ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXV — nr 1
1966

Chromosome maps in *Ascobolus immersus* (Rizet's strain)

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As an object of genetical investigations *Ascobolus immersus* was introduced by G. Rizet (1939). Since that time it has been used extensively for studying intragenic recombination (see review by Lissouba at al., 1962). A very high frequency of spontaneous mutations affecting ascospore pigmentation as well as the easiness of tetrad analysis on a great scale are most important characteristics of this fungus which make it useful for genetic studies. So far the work with *Ascobolus immersus* has been mainly concerned with studying recombination within short regions of chromosomes (Rizet et al., 1960, Lissouba et al., 1962, Gajewski et al. 1963, Makarewicz, 1964), in other words, within series — a term to describe a group of closely linked sites among which recombination appeares to be mainly of "conversion" type. There is no published data about the localization of the series in chromosomes, linkage, interference and the position of genes in relation to their centromeres. This refers, however, only to the strain coming from Rizet's laboratory, because such data have been obtained recently by C. C. C. Yu-Sun (1964) in the California Institute of Technology for another strain of *Ascobolus immersus* which failed to give fertile crosses with Rizet's strains. In this situation it is inevitable that chromosome maps for these two strains must be done separatly. It is the aim of the present work to make a beginning of chromosome mapping in Rizet's strain.

MATERIAL AND METHODS

Mutants. The following mutants of *Ascobolus immersus* were used in the experiments: *186(477, 231)*, *164*, *873*, *84W (936)*, *726(185)*, *713*, *Y (77, 1159)*, *(46/63, 1216)*, *XXVI*, *col-2* (col-colonial growth), wa-1 and wa-2 (wa-wavy type of growth). Numbers given in parentheses indicate mutants belonging to the same series. A series is named after the first mutant listed. Except for *col-2* and *wa-2* all these mutants are of spontaneous origin from wild stock S_{60}. All of them except *84W*, *col-2*, *wa-1* and *wa-2* were kindly offered to us by Professor Rizet. Mutants signed with arabic ciphers produce unpigmentated ascospores (wild type ascospores are darkly pigmentated), in mutant XXVI pigment appears in grains on the surface of ascospores. *Col-2* and *wa-2* were obtained following UV irradiation of ascospores. Both *col-2* and wavy mutants have characteristic patterns of ascospore germination so they can be distinguished from each other as well as from the wild type already at the beginning of mycelial growth (see Plate I).

Media. For vegetative growth of mycelium we used medium consisting of: 5g KH_2PO_4, 2.125g $NaNO_3$, 0.2g $MgSO_4 \cdot 7H_2O$, 0.1g $CaCl_2$, 20g agar, 20g glucose and sometimes 0.4 g yeast extract in 1000 ml H_2O. It was autoclaved for 15 min in 115°C. Medium for ascospore germination consisted of: 1.25g bacto pepton "Difco", 30g agar "Difco" and 1.5g NaOH in 1000 ml H_2O, autoclaved as above (Lissouba et al., 1962). Ascospores were put on this medium and subjected to temperature shock in 39—40°C for at least 10 hours. When germinated they were transferred on vegetative medium and grown in 25°C.

Crosses. Crosses were carried out on moist horse dung in Petri dishes. Sometimes dung was supplemented with yeast extract in the same concentration as in the vegetative medium. Autoclaving as described above. Two pieces of agar with mycelia of different mating type were put on a Petri dish with horse dung. To form apothecia this fungus needs light. We used normal fluorescent lamps kept 20 to 30 cm above the dishes. It is preferable to keep dishes with mycelium in the dark during the first two days and illuminate before apothecia are formed. First ascospores can be collected after 10 to 12 days. They are discharged in groups of eight from asci and can be easily collected on Petri dishes containing a thin layer of agar. Ascospores were scored under dissection microscope. Crosses were performed in 24 to 25°C. Sometimes variations in the proportions of different types of asci were observed, so they were collected and scored during 5 to 6 days for each cross and the data were pooled.

CALCULATING METHODS

As a basis of calculations we used the frequencies of the three groups of tetrads produced in a cross between two mutant stocks: PD (parental ditype), NPD (non-parental ditype) and T (tetratype). A distribution where PD>NPD and $T < 2/3$ is considered as a criterion of linkage (the L-distribution, Shult and Lindegren 1956). If PD=NPD and T=2/3 (the N-distribution), there are two possible arrangements of loci studied, namely, either they are both located in the same chromosome but more than 50 crossover units apart, or they are located in different chromosomes and at least one of them is situated more than 33.33 crossover units apart from its centromere. A distribution where PD=NPD and $T < 2/3$, indicates that both loci are situated in different chromosomes but less than 33.33 crossover units from their respective centromeres (the F-distribution).

The statistics:

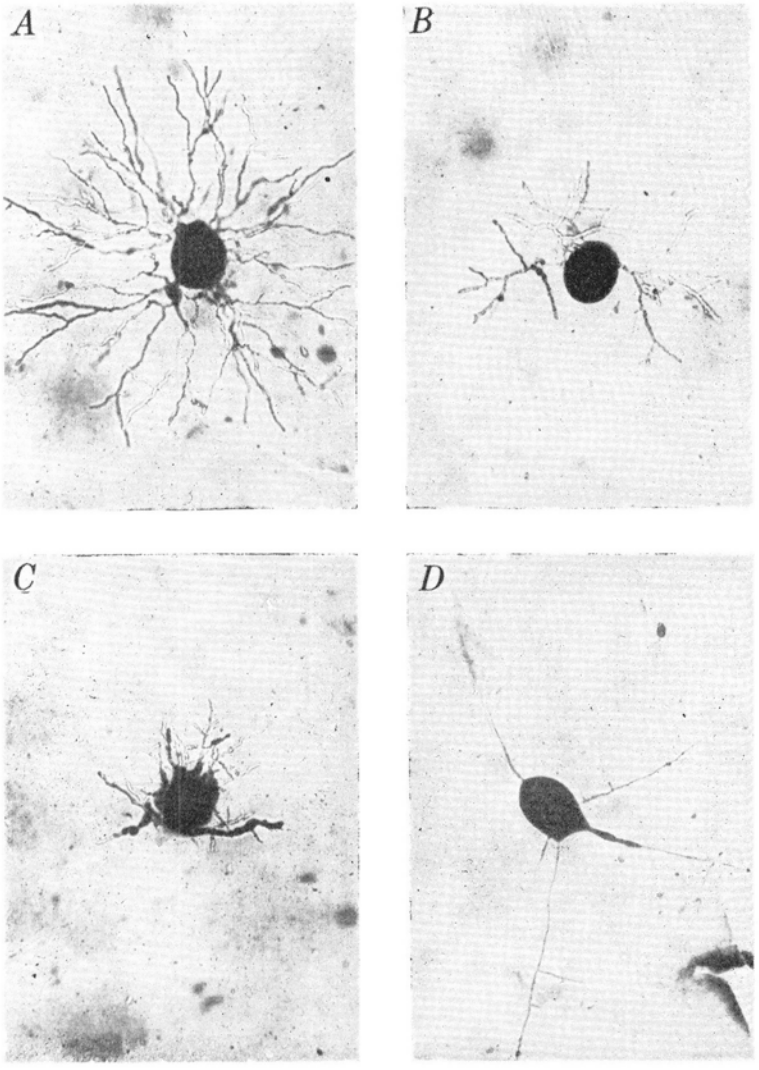
$$\chi_1^2 = \frac{(PD - NPD)^2}{PD+NPD} (1 \text{ d.f.})$$

and

$$\chi_2^2 = \frac{(2n - 3T)^2}{2n} (1 \text{ d.f.})$$

(Yun Lin Hwang et al. 1963) were used for testing the equality of frequencies between PD and NPD tetrads and the equality between the frequency of T tetrads

Plate I



Germination of spores of following mutants: *col-2* (*A*), *wa-1* (*B*), *wa-2* (*C*) and wild strain (*D*). (all four after being kept on germination medium in 39°C during 12 hours).

and 2/3 n, respectively (n- total number of tetrads). If χ_1^2 exceeds 3.84, PD deviates significantly from NPD at 5% level; if χ_2^2 exceeds 3.84, T deviates significantly from 2/3 n at 5% level.

Table 1

Segregation data from crosses involving two or three loci

No.	Cross	PD	NPD	T	Total	Frequ. of T	Distribution
1.	*164 × 713*	2085	1999	5715	9799	0.5832	F
2.	*713 × XXVI*	215	235	752	1202	0.6256	F
3.	*726.164 × ++*	1190	1184	570	2944	0.1936	F
4.	*726.XXVI × ++*	271	284	688	1243	0.5534	F
5.	*164 × 46*	1210	1176	4355	6741	0.6460	F
6.	*726 × 46*	418	445	885	1748	0.5063	F
7.	*wa-2 × 164*	22	21	59	102	0.5784	F
8.	*wa-1 × Y*	29	22	49	100	0.4900	F
9.	*wa-1 × 164*	23	23	56	102	0.5490	F
10.	*XXVI × 164*	3874	718	6328	10920	0.5795	L
11.	*col-2 × 164*	178	7	95	280	0.3395	L
12.	*col-2.164 × ++*	25	1	24	50	0.4800	L
13.	*col-2 × 936*	31	13	62	106	0.5849	L
14.	*84W × 873*	8555	2731	16183	27469	0.5891	L
15.	*84W × 164*	1892	1603	5548	9043	0.6135	L
16.	a) *wa-1 × 713*	36	15	50	101	0.4950	L
	b) *wa-1 × 713*	17	13	72	102	0.7058	N
	c) *wa-1 × 713*	17	14	42	73	0.5753	N
	d) *wa-1 × 713*	10	11	47	68	0.6911	N
	a+b+c+d	80	53	211	344	0.6133	L
17.	*wa-1 × 726*	107	8	118	233	0.5064	L
18.	*936.col-2 × wa-2*						
	wa-2 and *936*	15	13	77	105	0.7333	N
	wa-2 and *col-2*	16	18	71	105	0.6761	N
	col-2 and *936*	35	8	62	105	0.5904	L
19.	*873 × 164*	805	863	3497	5165	0.6770	N
20.	*873 × XXVI*	76	62	306	444	0.6891	N
21.	*873 × col-2*	42	38	142	222	0.6396	N
22.	*873 × 231*	163	144	563	870	0.6471	N
23.	*XXVI × 231*	524	470	1863	2857	0.6521	N
24.	*XXVI.186 × ++*	297	282	1161	1740	0.6672	N
25.	*477 × Y*	136	152	626	914	0.6849	N
26.	*231 × 713*	459	439	1883	2781	0.6777	N
27.	*873 × 726*	331	341	1369	2037	0,6720	N
28.	*84W × 726*	905	876	3621	5402	0.6702	N
29.	a) *wa-2 × 713*	28	18	64	110	0.5818	N
	b) *wa-2 × 713*	19	13	72	104	0.6923	N
	a+b	47	31	136	214	0,6355	N
30.	*wa-2 × 726*	13	13	56	82	0.6829	N
31.	*wa-2 × Y*	26	18	72	102	0.7058	N
32.	*wa-2 × 186*	17	21	74	112	0.6491	N
33.	*wa-1 × 186*	16	10	53	79	0.6708	N
34.	*wa-2 × 1216*	13	15	52	80	0.6500	N
35.	*wa-1 × 63*	9	9	56	74	0.7561	N
36.	*164 × 77*	763	1082	1445	3290	0.4392	R
37.	*1159 × 185*	229	617	281	1127	0.2493	R

Only crosses which gave clear F, L or N distributions are discussed in this paper together with two cases of the R-distribution (reverse linkage) in which PD < NPD and T < 2/3, because they can be used for estimation of the centomere-gene distances. The R-distribution results from preferential segregation of chromosomes.

This phenomenon has been found in *Ascobolus* (Surzycki and Paszewski 1964). Data from other crosses where it was manifested are not included in this paper since they need a special aproach.

RESULTS

The results of crosses are presented in Table 1. The F-distributions of tetrads (octads) are usually taken as a basis for centromere mapping. However, it is impossible to apply to the F-distributions given in the table, bacause there is no group of at least three mutants, which all crossed with each other gave F distributions (in some cases the L- or R-distributions were found, not described here). Thus the results of crosses 1, 2 and 10 were choosen to calculate centromere gene distances for mutants *164*, *713* and *XXVI* (Surzycki and Paszewski, 1964) using Whitehouse's method for two linked and the third unlinked loci (Whitehouse 1957). On the basis of these values other distances were calculated. They are given in Table 2. Because the calculated values for a given centromere marker differ from cross

Table 2

Gene-centromere distances for centromere markers

Cross No. from Tab. 1	Loci		T_{ab}	x_a	x_b	Gene-centromere distance for b in c/o units
	a	b				
1	*164*	*713*			0.5598	27.89
2	*713*	*XXVI*			0.4244	21.22
10	*XXVI*	*164*			0.1548	7.74
3	*164*	*726*	0.1936	0.1548	0.0505	2.5
4	*XXVI*	*726*	0.5534	0.4244	0.3558	17.74
5	*164*	*46*	0.6460	0.1548	0.6400	32.0
6	*726*	*46*	0.5063	0.0505	0.3234	16.17
9	*164*	*wa-1*	0.5490	0.1548	0.5136	25.64
36	*164*	*77(Y)*	0.4392	0.1548	0.5356	26.78
37	*185(726)*	*1159(Y)*	0.2492	0.0505	0.2154	10.77

Second division segregation frequencies of *164*, *XXVI* and *713* calculated according to Whitehouse (1957), for the rest of loci according to Perkins (1949) and Whitehouse (1949):

x_a = the frequency of 2nd division segragation of a

x_b = the frequency of 2nd division segregation of b

T_{ab} = the frequency of tetratypes in cross a×b.

$$x_b = \frac{T_{ab} - x_a}{1 - {}^3/_2\, x_a}$$

to cross they must be considered as approximations. Makarewicz (1964) found on the basis of mating type segregation in recombinant asci from crosses involving mutants from the series *726* that this locus is located about 5 crossover units apart from its centromere (in this calculation it was assumed that mating type segregated at the first meiotic division). This value is in good agreement with the value calculated from cross 3. Kruszewska (personal communication) found by the same way that series *Y* is about 10 units apart from its centromere what agrees with the value calculated on the basis of cross 37, assuming that the frequency of the second division segregation of locus *185* (*726*) equals 0.05.

It has been already found that loci *164* and *XXVI* are linked (Makarewicz, 1961) with the centromere located between them (Surzycki and Paszewski, 1964). Now it is evident that *col-2* is linked with *164* (18.46±1.6 units) and with *84W* (41.5±3.05 units). In turn the locus *84W* is linked with the locus *873* (39.39± ±0.187 units) and *wa-1* with *726* (28.28±1.25 units).

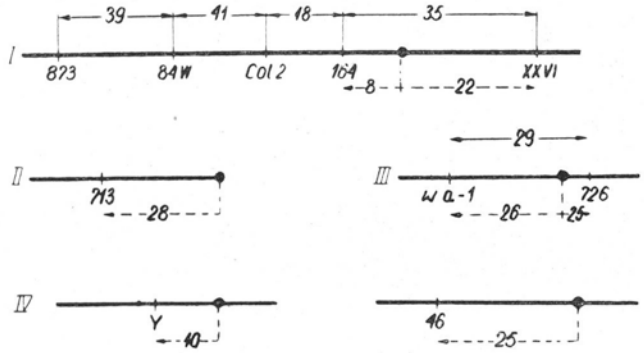


Fig. 1. Preliminary chromosome maps of *Ascobolus immersus* (Rizet's strain) based on data from Table 1 and Table 2. Dotted lines indicate gene-centromere distances

Preliminary chromosome maps constructed on the basis of calculated gene-centromere distances and linkage values are shown in Fig. 1. The localization of *wa-1* in relation to the centromere is still uncertain. The N-distributions found in a number of crosses presented in Table 1 confirm these maps.

DISCUSSION

The results of experiments described above indicate that the loci studied are located at least in four different chromosomes. It should be noted that Żuk and Swietlińska (1965) estimated the chromosome number in this mold by cytological studies to be 8 or 9. The chromosome maps given here are preliminary and many values, especially centromere-gene distances will be certainly corrected in the course of mapping of new loci. At present the chromosome I is the best marked one. It is evident that loci *726* and *wa-1* are linked. The pooled data of crosses between mutants *wa-1* and *713* suggest that these loci are linked, whereas three of the four crosses done point to the contrary.

The results of crosses between linked genes in chromosome I make it possible to check if there is chiasma interference. This can be done by calculating the frequencies of tetratypes (T) and nonparental ditypes (NPD) on the basis of recombination frequencies, assuming no interference ($T = 1/6\ [1 - (1 - 2r)^3]$ and $NPD = 6r - 1/2T$, where r is the recombination frequency; Whitehouse quoted by Holliday, 1961), and comparing them with values obtained in experiments. Theoretical curves for $k = 0$ and $k = 1$ are shown in Fig. 2 and experimental values are

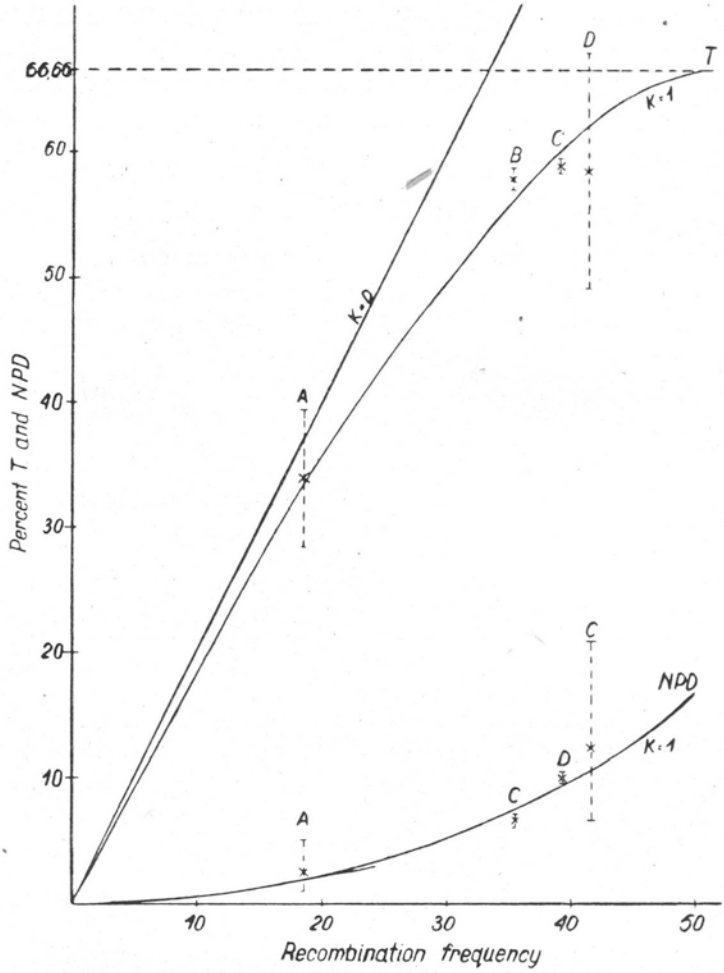


Fig. 2. Curves relating the frequencies of tetratype and nonparental ditype asci to the recombination frequency, with interference ($k = 0$) and without ($k = 1$). Experimental values and their standard deviations from crosses: *col-2* × *164* (*A*), *wa-1* × *726* (*B*), *XXVI* × *164* (*C*), *84W* × *873* (*D*), and *col-2* × *936* (*E*) are put on the graph

put on the graph. It is evident that the points corresponding to linkages in the chromosome I are distributed near the curve for $k = 1$ indicating that there is no or very slight interference.

SUMMARY

The results of crosses between some mutants of *Ascobolus immersus* (Rizet's strain) are presented. They are taken as a basis for the construction of the preliminary chromosome maps. The loci studied are distributed in at least four chromosomes from among eight (or nine) found by cytological analysis. Most of linkage data suggest that there is no or very slight chiasma inter-

ference in the best marked chromosome I. The crosses indicating that the chromosomes in question can segregate preferentially are not described in this paper.

Authors wish to thank Professor W. Gajewski for his advice offered during the course of this work and in the preparation of the manuscript.

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(Entered: 23.X.1965)

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Mapy chromosomów u *Ascobolus immersus* (szczep Rizet'a)

Streszczenie

W pracy przedstawiono wyniki krzyżówek pomiędzy rozmaitymi mutantami morfologicznymi workowca *Ascobolus immersus*. Na ich podstawie zrobiono wstępne mapy chromosomów dla tego organizmu. Stwierdzono, że badane loci są rozmieszczone w co najmniej czterech spośród ośmiu (lub dziewięciu) chromosomów opisanych przy analizie cytologicznej. Wyniki krzyżówek pomiędzy mutantami należącymi do sprzężonych loci wskazują na brak interferencji chiazmowej, względnie, że jest ona bardzo słaba.