Genet. Res., Camb. (1964), **5**, *pp.* 20–26
With 2 text-figures
Printed in Great Britain

Non-random segregation of chromosomes in *Ascobolus immersus*

By S. SURZYCKI and A. PASZEWSKI

Department of General Genetics, Polish Academy of Sciences al. Ujazdowskie 4, *Warsaw, Poland*

(*Received* 25 *March* 1963)

1. INTRODUCTION

Non-random segregation of chromosomes has been found by several workers. Michie (1953, 1955) and Wallace (1953, 1958*a*, *b*, 1959, 1961) observed non-random associations of unlinked markers in laboratory stocks of the house mouse. Wallace (1960*a*, *b*) found also a similar phenomenon in tomato and cotton. They proposed a hypothesis of centromere affinity at the first meiotic division, whereby centromeres of the same ancestral origin tend to travel to the same pole.

The same phenomenon was also described in *Saccharomyces* by Hawthorne & Mortimer (1960), Shult & Desborough (1960) and Shult, Desborough & Lindegren (1962) for a number of markers in different families, and by Prakash (1962) in *Neurospora crassa*.

We have encountered a similar phenomenon studying linkage in *Ascobolus immersus*. A high frequency of spontaneous mutations affecting ascospore pigmentation was found by Rizet (1939) in this mould. Spores are discharged from asci in groups of eight and can be easily collected and scored on Petri dishes with a thin layer of agar. This provides a good opportunity for tetrad analysis on a large scale. Methods for the handling of this organism were fully described by Rizet *et al.* (1960), Lissuba (1961) and Makarewicz (1961).

2. THEORY

Among tetrads obtained in a two-factor cross three types are distinguishable, namely: parental ditype (PD), non-parental ditype (NPD), and tetratype (T). Depending on the relative position of genes to each other and to their centromeres different frequencies of these tetrads can occur. Shult & Lindegren (1956) showed that the frequency of PD may exceed, equal, or be less than the frequency of NPD; and the frequency of T may exceed, equal, or be less than 2/3.

A basic distribution is the so called N-distribution (normal) where PD:NPD:T = 1:1:4 or 1/6:1/6:2/3. This distribution occurs when the two genes involved segregate independently of each other, and at least one of them must be situated as far as 33 cross-over units from its centromere. If the genes are located in different

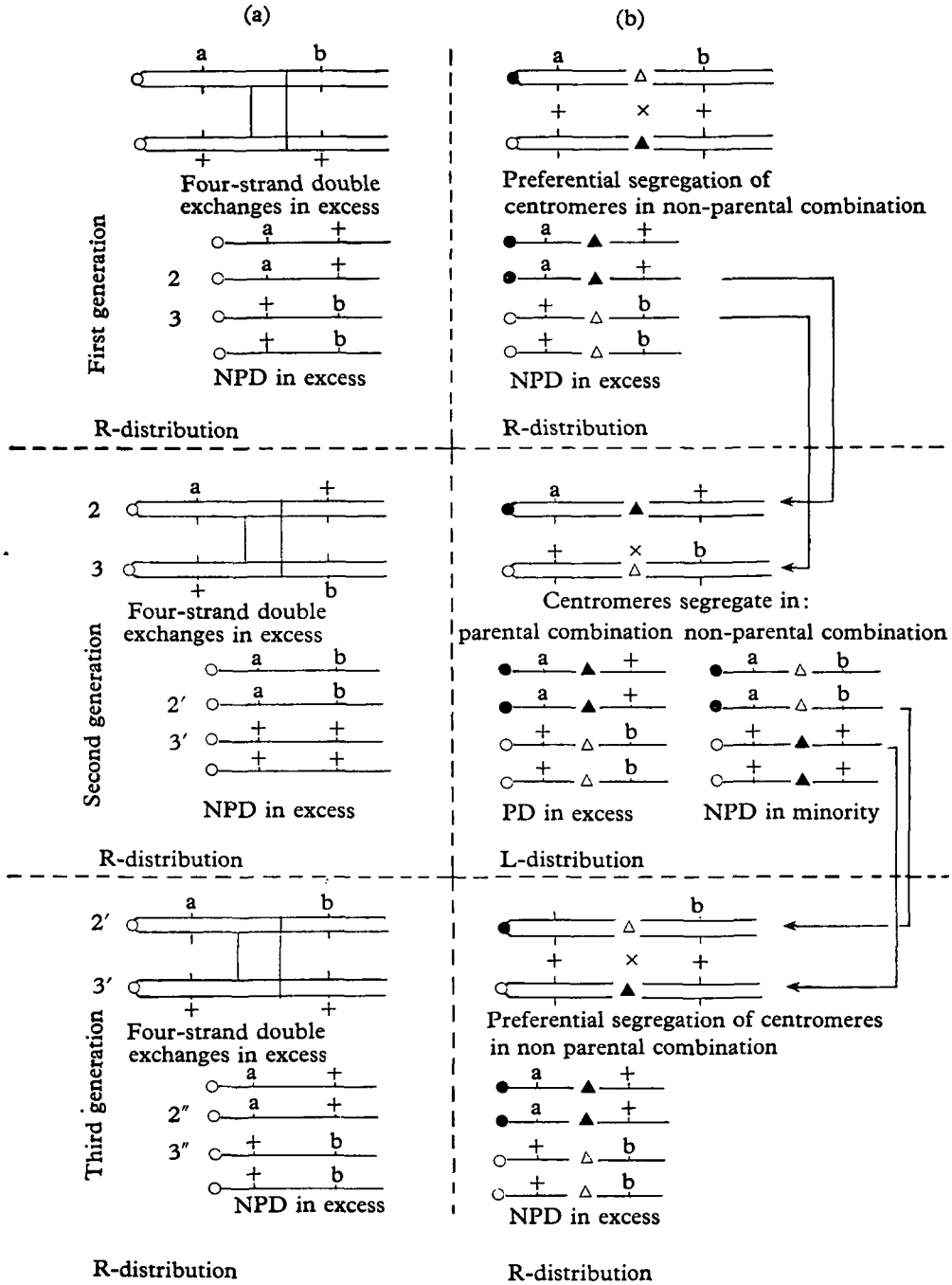

Fig. 1. Two possible explanations of R-distribution: (*a*) on the basis of an excess of four-strand double exchanges; (*b*) on the basis of preferential segregation of centromeres. ● ○, ▲ △ represent centromeres (or regions close to them) of two non-homologous chromosomes. In (*b*) centromeres marked by solid symbols (●, ▲) tend to the same pole and similarly with the open symbols (○, △).

https://doi.org/10.1017/S0016672300000999 Published online by Cambridge University Press

chromosomes but less than 33 cross-over units from their centromeres, the F-distribution is found (Shult & Lindegren, 1956). It is characteristic of this distribution that PD:NPD = 1:1, as in the N-distribution, but T < 2/3.

The distribution where PD > NPD and T < 2/3 was named L-distribution by the same authors.

In turn, the distribution where PD < NPD and T < 2/3 was named R-distribution (reverse linkage). This type of distribution may appear: (*a*) if there is an excess of four-strand double exchanges between two linked genes, or (*b*) if there is

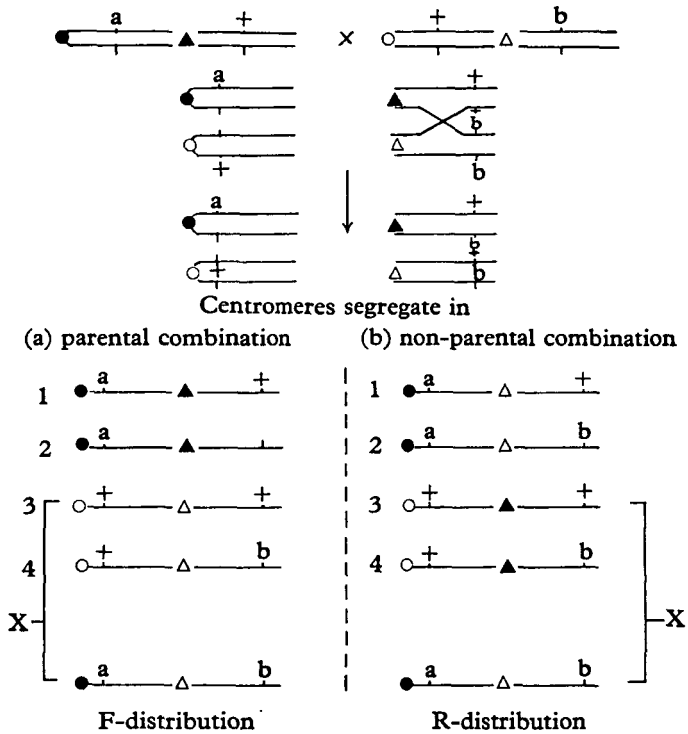

Fig. 2. Two types of T tetrad occur if there is preferential segregation of centromeres (or regions close to them). See text for full explanation.

preferential segregation of centromeres in non-parental combination at the first meiotic division when genes are located in different chromosomes.

One can distinguish these two explanations of reverse linkage by tetrad analysis. If the R-distribution is due to an excess of four-strand double exchanges, an R-distribution will always be found in the next and following generations (see Fig. 1).

If the R-distribution is due to preferential segregation of chromosomes, the markers are not really linked and, depending on the chromosome configuration, either R- or L-distributions will be found. In this situation an R-distribution gives rise to an L-distribution in the next generation, when spores from NPD asci are crossed. If, by chance, (as in our case) we start with an L-distribution we get an R-distribution in the next generation.

https://doi.org/10.1017/S0016672300000999 Published online by Cambridge University Press

Thus, if the R-distribution is due to preferential segregation of chromosomes, changes are observed from R-distribution to L-distribution and vice versa in successive generations. Besides, if the L-distribution is a result of preferential segregation of centromeres, then crosses between the majority of dark spores from T tetrads with double mutants from NPD tetrads will give an F-distribution. This is a consequence of random segregation of centromeres at the first meiotic division (see Fig. 2).

3. EXPERIMENTAL DATA

The following mutants of *Ascobolus immersus* were used: *164*, *726*, *231* and *XXVI*. All these mutants had been kindly offered us by Professor G. Rizet. The first three produce unpigmented ascospores; the last produces pigmented ascospores, but pigment appears in grains on the surface of spores. Wild-type spores are dark. Makarewicz (1961) found mutants *164* and *XXVI* to be linked (37 cross-over units apart). We carried out a number of crosses involving these two mutants and obtained a linkage value of $35{\cdot}43 \pm 0{\cdot}53$ (see Table 1). From the data presented in

Table 1. *The results of crosses involving mutants* 164, XXVI *and* 726

	Cross	Number of asci PD	NPD	T	Percentage recombination	Distribution
1.	*164* × *XXVI*	3874	718	6238	35·43	L
2.	*164* × *726*	1946	2513	1176	55·03	R
3.	*XXVI* × *726*	189	133	328	45·69	L

Table 2 we calculated using Whitehouse's method (Whitehouse, 1957) that the centromere is located between these markers and their distances from it are $7{\cdot}74 \pm 4{\cdot}62$ cross-over units for *164* and $21{\cdot}22 \pm 2{\cdot}2$ for *XXVI*. Mutant *726* behaved as linked with *XXVI* (see Table 1). It was known that *726* is located about 4 crossing-over units from its centromere (A. Makarewicz, personal communication). Therefore *726* was expected to be linked with *164*, but this was not in fact found.

Table 2. *The results of crosses involving mutants* 164, 713 *and* XXVI

Cross	Number of asci PD	NDP	T	Hypothesis PD = NPD χ^2	Prob.	Distribution
164 × *XXVI*	3874	718	6238	—	—	L
164 × *713*	2085	1999	5715	1·81	0·18	F
713 × *XXVI*	215	235	752	0·89	0·34	F

Thus, from the two linkages shown in Table 1, one was suspected to be actually quasi-linkage as in the situation presented in scheme 1*b* (second generation). To test this a cross between a double mutant and a wild-type spore, both isolated from

one NPD ascus, was carried out. If preferential segregation of centromeres is occurring, an R-distribution will result from such a cross, as shown in scheme 1*b* (third generation). Because linkage of genes *164* and *XXVI* had already been demonstrated (unpublished observations), only linkage between *726* and *XXVI* was tested. Three intra-ascal crosses were carried out (Table 3). In all three an R-distribution was found.

Table 3. *The results of intra-ascal crosses involving double-mutant* XXVI.726 *and wild-type spores derived from NPD asci produced in cross* 3 *of Table* 1

Cross	Number of asci			Hypothesis PD = NPD		Distribution
	PD	NPD	T	χ^2	Prob.	
1/XXVI.726 × ++	146	220	345	14·96	0·001	R
2/XXVI.726 × ++	149	187	278	4·3	0·04	R
3/XXVI.726 × ++	522	867	1613	85·7	< 0·001	R

To obtain additional evidence of non-random segregation of chromosomes, the double mutant spore *3/XXVI.726*, which was used in one of the intra-ascal crosses (Table 3), was crossed with each of two wild-type spores from tetratype tetrads coming from the same crosses (cross 3, Table 1). Results are presented in Table 4.

Table 4. *The results of crosses involving double-mutant spore* 3/XXVI.726 *and wild-type spores from tetratype asci from cross* 3 *in Table* 1

Cross	Number of asci			Hypothesis PD = NPD		Distribution
	PD	NPD	T	χ^2	Prob.	
1. *3/XXVI.762* × T_1	271	284	688	0·3	0·6	F
2. *3/XXVI.726* × T_2	192	239	475	5·13	0·02	R

F-distribution and R-distribution were found to correspond to (*a*) and (*b*) parts of the scheme 2, respectively. Evidently, the same double mutant spore (*3/XXVI.726*) may show F-distribution (random segregation of chromosomes) and R-distribution (non-random segregation of chromosomes), depending on which wild-type spore it is crossed with.

On crossing mutants *164* and *726* with *XXVI* in repulsion we obtained asci consisting of 4 white and 4 'granular' spores (PD); 4 white and 4 dark spores (NPD); and 4 white, 2 'granular' and 2 dark spores (T). PD asci from the cross in coupling correspond to NPD asci from the cross in repulsion. Therefore, the change from L- to R-distribution (and vice versa) in successive generations could be due to the selection of one type of ascus, namely, that consisting of 4 white and 4 dark spores (PD asci from Table 4). The F- and R-distributions obtained with the same double-mutant spore exclude the role of selection in this case. The ratio PD:NPD = 1:1

found in cross $3/XXVI.726 \times T_1(++)$ should be expected also in the next generation from intra-ascal crosses among spores from PD or NPD asci. Two crosses in coupling were carried out to check this assumption (Table 5).

Table 5. *The results of crosses involving progeny from cross* 2 *in Table* 4

	Number of asci			Hypothesis PD = NPD		
Cross	PD	NPD	T	χ^2	Prob.	Distribution
1.	348	305	725	2·83	0·09	F
2.	130	120	367	0·4	0·5	F

Table 6. *The results of crosses involving mutants* 231 *and* 726

	Number of asci			Hypothesis PD = NPD	
Cross	PD	NPD	T	χ^2	Prob.
231a × *726*	135	143	825	0·23	0·6
231b × *726*	402	383	1714	0·46	0·5

According to the theory of preferential segregation of centromeres at the first meiotic division, a gene located more than 33 cross-over units from its centromere should not segregate preferentially. In fact, on crossing *726* with *231* (which recombines with *164* in 48 per cent of the progeny) we obtained the ratio PD:NPD = 1:1. Two experiments were carried out, the results of which are presented in Table 6.

4. DISCUSSION

The preferential segregation of the non-homologous chromosomes may be explained either by complete or partial centromere affinity; or by assuming that some other chromosomal region is responsible for this phenomenon.

The possibility of complete centromere affinity is excluded because in every cross described both PD and NPD asci have occurred. The ratio NPD:PD in the cross *164* × *726* (cross 1, Table 1,) and in the cross $XXVI.726 \times ++$ (cross 3, Table 3), is equal to 1·29 and 1·66, respectively. Because *164* is located nearer to the centromere than *XXVI* (7·74 ± 4·62 and 21·22 ± 2·2, respectively), one would expect a higher proportion of NPD with *164* than with *XXVI* if centromere affinity is involved, but the opposite is found. Comparing the ratios given above one suspects that the 'affinity region' of the chromosome *164-XXVI* is not absolutely restricted to the centromere. Indeed, the data would suggest tentatively that the centre of affinity was situated at a point which divided the interval between the loci of *164* and *XXVI* in the ratio 1·66:1·29. This would place it about 8·5 units from the centromere in the *XXVI* arm.

https://doi.org/10.1017/S0016672300000999 Published online by Cambridge University Press

SUMMARY

Genetic consequences of non-random segregation of chromosomes are discussed. In the experimental part preferential segregation of two linkage groups in *Ascobolus immersus* is described. This was established in several crosses involving mutants *164*, *XXVI* and *231* (all in the same linkage group) and mutant *726*.

Our most sincere gratitude is due to Professor W. Gajewski for his generous help and stimulating criticism in the course of this work. It is a pleasure to thank Dr M. E. Wallace for the most interesting discussion about the discovery of the phenomenon of affinity.

We wish to thank Mr K. Lenk for help in the preparation of the schemes.

REFERENCES

HAWTHORNE, D. C. & MORTIMER R. K. (1960). Chromosome mapping in *Saccharomyces:* centromere-linked genes. *Genetics*, **45**, 1085–1110.

LISSOUBA, P. (1961). Mise en evidence d'une unité génétique polarisée et essai d'analyse d'un cas d'interference negative. Théses Science, Paris.

MAKAREWICZ, A. (1961). Preliminary results of genetic analysis in *Ascobolus immersus*. *Ac. Soc. Bot. Polon.* **30**, 271–283.

MICHIE, D. (1953). Affinity: a new genetic phenomenon in the house mouse. *Nature, Lond.*, **171**, 26–27.

MICHIE, D. (1955). 'Affinity.' *Proc. roy. Soc.* B, **144**, 241–259.

PRAKASH, V. (1962). Ph.D. Thesis, Univ. Library, Cambridge.

RIZET, G. (1939). Sur les spores dimorphes et l'hérédité de leur caractère chez un nouvel *Ascobolus* hétérothallique. *Ct. R. Acad. Sci., Paris*, **208**, 1669–1671.

RIZET, G., LISSOUBA, P. & MOUSSEAU, J. (1960). Les mutations d'ascospores chez l'Ascomycete *Ascobolus immersus* et l'analyse de la structure fine des génés. *Bull. Soc. franç. Physiol. Veget.* **6**, 175–193.

SHULT, E. E. & DESBOROUGH, S. (1960). The application to tetrad-analysis-data from *Saccharomyces*, of principles for establishing the linear order of genetic factors. *Genetica*, **31**, 147–187.

SHULT, E. E., DESBOROUGH, S. & LINDEGREN, C. C. (1962). Preferential segregation in *Saccharomyces*. *Genet. Res.* **3**, 196–209.

SHULT, E. E. & LINDEGREN, C. C. (1956). Mapping methods in tetrad analysis. I. Provisional arrangement and ordering of loci preliminary to map construction by analysis of tetrad distribution. *Genetica*, **28**, 165–176.

WALLACE, M. E. (1953). Affinity: a new genetic phenomenon in the house mouse. *Nature, Lond.*, **171**, 27–28.

WALLACE, M. E. (1958*a*). Experimental evidence for a new genetic phenomenon. *Phil. Trans.* B, **241**, 211–254.

WALLACE, M. E. (1958*b*). New linkage and independence data for ruby and jerker in the mouse. *Heredity*, **12**, 453–462.

WALLACE, M. E. (1959). An experimental test of the hypothesis of affinity. *Genetica*, **29**, 243–255.

WALLACE, M. E. (1960*a*). A possible case of affinity in tomatoes. *Heredity*, **14**, 275–283.

WALLACE, M. E. (1960*b*). Possible cases of affinity in cotton. *Heredity*, **14**, 263–274.

WALLACE, M. E. (1961). Affinity: evidence from crossing inbred lines of mice. *Heredity*, **16**, 1–23.

WHITEHOUSE, H. L. K. (1957). Mapping chromosome centromeres from tetratype frequencies. *J. Genet.* **55**, 348–360.