First results of genetic analysis in series 726 of *Ascobolus immersus*

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Following Rizet (Rizet et al. 1960) series mean compound loci determining in *Ascobolus immersus* white ascospores; wild-type ascospores are brown-violet. One of such series called 726 was studied.

MATERIAL AND METHODS

Five mutants of series 726 we owe to Rizet who was the first to carry on genetic studies on *A. immersus* (Rizet 1958). Later on the number of mutants raised up to 17 but after preliminary trials only six of them were left.

After crossing mutants of the same series produce asci with eight white ascospores (8:0). Besides these irregular asci appear containing either wild-type spores — two per ascus (6:2), one, three, four per ascus (7:1, 5:3, 4:4) — or granular ascospores, where the pigment is not smoothly spread. (6:2g, 7:1g, 5:3g, 4:4g) (Rizet et al. 1960, Makarewicz 1961). The frequency of irregular segregation and the types of irregular asci vary greatly in different crosses. Some of them produce no more than several irregular asci per thousand, the irregularity consisting solely or nearly so in asci of type 6:2. Other crosses show more than 10% of irregular segregation producing a whole gamut of irregular asci: the most frequent are the 6:2, 7:1, 6:2g, and 7:1g asci. The 5:3, 4:4, 5:3g and 4:4g asci are rarer. Crosses which give intermediate results were also found but in all cases greater frequency of irregular segregation goes together with a variety of irregular asci.

In preliminary trials wild-type recombinants and granular ascospores were tested to verify if they corresponded to their phenotypes. In some of the tested 6:2 asci the wild-type spores were genetically white mutants. These asci resulted from crosses showing high frequency of irregular segregation. In 15 from 29 tested 7:1 asci the wild-type ascospores showed correspondence between phenotype and genotype, the remaining 14 did not. The same crosses always gave the same results.

In some crosses the occurrence of irregular asci (6:2g and 7:1) was observed in several generations. The obtained data were very similar

and can suggest that 6:2g and 7:1 asci resulted from some chromosomal aberrations.

Taking into account that in series 726:

1) the 6:2g and 7:1 asci always appear in crosses with high frequency of irregular segregation, when, however, this frequency is low, solely or almost solely 6:2 asci are found,

2) the 7:1 asci often prove to be really 8:0 in genetic analysis,

3) the 6:2 asci are usually genuine except the crosses which show high frequency of recombination,

only those mutants which produce 6:2 asci were selected for further work.

It appeared later that when mutants showed high frequency of irregular segregation in crosses at least one of them also showed recombination when selfed. Therefore the procedure adapted in selecting mutants for further work was as follows: each new mutant was selfed and eliminated if any recombinant appeared. When, after scoring about 20 thousand asci, no irregular spores were found, the new mutant was crossed with one or two selected mutants. If only 6:2 asci appeared it was selected. In this way following six mutants remained: 113, 782, 1056, 784, 709 and 726.

RESULTS

Crosses between the six mutants showed frequency of 6:2 asci ranging from 0.17 to 2.30 per thousand (Table 1). Mutants 782, 1056 and 784 that at first did not give any recombination, behaving like homoalleles, were treated as one group (784A), although in the last time rare recombinants were found.

Table 1

Frequency of the 6:2 asci per thousand in crosses among sites of the 726 series

1056 × 709 — 2.30	113 × 709 — 1.70
782 × 709 — 2.00	113 × 726 — 1.00
784 × 709 — 1.90	709 × 726 — 0.40
1056 × 726 — 1.11	113 × 784 — 0.25
784 × 726 — 0.96	113 × 782 — 0.19
782 × 726 — 0.73	113 × 1056 — 0.17

The results of the genetic analysis of 90 6:2 asci agree in substance with the data of Lissouba and Rizet (1960). There are namely two kinds of 6:2 asci. In one of them among the six white ascospores two represent the double mutant; so, they can be interpreted as resulting from reciprocal exchange (crossing-over). In the other kind there are no double mutants — four of the six white ascospores represent the first mutant and the remaining two the second one — so, they could

be caused by unreciprocal exchange (conversion). The occurrence of conversion and crossing-over shows some regularity: in some crosses conversion and crossing-over was found, in others only conversion (Table 2). The fact that crossing-over was not stated in some crosses, of course, does not prove that it did not occur, especially in these crosses in which a relatively small number of asci was analysed. In spite of this reservation it is surprising that crosses showing the highest frequency of recombination, namely $784A \times 709$, don't give crossing-over.

Table 2
Genetic analysis of the 6:2 asci

Crosses	Number of asci tested	Conversion asci				Crossover asci	
		Cosegregation				Cosegregation	
		observed		not observed		observed	not observed
		←	→	←	→		
113 $\times$ 726	7	2		2		3	
113 $\times$ 709	9	2	5			2	
113 $\times$ 782	5		5				
784 $\times$ 726	9	4				5	
1056 $\times$ 726	9	2				6	1
782 $\times$ 726	6	1	1			4	
784 $\times$ 709	12	4	7	1			
1056 $\times$ 709	16	9	5		2		
782 $\times$ 709	4	3	1				
709 $\times$ 726	7	5		2			
Other, involving eli- min. mutants	6	2	4				
	90	34	28	5	2	20	1

Arrow heads mark the minority mutants.

This fact may be, perhaps, explained when we take in account that within series 726 conversion takes place in a double way. In some crosses e.g. $113 \times 784A$ and 709×726 it is always one site which segregates normally 2:2 (the mutants 113 and 726, the so called majority mutants) when the other segregates 1:3 (the mutants 784A and 709, the minority mutants). In other crosses the relation between the majority and minority mutants does not hold, both sites are able to convert (inversion of majority). In 17 out of 32 conversion asci resulting from crosses $784A \times 709$ it was 784A that showed segregation 1:3, in the remaining 15 it was the 709 which was the minority mutant. Thus, when comparing the frequencies of recombination between the crosses $113 \times 784A$, $784A \times 709$ and 709×726 we can, it seems, in the cross $784A \times 709$ reduce the actual figures of the 6:2 asci to only half of them. The crosses 113×709 and $784A \times 726$ could be treated in a similar way, but in these cases, as

crossing-over occurs besides of conversion, such a big adjustment cannot be applied. Respective adjustments being made, the arrangement of sites as well as the occurrence of crossing-over and conversion within the series 726 may be illustrated as in Fig. 1.

Data from three point crosses confirm the above results. The double mutant 113.726 gives recombination both with 784A and with 709 i.e.

Fig. 1. Scheme of the series 726. The number of asci tested is given in parantheses. Arrow heads mark the minority mutants. $\square$ — the postulated points.

with two mutants able to convert. The double mutant 784A.726 gives recombination only with 709 and does not give with 113 as this mutant shows regular segregation. Similarly, the double mutant 113.709 does not give recombination with 726.

Crosses between mutants and the wild-type brought some additional data. In such crosses, as usual, besides the vast majority of 4:4 asci, rare 6:2 and 2:6 asci were found. The 2:6 asci, which correspond to the 6:2 asci from crosses between mutants, can be interpreted as resulting from conversion of the investigated site to the wild-type. The frequency of such asci was as follows:

113	×	wild-type	—	0,05 per thousand
784A	×	wild-type	—	0,13 per thousand
709	×	wild-type	—	0,33 per thousand
726	×	wild-type	—	0,09 per thousand

The above figures show that three of the four investigated sites, namely 113, 784A and 709 can be arranged one after another according to their increasing recombination frequencies and to the previous data. It is not the case with site 726 which shows decrease of recombination frequency. These facts may lead to the conclusion that we have to do with two points annullating the preceding conversion switches: one on the left of site 113 and very near to it and one between 709 and 726, very near to 726 (Fig. 1).

This last assumption fits well the suggestion of Rizet and Rosignol (1963, in press) about some points on chromosomes being limits for conversion switches.

Some additional light on the series 726 was thrown when a simultaneous analysis of sites and signs was undertaken. *Ascobolus immersus* as a heterothallic organism, shows segregation of the mating types into sign „+“ and sign „—“. It was found in asci of the conversion type that the same mutants are, usually, of the same sign, while there is no linkage between mutant and sign. This may be the result of the sign and the series 726 being located close to their respective centromeres and therefore segregating usually in the first division of meiosis.

100 asci obtained in crosses between mutants and wild-type, where recombination did not occur (4:4), were tested for the distribution of signs. In all instances signs and the investigated sites segregated together. Further on this phenomenon will be called cosegregation. Asci with crossovers showed also cosegregation; in 20 out of 21 analysed asci the single mutants were of different signs, just as the double mutants and the recombinants were.

Lastly we got numerous data* about the distribution of signs in asci resulting from crosses 784A $\times$ wild-type which confirm our results. Reassuring the results are as follows:

	Number of asci tested	Cosegregation of signs and mutants	
		observed	not observed
Asci without recombination	726	717	9
Asci with recombination:			
Conversion asci	69	62	7
Crossover asci	21	20	1

It is, thus, evident that not only sign and the series 726 are located near the respective centromeres, but that the sign is nearer to the

* We owe these data to Mrs. C. Lefort from the Rizet's Laboratory (Laboratoire de Génétique Physiologique C.N.R.S., Gif-sur-Yvette) who kindly has sent them to us for use. We should like to express on this occasion our best thanks.

centromere than the series 726; the frequency of asci in which cosegregation was not observed is significantly lower in asci without recombination ($\chi^2 = 19.4$, $P < 0.005$).

Assuming that the sign segregates almost solely at the first division, we may expect that cosegregation will not appear only after crossing-over between the centromere and the series 726. This is due to the fact that the probability of two events within the series 726, which occupies a rather short chromosome segment, is very low; not more than 4 asci in which the occurrence of two events could be suspected were met as yet. On the other side simultaneous conversion on two sites of the series 726 was never found; it must be very rare, if any.

According to what was said above, the probable mode of derivation of the asci showing no cosegregation can be suggested. Seven of them being of the conversion type may be the result of conversion within the series and of crossing-over in the proximal segment of the chromosome. One ascus with crossovers may be the result of double three strand crossing-over (Fig. 2).

The phenomenon of cosegregation made it possible to determine the position of the series 726 in relation to the centromere. The centromere is located nearer to 113 than to 726. This results from the analysis of signs of double mutant; in 19 out of 20 asci carrying crossovers and showing cosegregation the double mutants are of the same sign as the mutants located on the side of the mutant 113. In other words, when crossing-over between series 726 and the centromere does not take place, the double mutant deriving from crossing-over within the series segregates together with the mutant located nearer the centromere i.e. 113.

The twentieth ascus in which the double mutant is of different sign than the proximal mutant may be caused by a double two-strand crossing-over (Fig. 3).

The last case indicates that even when crossing-over between the centromere and the series does not touch cosegregation, it can be known that it took place by comparing the signs of the double and the single mutants. It is thus possible to estimate the frequency of crossing-over and the distance between the centromere and the series 726. Conversion asci show 10.2% of recombination (7 out of 69), crossover asci — 9.5% (2 out of 21), which corresponds to about 5 crossover units.

If conversion is the result of copy choice during replication of DNA then, on the whole, the data about the series 726 permit to make some assumptions concerning the direction of replication. Rizet and Rosignol (1963) as well as Surzycki (personal communication) distinguish between starts and returns of conversion which cannot be discerned as long as the direction of replication is unknown. As mentioned above, between the mutants 709 and 726 a point is postulated where copying is interrupted. It may be said that between those mutants only returns of conversion are possible which may be related with the postulated point. On the other side, between the mutants 113 and 784A only starts

Fig. 3. The probable mode of derivation of one crossover ascus in which the double mutants were of the same sign as the distal mutant; double two strand crossing-over.

of conversion occur, while between 784A and 709 starts and returns occur with the same frequency. If the assumed arrangement of mutants (Figure 1) is right, it may be said that replication proceeds from centromere to the end of the chromosome.

SUMMARY

The white ascospore mutants of *Ascobolus immersus* belonging to one series, when intercrossed, rarely give wild-type recombinants, i.e. dark spores in 6:2 asci. These asci result either from crossing-over either from conversion. Crosses between mutants and the wild-type rarely give asci 2:6 resulting from conversion. Data obtained in intercrosses, in crosses with wild-type and in three point crosses were compared with the data from genetic analysis in which segregation of the mating type was investigated simultaneously with the segregation of sites. After some adjustment of the recombination frequencies the most probable scheme of the series 726 was outlined, namely:

1. The series 726 is located approximately 5 crossover units from the centromere, the sequence of mutants is determined;
2. On some regions only the segregation of conversion type, while on others both conversion and crossing-over were found;
3. On one region only starts of conversion occur, on others only returns and on some both starts and returns take place.

It was stated that the sign is located on a different chromosome than the series 726 and very near to the centromere.

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Pierwsze wyniki dotyczące analizy genetycznej serii 726 u Ascobolus immersus

Streszczenie

U *Ascobolus immersus* mutanty o bezbarwnych sporach należące do jednej serii dają po skrzyżowaniu ze sobą, z niewielką częstością, rekombinanty typu dzikiego. Są to dwie ciemne spory w worku (6:2). Worki 6:2 powstają bądź wskutek crossing-over, bądź wskutek konwersji. Krzyżowanie mutantów z typem dzikim daje, z niewielką częstością, worki typu 2:6 będące wynikiem konwersji. Zestawiono dane, dotyczące częstości rekombinantów, otrzymane z krzyżowania mutantów między sobą, z krzyżowania mutantów z typem dzikim oraz z krzyżowania podwójnych mutantów z pojedynczymi, z wynikami analizy genetycznej worków 6:2. Podczas analizy genetycznej, obok segregacji mutantów, uwzględniano segregację znaków. Po wniesieniu pewnych poprawek do częstości rekombinacji opracowano najbardziej prawdopodobny układ serii 726, a mianowicie:

1. Seria 726 znajduje się w odległości około 5 jednostek crossing-over od centromeru, kolejność mutantów jest ustalona;
2. Na pewnych odcinkach zachodzi jedynie konwersja, na innych konwersja i crossing-over;
3. Na jednym z odcinków możliwe są jedynie początki konwersji, na innych jedynie zakończenia, na niektórych zaś zachodzą zarówno początki, jak i zakończenia.

Stwierdzono, że znak u *Ascobolus immersus* mieści się na innym chromosomie niż seria 726, bardzo blisko centromeru.