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The genetics of *Phycomyces blakesleeanus*

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SUMMARY

The genetic relationship between germspores and parental mycelia has been investigated in crosses involving multiply-marked heterokaryons and in simple difactor crosses.

It is concluded that for each zygospore the number of different diploid nuclei undergoing meiosis is often more than one, but generally not more than two. The meiotic products divide to produce the nuclei in the usually uninucleate germspore primordia and then continue dividing to produce germspores. Unmated nuclei do not pass directly from parents to progeny.

Each germsporangium contains differing numbers of viable germspores, with an average of about 7000; different genotypes are represented by varied numbers of germspores and often expected genotypes are missing. When the results of several germsporangia are added together or germspore stocks from many germsporangia are analysed, reciprocal genotypes appear in equal numbers and reliable recombination frequencies may be calculated.

1. INTRODUCTION

The mycelia of *Phycomyces* and other Mucorales may be classified in mating types (+) and (−) (Blakeslee, 1904). When mycelia of different mating types grow near each other, a series of biochemical and morphological changes occurs (Van den Ende & Stegwee, 1971; Sutter, 1975), leading to the development of specialised hyphae and the formation of separate cells, the gametangia, at their tips. The fusion of two gametangia of opposite mating type forms the zygospore. After a long dormancy period the zygospore germinates, producing a large aerial hypha, the germsporangiophore, which carries a germsporangium at the tip, containing the germspores. Germspores are similar in appearance to the regular vegetative spores (sporangiospores) of the asexual cycle and are able to produce normal mycelia.

The genetic problem is to relate the genetic constitution of the many nuclei in the gametangia with the genetic constitution of the germspores. This problem was attacked by Hans Burgeff using a number of spontaneous genetic variants (Burgeff, 1915, 1928). He proposed the hypothesis that a single diploid nucleus, formed by the fusion of one haploid nucleus from each parent, undergoes meiosis to form four haploid products, which, after many divisions, become the nuclei of germspore

primordia. Most germspores begin as uninucleate cells and become multinucleate after further nuclear divisions. Germspore formation thus differs from sporangiospore formation, which consists in the packing together of several preformed nuclei, without subsequent mitosis (Swingle, 1903; Heisenberg & Cerdá-Olmedo, 1968). I have previously reviewed the cytological and genetical research on the sexual cycle of *Phycomyces* (Bergman *et al.* 1969; Cerdá-Olmedo, 1974), including some of the results presented in this paper.

2. MATERIALS AND METHODS

(i) *Strains*

Wild-type strain NRRL1555, mating type (−) is the standard strain for behavioural and biochemical studies. Other wild types are NRRL1554 and UBC21, both mating type (+), and UBC24, mating type (−). Strains C2, genotype *carA5* (−), C5, genotype *carB10* (−), and C9, genotype *carR21* (−), derive from NRRL1555. Strain C63, genotype *mad-54* (+) derives from NRRL1554. Strain C170, genotype *carA5* (+), derives from the cross UBC21 × C2, carried out by S. Trubatch. Strain S18, genotype *carA57 madC209* (−), derives from UBC24; strain S80, genotype *carR21 aux* (−), derives from C9; strain S82, genotype *xtv-51* (+), derives from UBC21. C strains are from the collection at the California Institute of Technology, S strains from the Departamento de Genética, Universidad de Sevilla. The symbol *car* refers to mutations affecting the synthesis of carotenoids; mutation *carR21* results in red colour, and the others in white colours. The symbol *xtv* indicates resistance to crystal violet; *aux*, an undefined auxotrophy, allowing growth in medium supplemented with yeast extract, but not on minimal medium; *mad*, mutations altering the phototropic behaviour of the sporangiophores.

(ii) *Crosses*

Table 1 indicates the parental strains used in the five crosses studied and their genotypes; stars separate heterokaryon components. Spores or mycelial bits of the parents were inoculated on opposite sides of a plate containing potato dextrose agar (Difco, or prepared in the laboratory), and incubated at 17° C, under constant dim illumination. Zygospore age is measured from the day when parental strains were innoculated, even though zygospores actually formed from four to eight days later. At an age of 2–8 weeks the zygospores were scraped from the plates with a spatula, cleaned of adherent mycelia, placed one by one on germination plates, and incubated at room temperature in a translucent box containing water jars to increase humidity and slow desiccation of the plates. Germination plates contained 10 g of Ionagar or Noble agar (Difco) per litre distilled water. The germination plates for Cross 5 were obtained by mixing autoclaved solutions of SiO_3H_2 and SiO_3Na_2 at a final pH of about 6 and a final SiO_2 concentration of about 15 g/l. If the plates became very contaminated, zygospores were removed to new germination plates.

Zygospores close to germination were often moved to agar- or silicate-containing

vessels deeper than Petri plates so that germsporangia would not collide with the cover of the plates. Individual germsporangia were collected by picking them up with tweezers carrying a drop of sterile water, crushing them and dropping them into a tube containing 1 ml of distilled sterile water. In the tables they are numbered in their relative order of germination. Mixed stocks were obtained by adding many individual germsporangia to the same tube.

Table 1. *Crosses studied in this work*

Cross	Strains	Genotypes
1	NRRL1554 × (C2 * C9)	(+) × {*carA5* (−) * *carR21* (−)}
2	C63 × (C5 * C9)	*mad-54* (+) × {*carB10* (−) * *carR21* (−)}
3	S82 × (S18 * S80)	*xtv-51* (+) × {*carA57 madC209* (−) * *carR21 aux* (−)}
4	C170 × NRRL1555	*carA5* (+) × (−)
5	S116 × NRRL1555	*carA5* (+) × (−)

(iii) *Germspore analysis*

Aliquots of the germspore suspension were plated on glucose–asparagine–yeast extract agar (Heisenberg & Cerdá-Olmedo, 1968), on which mycelia spread freely, or on the same medium acidified to pH 3·3, on which well-defined colonies were formed. Upon incubation at room temperature (20–25 °C), mycelia were marked as they became visible to the naked eye. Final counting and colour attribution were done after 4 days. Approximately the same viable counts were obtained on normal and acidified media or following the shell vial technique (Heisenberg & Cerdá-Olmedo, 1968).

To determine mating type, spore stocks of wild-type strains NRRL1555 and NRRL1554 were placed on opposite sides of a potato dextrose agar plate, and a piece of the problem mycelium was transplanted to the centre of the plate; sexual reactions were observed after 4–6 days' incubation at 17 °C. To prepare these plates, some 10^2 spores of the mating type standards can be placed on opposite sides of a sterile cloth and pressed against a series of plates following the replica plate technique. Phototropic behaviour can be tested by growing the problem mycelia in a glass-bottom box, where all light comes from below, and observing the direction of sporangiophore growth. Auxotrophy and crystal violet resistance can be tested by transplanting pieces of mycelium to medium lacking yeast extract or containing crystal violet (1 μg/ml). In the difactor crosses, viability and colour distribution were obtained by plating an aliquot of the germspore suspension, and a sample of the resulting mycelia, not necessarily random for colour, was then tested for mating type.

Heterosexual heterokaryons, involving nuclei of both mating types, characteristically exhibit short aerial hyphae (pseudophores) and few sporangiophores, especially on potato dextrose agar. Heterokaryons segregate the constituent types and thus may be detected by spreading their sporangiospores on acid medium and testing the resulting mycelia. Heterokaryons containing complementing *car* mutations are yellow, like the wild type, but they often synthesize peculiar carotene mixtures and have a recognizable appearance (De la Guardia *et al.* 1971). Hetero-

karyons are quite stable and maintain their nuclear proportions during vegetative growth (Heisenberg & Cerdá-Olmedo, 1968).

3. RESULTS

(i) *Zygospore germination*

When the parents were derived from strains NRRL1554 and NRRL1555, the zygospores remained dormant for more than 5 months. Thus 400 zygospores from Cross 1 were picked out of the cross plates at an age of 2 months and placed on

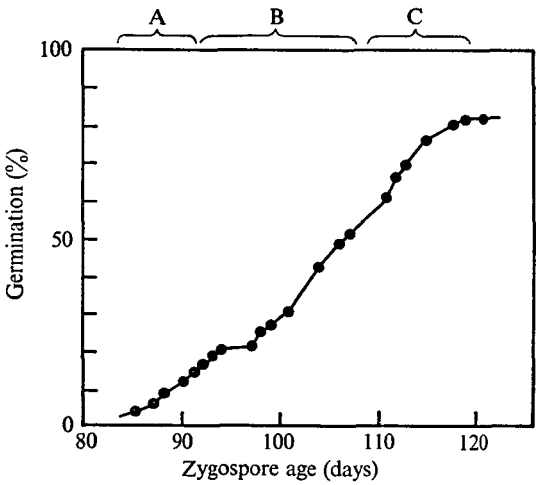

Fig. 1

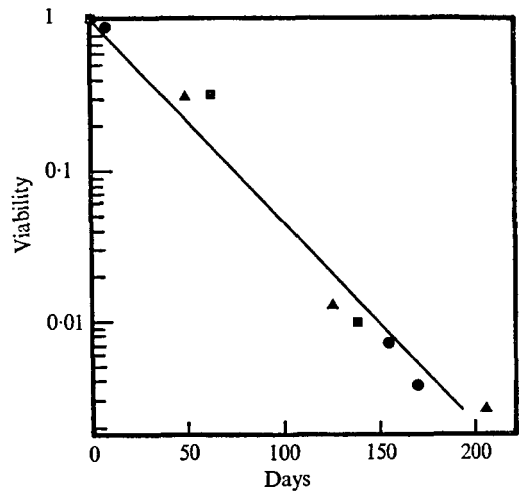

Fig. 2

Fig. 1. Cumulative distribution of germination of 200 zygospores from cross 4, harvested at an age of 12 days, refrigerated for another week, and placed on germination plates. Final germinability was 80 %.

Fig. 2. Viability of concentrated germspore stocks kept in the refrigerator in screw-capped tubes with their caps slightly unscrewed. The germspores were collected in the periods indicated by brackets in Fig. 1. The number of germsporangia and the initial absolute viability are given in Table 8. ●, stock A; ■, stock B; ▲, stock C.

germination plates; 200 of them had just been freed from the suspensors. Zygospores started germination at an age of $5\frac{1}{2}$ months and most of them germinated within another month. At an age of 9 months 140 suspensor-covered zygospores and 143 suspensor-freed zygospores had germinated. Thus average germinability was 70 %, and the removal of the suspensors, while conducive to greater cleanliness and lower plate contamination, was shown to have no influence on dormancy duration.

E. W. Goodell, at the California Institute of Technology, studied zygospore dormancy in many pairs of wild-type strains of *Phycomyces* and concluded that the zygospores of Crosses UBC21 × UBC24 and UBC21 × NRRL1555 had the shortest dormancies (unpublished results). In our experimental conditions these

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zygospores started germination at an age of less than three months (Fig. 1). Dormancy duration is influenced by temperature; zygospores exposed to room temperatures above 26 °C never germinated.

Sometimes zygospores are contaminated by sporangiospores, which may germinate and even produce sporangiophores in 2 or 3 weeks. The resulting spores are all identical to one of the parental types. This may have been the basis for the early zygospore germination reported by Hocking (1967).

Germspores were plated either immediately after collection or within a few days; there were indications that very diluted germspore suspensions rapidly lose viability even when kept in the refrigerator. The viability of concentrated germspore stocks is depicted in Fig. 2.

The number of viable germspores per germsporangium was extremely variable; for 157 germsporangia of Crosses 4 and 5 the mean was about 7000, but the standard variation was close to the mean.

Very few viable germspores were recovered when zygospores were allowed to germinate in Petri plates, crashing their germsporangia against the lid, from which they were later washed off. Thus, from the lid of a plate with 15 germsporangia, 2000 viable germspores were collected; from the lids of four plates totalling 69 germsporangia, only 400 viable germspores were collected.

Table 2. *Mycelial colours from germspores of three-colour crosses of the type Yellow × (White * Red)*

	Number of germsporangia			
Colours	Cross 1	Cross 2	Cross 3	Total
Yellow, white and red	1	2	1	4
Yellow and white	5	8	8	21
Yellow and red	0	0	2	2
White and red	0	0	0	0
All yellow	25	2	7	34
All white	0	0	3	3
All red	0	0	0	0
Sterile	7	?	11	?

(ii) *Three-colour crosses*

In these crosses the (+) parent is wild-type for colour, while the (−) parent is a heterokaryon carrying markers for albino and red mycelial colours in separate nuclei. Judging by their colour, the heterokaryons used in this work contained many more albino-marked nuclei than red-marked nuclei.

Table 2 indicates the mycelial colours produced by germspores from individual germsporangia of the three different crosses analysed. Many of the germsporangia were completely sterile and others contained a small number of viable germspores.

Tables 3 and 4 give the results of detailed genotypic analyses of germspores from individual germsporangia of Crosses 1 and 2, respectively. Heterokaryosis for mating type and colour markers was investigated. Some mycelia were pheno-

typically abnormal. Thus a germsporangium from Cross 2 gave rise to about 1000 mycelia, 79% yellow and the rest white, but they all grew very slowly and eventually had a very irregular texture with many bumps and creases, and their sporangia were nearly sterile.

Table 3. *Genotypes of germspores from individual germsporangia of Cross 1*

	Germsporangium number			
Homokaryons	1	2	3	4
(+)	27	16	6	12
(−)	41	0	4	4
carA5 (+)	0	0	0	1
carA5 (−)	3	4	2	1
Heterokaryons	None	†	None	‡

† 8 (+) * *carA5* (+), 1 (−) * *carA5* (−).
‡ 2 (+) * (−), 1 (+) * *carA5* (−).

Table 4. *Genotypes of germspores from individual germsporangia of Cross 2*

	Germsporangium number						
	1	2	3	4	5	6	7
Viable germspores	20	550	200	80	35	100	6
Homokaryons:							
(+)	1	0	0	2	2	1	1
(−)	2	0	0	1	0	49	1
carB10 (+)	0	0	26	3	6	6	2
carB10 (−)	3	65	7	2	1	0	0
mad-54 (+)	0	49	21	7	9	0	0
mad-54 (−)	0	0	28	3	6	0	0
carB10 mad-54 (+)	0	0	2	0	1	0	0
carB10 mad-54 (−)	0	0	7	18	0	0	0
carR21 (−)	2	0	0	0	0	0	1
Heterokaryons	†	‡	§	0	‖	0	¶

Other possible genotypes were not found.
† 12 *carB10* (−) * *carR21* (−).
‡ 1 *carB10* (−) * *mad-54* (+).
§ 1 *carB10* (−) * *mad-54* (+).
‖ 5, genotype undetermined.
¶ *1 carB10* (−) * *carR21* (−).

Many germspores from Cross 3 were only partially analysed, but in most germsporangia at least one allele was present in more than two genotypic combinations. For example, in a germsporangium, 22 germspores were found with the genotype *madC209* (+), 9 *carA57* (+), and 4 *carA57 madC209* (−), and in another, 28 *carA57 madC209 xtv-51* (+), 3 *carA57 madC209 xtv-51* (−), 1 *carA57 madC209* (−), 1 *carA57 xtv-51* (+), 2 *madC209 xtv-51* (+), 4 *madC209 xtv-51* (−), 2 *madC209* (−), and 1 *xtv-51*(+).

Strain S18, involved in Cross 3, was obtained from wild-type UBC24 after a single mutagenesis step. It was thus interesting to find out whether its two phenotypic traits, white colour and abnormal phototropism, were due to a single mutation. Table 5 analyses the germspores in seven germsporangia of Cross 3 as to these characteristics and shows that they must be due to separate mutations, lying presumably in the same chromosome.

(iii) *Difactor crosses*

The strains involved in these crosses differ as to sex and colour. Strain C170 derives from Cross UBC21 × NRRL1555, and strain S116 from Cross C170 × NRRL1555. These two (+) strains are thus expected to be more akin to the (−) strain than the original wild-type UBC21.

Table 5. *Recombination between genes* carA *and* madC *in Cross 3*

(Number of germspores with the indicated partial genotype; all germsporangia given equal weight for the average proportion).

Partial genotype	Germsporangium number 1	2	3	4	5	6	7	Average proportion
carA57 madC209	4	17	33	1	1	0	56	0·38
carA57	9	0	0	23	0	0	4	0·12
madC209	22	0	2	4	0	0	24	0·15
Wild type	0	0	4	14	23	58	2	0·35

Only 3 germsporangia out of 20 in Cross 4 were completely sterile; one germsporangium produced only 15 very slow-growing mycelia; each of the others produced apparently normal mycelia of both colours. Table 6 analyses the germspores in 14 of these germsporangia coming from all periods of the germination process described in Fig. 1. No heterokaryons were found in any of them. The yellow (−) mycelia from germsporangium number 14 reacted normally with strain NRRL 1554 but the yellow (+) mycelia reacted very weakly with strain NRRL1555 and five yellow mycelia failed to react altogether with either of the standard mating types

Only one germsporangium out of 16 in Cross 5 was completely sterile; Table 7 analyses the germspores in 12 of the other germsporangia. Very few heterosexual heterokaryons were detected: one each in germsporangia number 1 and 4. Again the whole contents of some germsporangia were 'abnormal'. Thus, one germsporangium contained some 7000 viable germspores; 34% of them gave rise to yellow mycelia, and the others to white mycelia, but all grew very slowly, seldom branching and their sexual reactions were incomplete. Another germsporangium contained about 200 germspores, all giving rise to white mycelia with the characteristic surface of heterosexual heterokaryons and failing to react sexually with either of the two opposite mating types; their sporangiospores produced normal yellow (+) and (−) mycelia and white mycelia with the same abnormalities. No

Table 6. *Genotypes of germspores from individual germsporangia of Cross 4*

	Germsporangium number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Viable germspores	50	300	400	300	10000	8000	10000	6000	2000	20000	8000	30000	20000	10000
Phenotypes														
Yellow	4	92	19	58	279	98	136	163	9	137	66	73	116	195
White	18	75	233	48	176	252	199	64	357	65	143	61	89	94
Yellow (+)	3	7	6	18	24	18	30	31	6	0	29	17	19	14
Yellow (−)	0	16	0	15	0	11	0	0	0	22	0	11	11	15
White (+)	0	17	24	26	0	18	0	0	0	19	0	12	29	0
White (−)	17	1	17	1	30	12	29	26	28	0	28	18	0	13
Genotype proportions†														
(+)	0·18	0·17	0·08	0·30	0·61	0·17	0·41	0·72	0·02	0	0·32	0·33	0·36	0·33
(−)	0	0·38	0	0·25	0	0·11	0	0	0	0·68	0	0·21	0·21	0·34
carA5 (+)	0	0·42	0·54	0·44	0	0·43	0	0	0	0·32	0	0·18	0·43	0
carA5 (−)	0·82	0·02	0·38	0·02	0·39	0·29	0·59	0·28	0·98	0	0·68	0·27	0	0·33

† Wild-type and *carA5* genotypes were assigned according to the observed yellow and white phenotypes. The resulting proportions were distributed as to mating type according to the tests carried out separately on samples of each colour.

segregation or sectoring was observed by growing these white mycelia on media containing different concentrations of *p*-fluorophenylalanine, a compound which might induce the somatic segregation of heterozygous diploids. Still another germsporangium contained about 100 viable germspores, all giving rise to yellow mycelia, with few short and thick sporangiophores, and failing to react sexually with either

Table 7. *Genotypes of germspores from individual germsporangia of Cross 5*

	Germsporangium number											
	1	2	3	4	5	6	7	8	9	10	11	12
Viable germspores	40	4000	2000	8000	4000	1000	4000	100	8000	2000	100	1000
Phenotypes:												
Yellow	7	138	50	127	124	20	125	30	232	38	62	88
White	15	74	50	92	0	37	79	20	60	53	0	66
Yellow(+)	6	16	8	8	40	11	12	0	0	20	0	29
Yellow (−)	0	22	16	22	0	5	17	23	40	6	30	0
White (+)	6	21	12	13	0	14	10	17	20	1	0	0
White (−)	11	0	12	16	0	14	15	0	0	32	0	19
Genotype proportions†												
(+)	0·32	0·27	0·17	0·15	1	0·24	0·25	0	0	0·32	0	0·57
(−)	0	0·38	0·33	0·43	0	0·11	0·36	0·60	0·79	0·10	1	0
carA5 (+)	0·24	0·35	0·25	0·19	0	0·32	0·15	0·40	0·21	0·02	0	0
carA5 (−)	0·44	0	0·25	0·23	0	0·32	0·23	0	0	0·56	0	0·43

† Calculations as in Table 6.

Table 8. *Genotypes of germspores from mixed stocks and averages from individual germsporangia analyses*

	Germspore stocks			Average	Average from Table 6	Average from Table 7
	A	B	C			
Pooled germsporangia	17	59	45	—	—	—
Viable germspores	$2{\cdot}6 \times 10^4$	$6{\cdot}2 \times 10^5$	$3{\cdot}3 \times 10^5$	—	—	—
Phenotype						
Yellow	147	137	53	—	—	—
White	135	176	46	—	—	—
Yellow (+)	14	16	11	—	—	—
Yellow (−)	20	13	16	—	—	—
White (+)	13	17	4	—	—	—
White (−)	13	12	17	—	—	—
Genotype proportions†						
(+)	0·21	0·24	0·22	0·22	0·29	0·27
(−)	0·31	0·20	0·32	0·28	0·16	0·34
carA5 (+)	0·24	0·33	0·09	0·22	0·20	0·18
carA5 (−)	0·24	0·23	0·37	0·28	0·36	0·21
Recombination frequency	0·45	0·47	0·59	0·50	0·65	0·48

† Calculations as in Table 6.

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of the opposite mating types. Their sporangiospores segregated out normal yellow (+) and (−) mycelia, normal white (+) mycelia and white mycelia reminiscent of those described for the previous germsporangium.

Table 8 indicates the genotypes in germspore stocks from many germsporangia of Cross 4, collected at the times indicated in Fig 1. Again, heterosexual heterokaryons were rare: two, genotype (+) * (−), out of 124 germspores of stock A, and another out of 65 germspores of stock B. No colour heterokaryons were detected. The same table includes the averages from Tables 6 and 7, obtained by giving equal weight to all germsporangia; if these are counted in proportion to their viable germspore content, the final averages are not very different. The recombination frequencies, around 50 %, indicate that gene *carA* and the mating-type determinant are unlinked.

4. DISCUSSION

(i) *The genetic system of* Phycomyces

The results are incompatible with the hypothesis that the meiosis of a single diploid nucleus always originates all the germspores in a germsporangium. In the three-colour crosses, the progeny of a single diploid nucleus should be of two different colours, presumably yellow and white, or yellow and red, but four germsporangia have been found containing all three colours. At least for these cases, the progeny must have resulted from more than one haploid nucleus from each parent.

With n allele pairs, a total of 2^n genotype combinations are possible. A single meiosis, however, cannot lead to more than four different genotypes, and each allele cannot be found in more than two different genotypes. Most germsporangia from Crosses 2 and 3 contradicted these criteria, requiring the occurence of more than one meiosis.

The existence of a single meiosis is contradicted by more germsporangia than is the existence of a single diploid nucleus. It is possible that diploid nuclei divide mitotically before undergoing meiosis. It is more likely, however, that the proportion of three-colour germsporangia has been underestimated, since many germsporangia contain a single colour, instead of the expected two or three, and since the heterokaryotic parents were imbalanced, containing few red-marked nuclei.

The results are not as beautifully symmetric as characteristic meiotic results. Expected alleles and allele combinations are missing from the progeny of many germsporangia and relative frequencies of different genotypes are very irregular.

These irregularities may be due to the existence of a period of mitotic divisions converting haploid meiotic products into germspore nuclei, if the different genotypes exhibited very different growth rates.

It is more likely that the wild types used in our investigation, probably coming from different continents, differ in many gene mutations and chromosome rearrangements; meiosis would then often result in lethal or sublethal combinations. A detailed and illuminating study of chromosome rearrangements in *Neurospora* has been carried out by Perkins (1974). Many of Burgeff's 'mutants' were isolated among the progeny of sexual crosses and may contain chromosome rearrangements

or new gene combinations, rather than specific gene mutations, adding a new difficulty to the interpretation of his results. It is thus important that (+) and (−) isogenous strains be developed by repeated back-crossing of strain UBC21 to the standard wild-type NRRL1555, while selecting for short dormancy. Alternatively (+) and (−) strains with short dormancies could be isolated from the same locality.

Crosses 4 and 5, involving partially consanguineous parents, yield more regular results than crosses involving wild types. In Tables 6 and 7, three germsporangia may be classified as parental ditypes, 7 as nonparental ditypes, 9 as tetratypes, and 7 as irregular (2 monotypes and 5 tritypes). Probably some of the irregular types would have been classified as regular if more germspores would have been analysed. The existence of ditypes proves that the number of separate meiotic processes per zygospore must be small: even if no tetratypes were formed at meiosis (markers at centromeres of different chromosomes), the occurrence of just two meiotic processes would result in 50 % tetratypes. Thus two may be taken as a likely upper limit for the usual number of meiotic processes per germsporangium.

The irregular results of the three-colour crosses suggested that the zygospore of *Phycomyces* might encompass a parasexual cycle involving diploidization, mitotic recombination, and haploidization. This suggestion must be rejected, since reciprocal genotypes, particularly non-parental ditypes, are very unlikely to result from random haploidization.

The idea that unmated nuclei pass from parents to progeny is excluded by the finding of nonparental ditypes and overall recombination frequencies of 50 %.

Most of the germspores appear to be haploid homokaryons, even in germsporangia with many different kinds of germspores. This indicates that the process of germspore formation differs from the process of sporangiospore formation in that the first begins with usually uninucleate primordia, while the second begins with multinucleate primordia. A variable proportion of germspores, however, appears to be heterokaryotic, since they segregate in the next vegetative cycle as described by Heisenberg & Cerdá-Olmedo (1968). The heterokaryotic germspores could be formed by packing together two different nuclei in a single germspore primordium. This simple hypothesis does not explain why some germsporangia contain only 'abnormal' germspores, which may still be diploid or aneuploid. The study of such germsporangia should yield important information on the genetic process.

(ii) *The practical use of* Phycomyces *genetics*

Current research on the biochemistry and behaviour of *Phycomyces* would greatly benefit from genetic analysis. The results in this paper indicate that this task is harder than with other fungi, but not forbiddingly difficult. The analysis of a cross requires three or four months, but little attention is necessary during the long dormancy. The analysis of single germsporangia does not exactly correspond to tetrad analysis in other organisms, and the results are not commensurate with the work involved. Less laborious is the analysis of several hundred germspores from mixed stocks obtained by suspending together the germspores from many germsporangia. Such analysis should not be delayed too long, since germspores

do not survive well in the refrigerator, and since survival may depend on their genotype. Survival is much lower when germspores are left adhering to the lid of Petri plates.

The formation of genetic maps for *Phycomyces* now appears feasible.

Note added in proof: A report of recent investigations on the same subject by A. P. Eslava, M. I. Alvarez, and M. Delbrück is due to appear shortly in *Genetics*.

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