NON-RANDOM CROSSING OVER IN THE SECOND CHROMOSOME OF NEUROSPORA CRASSA[1]

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INTRODUCTION

CYTOLOGICAL analysis (LINDEGREN and RUMANN, in press) has shown that *Neurospora crassa* probably contains nine chromosomes. These chromosomes were found to contain respectively, 29, 18, 13, 9, 5, 3, 3, 2, and 1 chromomeres; but since they were not completely uncoiled, the number of ultimate chromomeres is probably at least twice as great. They are certainly the smallest chromosomes in which crossing over has ever been analyzed. The sex chromosome in Drosophila, in which comparable studies have been made, contains at least twenty times more chromomeres. In Neurospora, one linkage group containing six loci has already been established (LINDEGREN 1936). If mutations occur at random, about 35 percent of the mutants should be found in the first chromosome and it seems probable that the sex chromosome, which is the longest genetically, corresponds to the longest cytological chromosome.

The present paper reports a second linkage group containing four loci. The second longest chromosome contains 22 percent of the chromomeres and probably contains the genes of this second linkage group. About half of the genes should be distributed among seven other linkage groups and many other mutants which have not yet been mapped are being carried in stock. None of these has been found to be linked in those tests which were made. All the mutants used in these studies appeared spontaneously.

EXPERIMENTAL

Four loci located in the second chromosome are the spindle fiber attachment (SFA), peach (*Pe*), tuft (*Tu*), fluffy (*F*). Peach (LINDEGREN 1936) differs from normal in its lighter color. The conidia in tuft are clustered together in large bunches. It grows slightly slower than normal. The color of the mature culture is considerably lighter than normal. The conidiophores grow to nearly the same height as normal, but the lower part of the aerial growth is almost white and the conidia are mostly borne at the top in masses separated irregularly from each other by relatively large "moth-eaten" open spaces. Fluffy is a non-conidial mutant, and has already been reported (LINDEGREN 1933).

The genes differentiating these mutants are all distributed in the same

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arm of the second chromosome. Peach is 11.3, tuft is 19.8, and fluffy is 38.7 units from the spindle fiber attachment. It has already been shown that neither peach nor fluffy are in the sex chromosome. A preliminary cross of peach by fluffy indicated that these two genes are linked. A second cross of tuft by fluffy indicated that tuft was also in this linkage group. Tuft, (like peach and fluffy) was shown to segregate independently of the sex differentiating genes. The exact relationships were established by three different crosses, namely, *PeF*×*Tu*, *PeTu*×*F*, and *TuF*×*Pe*. In table I, 278 asci from these crosses are analyzed.

In measuring distances between different loci, advantage is taken of the fact that the arrangement of the spores in the ascus makes it possible to distinguish asci in which the gene pairs were segregated from each other at the first reduction division from those in which second-division segregation occurred. This makes it possible to determine the amount of recombination occurring between the SFA and other loci. For example, in calculating the distance of peach from the SFA, the total number of exchanges in region I is determined and this number is divided by the total number of chromatids. There were 34 asci in which an exchange occurred in region I, but in neither of the other regions. In each exchange two of the four strands interchange; in the 34 asci, 68 of the 136 strands crossed over. The exchanges involving region I (either in I only, or in one or more of the other regions as well) are listed below with the number of crossover chromatids which each contribute to the total number in region I.

Region	*Asci*	*Crossover chromatids*
I	34	68
I & II	5	10
I & III	9	18
I & II	6	12
I & III	6	12
I & II & III	3	6
		126

Since each ascus produces four chromatids; the total number of chromatids is 4 times the total number of asci (278×4=1,112). To obtain the distance of peach from SFA, 126 is divided by 1,112 which equals 11.3. In the same way the total number of crossover chromatids in the other two regions is determined and the distances evaluated (table I).

If no exchange occurs, the heterozygous tetrad in the first reduction division separates into two homozygous dyads, each held together by the SFA (fig. 1). When the SFA splits at the second division, each pair of homozygous chromosomes is separated, but the narrow ascus prevents overlapping of the spindles. Four homozygous chromosomes are formed into nu-

clei in the upper half of the ascus and the four oppositely homozygous chromosomes (containing the corresponding alleles) are lined up in the lower half. Thus no exchange results in a first-division segregation of all the alleles in the chromosome. Since the tetrads are oriented at random on the spindle, there are two possible arrangements of the spores in these *no exchange* asci, either 1 to 4 are A B C and 5 to 8 are a b c, or 1 to 4 are a b c, and 5 to 8 are A B C.

TABLE 1

Classification of type of exchange and calculation of number of crossover chromatids.

			CROSSOVER CHROMATIDS		
			REGIONS		
			I	II	III
No Exchanges		104			
One-Region Exchange in I		34	68	0	0
One-Region Exchange in II		26	0	52	0
One-Region Exchange in III		76	0	0	152
Two-Region Exchanges	2-Strand I & II	5	10	10	0
	2-Strand II & III	2	0	4	4
	2-Strand I & III	9	18	0	18
	3-Strand I & II	6	12	12	0
	3-Strand II & III	3	0	6	6
	3-Strand I & III	6	12	0	12
	4-Strand II & III	2	0	4	4
One-Region	4-Strand III	2	0	0	8
Three-Region Exchanges	2-Strand, 2-Strand	1	2	2	2
	3-Strand, 2-Strand	2	4	4	4
			126	94	210

If an exchange occurs in the first region, there is no recombination of characters, but the dyads on the second spindle are heterozygous instead of homozygous. In the second division each of the heterozygous dyads is split to form two homozygous chromatids. These are then arranged alternately in the ascus in one of the four arrangements shown in figure 2, as a result of the random orientation of the spindles in the two divisions. Similarly a single exchange in region II results in producing four genotypes and there are eight different possible arrangements of the recombined genotypes in the ascus. A study of figure 2 shows several of the other possible arrangements.

The distance of peach from the SFA (11.3 units) indicates that in 22.7 percent of the asci, a single exchange has occurred in region I, similarly the distance of 8.5 between peach and tuft, indicates that in 17.0 percent

of the asci, a single exchange has occurred between peach and tuft. The product .227×.170=.039 gives the fraction of asci in which a simultaneous exchange in both regions may be expected. Therefore, in about 3.9 percent, or 11 of the 278 asci, it would be expected that simultaneous exchanges in regions I and II would be found. The observed number was 14.

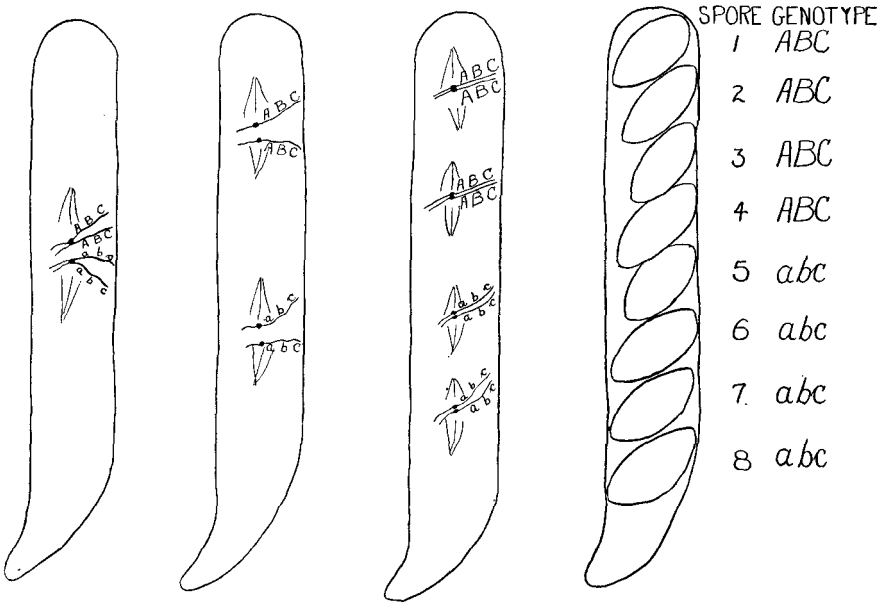

FIGURE 1.—Orientation of spores in the ascus when no exchange occurs.

Comparison of the numbers of calculated and observed exchanges is shown below:

Region	*Calculated*	*Observed*
I & II	11	14
I & III	24	18
II & III	18	10

These data show a deficiency rather than an excess of the number of expected multiple exchanges. An excess of multiple exchanges was found in a study of crossing over in the sex chromosome. However, an examination of the data on the sex chromosome show that this excess only occurs between regions symmetrically placed on opposite sides of the spindle fiber attachment. When regions on one side of the SFA are considered there was not an excess but a deficiency. Thus the present data are in agreement with those already reported. If regions on both sides of the SFA had been studied, it is probable that an excess of multiple exchanges would have been found in the second chromosome also.

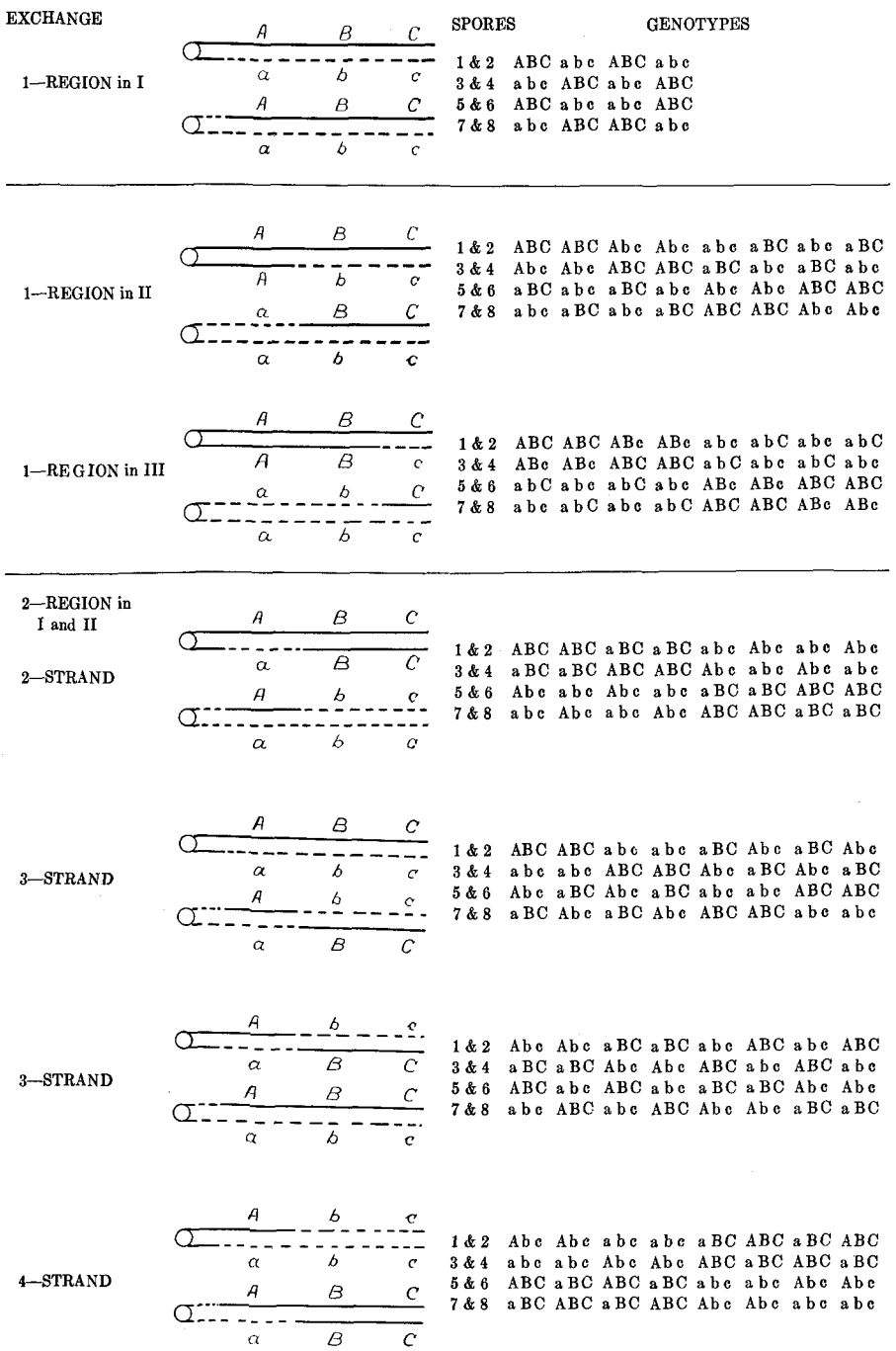

FIGURE 2.—Diagram showing orientation of genotypes in the ascus due to various types of exchanges. Loci *Pe/pe*, *Tu/tu*, and *F/f* are indicated by *A/a*, *B/b*, and *C/c*. The SFA indicated in the diagram is not recognizable in the genotypes, but first division segregation of the SFA means that chromatids in spores 1 to 4 contain SFA's of similar origin, as compared to those in spores 5 to 8.

It is not possible in this study of four-strand crossing over to determine which two of the four strands are involved in any given exchange. In the diagram, the first exchange is arbitrarily designated as always occurring between strands 2 and 3 (fig. 2) actually there are three other possibilities, namely, 1 and 3, 2 and 4, 1 and 4. If this first exchange is followed by a second exchange in another region, the ascus is classified as a two-region exchange ascus. If this second exchange involves the same two strands as the first, that is, strands 2 and 3, this ascus is called a 2-region, 2-strand, ascus,

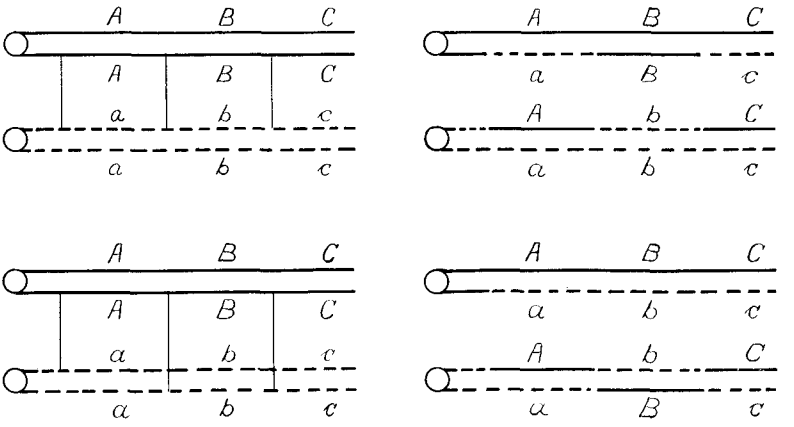

FIGURE 3.—Chromatids produced by the 3-region exchanges. The first is a 2-strand, 2-strand; the second is a 3-strand, 2-strand multiple exchange.

because only 2 of the 4 strands (2 and 3) have participated in the exchanges. Strands 1 and 4 have not been involved in any exchange at all. If the second exchange involves 1 and 4, then the ascus exchange is called a 2-region, 4-strand ascus because all 4 strands, 1, 2, 3, and 4, are crossover strands. Strand 2 crossed over with 3; and strand 1 crossed over with 4. There are two possible kinds of 2-region, 3-strand exchanges: (1) those in which the exchange between strands 2 and 3 is followed in the second region by an exchange between strand 2 and 4; (2) those in which the original exchange between strands 2 and 3 is followed by an exchange between strands 1 and 3. In the former case, strand 1 is a non-crossover strand and in the latter case, strand 4 is a non-crossover strand.

If the second crossover occurs at random with respect to the first, then the four different types of 2-region exchanges shown in figure 2 will occur in equal numbers. That is, the ratio of 2-strand to 3-strand to 4-strand exchanges should be 25 to 50 to 25. In the present paper, there are reported sixteen 2-strand, fifteen 3-strand, and two 4-strand, two-region exchanges. There are three 3-region exchanges, one a double 2-strand as shown in figure 3, the other two were 3-strand, 2-strand exchanges as

shown in the same figure. Therefore, the total ratio of 2-, to 3-, to 4-strand exchanges is 20 to 17 to 4 in the second chromosome. LINDEGREN and LINDEGREN (1937) reported a ratio of 27 to 14 to 8 in the sex chromosome, which is statistically equivalent to the present ratio. Thus, of a total of 90 double exchanges, the ratio is 52 to 34 to 13 and in striking agreement in the two different chromosomes.

In the earlier study the regions were short so that practically no undetected exchanges could have occurred. In the present study, there may have been about ten undetected multiple exchanges in the long region between tuft and fluffy. If a 2-strand multiple exchange occurred in this region it would be undetected. Therefore, although the number of 2-strand exchanges are in excess of those expected, this long region reduces rather than increases their apparent number. A 3-strand exchange occurring within this long region would appear to be a single crossover and would be recorded as such. The 4-strand exchanges can be detected even if they occur in a single region, because each one of the four strands is a crossover. Therefore, the deficiency of 4-strand exchanges like the excess of the 2-strand exchanges is real and not the result of using data involving one long region.

SUMMARY

The discovery of non-random crossing over in the second chromosome, essentially conforming to the type of non-randomness reported in the first chromosome, offers further support to the modification of Belling's theory of crossing over already suggested by the writers.

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