsporangia[c]		
Markers	Parentals	Recombinants	P/R	Parentals	Recombinants	P/R
leu-51 and mt[a]	123	155	0.80	281	218	1.29
nicA101 and *leu-51*	118	160	0.74	248	251	0.99
carA5 and *nic A101*	142	136	1.04	230	269	0.86
carA5 and mt	135	143	0.94	249	250	1.00
carA5 and *leu-51*	132	146	0.90	277	222	1.25
nicA101 and mt	153	125	1.20	246	253	0.97
Total	803	865		1531	1463	

[a] Mating type.
[b] For each allele combination and each germsporangial sample the number of genotypes containing that combination is counted, irrespective of the number of representatives of that genotype in the sample.
[c] For each allele combination the number of germspores is counted whose genotype contains that combination.

In this way we obtained the tetratype frequencies listed in Table 4. The computer program then yielded the following frequencies of second division segregation for each factor:

	Case (A)	Case (B)
s_{mt}	0.160 ± 0.039	0.309 ± 0.013
s_{leu}	0.257 ± 0.032	0.224 ± 0.015
s_{nic}	0.426 ± 0.027	0.418 ± 0.011
s_{car}	0.190 ± 0.036	0.200 ± 0.016

In view of the small sample (38 and 65 crosses for each factor pair), the fit is fortuitously good. The two calculations give similar results. It is reasonable to conclude that all s_i are

Table 4. Tetratype frequencies for each factor pair

$t_{leu, nic}$	$t_{leu, car}$	$t_{nic, car}$	$t_{mt, leu}$	$t_{mt, nic}$	$t_{mt, car}$
(A) Omitting 1T, 2T nonreciprocal, and 3T cases					
21/38	14/38	18/38	13/38	18/38	12/38
0.553	0.368	0.474	0.342	0.474	0.316
(B) Including 1T, 2T nonreciprocal, and 3T cases					
32.5/65	23.5/65	31.5/65	27.5/65	35.5/65	27/65
0.500	0.362	0.485	0.423	0.546	0.415

around 0.3, and the corresponding centromere distances around 15 map units. These results reinforce the nonlinkage between the factors, as inferred in the preceding section from factor recombination frequencies.

Genotype losses

The cross reported here shows conspicuously better genotype recovery than earlier ones (refs. 9 and 10, and unpublished ones). Possibly this better recovery is due to greater isogenicity of genetic background (see pedigree, Fig. 1). In fact, the losses are so modest that one feels encouraged to attempt a closer analysis of them. It turns out that the data cannot be fitted satisfactorily by assuming random losses of the four products of the second meiotic divisions. They can be fitted, however, by assuming that, in addition, random losses of the two nuclei produced by the first meiotic division occur with a uniform probability of 0.1–0.2 for loss of any one nucleus throughout the meiotic cycle; a good fit is obtained for all classes of samples.

This loss rate cannot account for the 25% fraction of germsporangia found to be sterile. A separate loss mechanism, applicable to diploid nuclei, appears to be operative. Perhaps this mechanism is the same as that which reduces the average number of meioses so close to one.

Postmeiotic mitoses

The average number of germspores is about 15,000, of which 40% are viable. Since these spores originate in most cases from one meiosis, each primary meiotic product must go through about 12 postmeiotic mitoses if all four products proliferate. If some genotypes are lost, the remainder seem to go through perhaps one more mitotic cycle, since the germsporangia yielding fewer genotypes give very similar numbers of viable spores (Table 1). The postmeiotic proliferation appears to be remarkably synchronous in those cases (the majority) where no genotypes are lost (the ten germsporangia with two reciprocal genotypes and the 28 with four types compatible with one meiosis). In fact, in these cases the disparities between the numbers found could be due almost entirely to the small sample size.

Mating type heterokaryons

In most of the germsporangia mating type heterokaryons are absent, or present in very small numbers, as reported by most earlier observers. These mating type heterokaryons have been interpreted by the assumption that occasionally the protospore encloses two nuclei and these two nuclei may then be of different genotype. However, a second mechanism of origin is suggested by the finding that in two germsporangia mating type heterokaryons occurred as a large fraction of the germspores (50% and 100%, Table 2). We have encountered this phenomenon in our earlier, less isogenic crosses and had tended to interpret it as indicating the production of many recessive lethal recombinants. This explanation seems now unlikely, in view of the low loss rate of genotypes in the present cross.

DISCUSSION

Our four-factor cross shows clearly that in the zygospores of *Phycomyces* there occurs a standard meiosis yielding ditype and tetratype tetrads, implying four-strand crossingover. In the majority of the cases only one meiosis per zygospore contributes to the germspores, in agreement with Burgeff's (7) conjecture. Both conclusions are reached "blindly," on the basis of recombination data for single germsporangia. The germsporangium serves, as it were, as an enlarged tetrad, so much so, in fact, that it would now seem profitable to look for spore color or shape markers. *Phycomyces*, like most fungi, has chromosomes too small to be made visible by conventional means. Indeed, the where and when of karyogamy and meiosis are still unknown. Perhaps the newer methods for visualizing the synaptinemal complex (13), a universal and distinct feature of meiosis, could be applied to search for zygotene nuclei in zygospores of various stages of development.

Phycomyces vies with other fungi for having the smallest eukaryotic DNA complement per genome, about seven times that of *Escherichia coli* (14). That it also has, like yeast, quite a few chromosomes, is suggested by the fact that no linkage was found for the present factors, nor among those reported by Cerdá-Olmedo (10) with two possible exceptions. It would appear that once the genome size is increased to an extent requiring more than one chromosome, the gulf from the nonnucleated to the nucleated organism must be crossed and that then chromosome sizes may again decrease.

The gap that separates the prokaryotes from the eukaryotes is getting wider as research probes more deeply. As soon as more than one chromosome is needed to carry the genome we find, all at once: the nuclear envelope, the mitotic apparatus, and the sexual processes of karyogamy and reduction. Reduction occurs, in fact, in the very rigid form leading from a four-strand mitotic prophase to a meiotic tetrad. Why this particular form for recombination evolved to replace the various prokaryotic forms of genetic exchange is still unexplained, and may be expected to remain so until its mechanisms, especially the synaptinemal one, are better understood.

We thank E. D. Lipson, J. Reissig, and E. Cerdá-Olmedo for helpful discussions, and Jeannette Navest and Doris Tsongas for technical assistance. A.P.E. was supported by a fellowship of The Commission for Educational Exchange between Spain and the U.S.A. The research was supported by N.S.F. Grant no. BMS 75-11316.

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