GENETIC FACTORS INFLUENCING CROSSING-OVER FREQUENCY IN NEUROSPORA[1,2]

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DIFFERENT crosses involving the same marked region in *Neurospora crassa* may yield markedly different frequencies of recombinants. It has been suggested that the fluctuations in recombination frequency result from inherent differences between the various wild-type strains from which the stocks descended (BARRATT 1954; FROST 1961). However, the nature of these differences and the ways in which they may influence the crossing-over process remain unknown.

The present report deals with experiments designed to pursue the following questions about genetic factors which influence the frequency of crossing-over:

(1) Are factors at a single genetic locus responsible for these effects?

(2) Is the frequency of crossing-over influenced by the presence of particular genetic factors or by heterozygosity *per se*?

(3) Are the effects on crossing-over localized or diffuse?

METHODS

In this study use was made of the ascospore mutant *asco*, which causes spores to remain colorless (wild-type spores turn black). *asco* is linked to the centromere of linkage group VI (STADLER 1956). In a cross segregating for *asco* the frequency of asci with second division segregation for spore color is a measure of crossing-over between *asco* and the centromere. This provides a quick assay for crossing-over frequency; it is possible to score 300 asci per hour.

In all crosses in which *asco* was segregating, the *asco*+ strain served as protoperithecial parent. It was inoculated onto slants of crossing medium at 25C five days before fertilization with conidia of the *asco* parent. After fertilization the crosses were allowed to mature for about ten days at 25C before they were examined for segregation patterns.

Spores carrying *asco* are seldom viable. In crosses segregating for *asco* we are limited to the *asco*+ spores for information about other segregating genes. To classify these, the two *asco*+ spore pairs of each ascus were isolated on slants of complete medium and tested for nutritional requirements by drop tests of conidial suspensions on plates of sorbose minimal medium with specific supplements.

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[2] Some of the data reported here have previously appeared in abbreviated form in the Microbial Genetics Bulletin (Number 13 and Number 16), a mimeographed nonpublication.

EXPERIMENTAL RESULTS

Crosses of various strains to *asco* give frequencies of second division segregation ranging from ten percent to 58 percent (STADLER 1956). The frequencies are distributed fairly evenly throughout the range. This shows that the frequency is not controlled by a single pair of alleles, as this type of control would result in a bimodal distribution. It could mean that genes at several distinct loci can affect the frequency, or that a multiple allelic series at a single locus is responsible. To explore this point, a cross was made between two strains which, when crossed to *asco*, gave high and low frequencies of crossing-over, respectively. Asci were dissected from this cross, and the component strains were all crossed to *asco* to score for crossing-over between the *asco* locus and the centromere. If crossing-over frequency were controlled at a single locus, each ascus should contain two spore pairs like each parent in this characteristic. The results (Table 1a) indicate that control is manifested at more than one locus.

TABLE 1a

Analysis of tetrads from a cross between a low-crossover strain and a high-crossover strain

	Strain crossed to *asco*	Total asci	Second division segregation asci	Frequency of second division segregation	Standard error
Parents:					
	4A	5576	556	.100	.004
	al_2a	695	252	.363	.018
Progeny:					
ascus 1					
	1–1a	197	34	.173	.027
	1–2a	203	34	.167	.026
	1–3A	131	29	.221	.036
	1–4A	285	59	.207	.014
	1–5a	214	53	.248	.030
	1–6a	232	54	.233	.028
	1–7A	260	53	.204	.025
	1–8A	324	66	.204	.022
ascus 2					
	2–1A	183	56	.306	.034
	2–3A	167	40	.240	.033
	2–5a	183	28	.153	.027
	2–7a	164	34	.207	.032
ascus 3					
	3–1a	253	61	.241	.027
	3–3a	195	51	.262	.031
	3–5A	201	33	.164	.026
	3–7A	184	39	.212	.030
ascus 4					
	4–1A	220	36	.164	.025
	4–3A	230	49	.213	.027
	4–5a	223	69	.309	.031
	4–7a	144	45	.313	.037

TABLE 1b

Analysis of a tetrad from a cross between two low-crossover strains

	Strain crossed to *asco*	Total asci	Second division segregation asci	Frequency of second division segregation	Standard error
Parents:					
	854a	313	40	.128	.019
	4A	5576	556	.100	.004
Progeny:					
	1–1	128	17	.133	.030
	1–3	145	16	.110	.026
	1–5	207	23	.111	.022
	1–7	124	15	.121	.029

The interpretation of this experiment requires the assumption that the two *asco* stocks of opposite mating type were identical for genetic factors influencing crossing-over. Some justification for this assumption is provided by the analysis of an ascus from a cross between two low-frequency strains (Table 1b); there is no indication of segregating factors.

Heterozygosity and crossing-over frequency: The cross of *rib-1 A* to *asco a* results in second division segregation at the *asco* locus in 12.6 percent of the asci (Table 2). This represents a map distance of 6.3 units between *asco* and the centromere. The *rib-1* locus is in the opposite arm of linkage group VI, very close to the centromere, so the map distance can be checked by counting prototrophs among germinated random spores. (The frequency of prototrophs is a direct measure of map distance in this case, as the germinated (*asco*+) spores include one recombinant class and one parental class.)

Progeny of this cross with the genotype *rib A* were crossed back to the *asco* parent. Frequencies of second division segregation for *asco* in these backcrosses are consistently as high as, or higher than, the frequency in the parent cross. A second generation of backcrossing gives the same result. Inbreeding often results in an increase but never in a decrease in frequency of second division segregation. That this represents an increase in crossing-over frequency (rather than nuclear passing or spore slippage) is verified by the corresponding rise in frequency of prototrophic spores.

The result indicates that crossing-over in the parent cross was suppressed by heterozygous elements at numerous loci. It seems probable that heterozygosity itself was responsible for the effect. Alternatively, it is possible that there are genes for high crossing-over and genes for low crossing-over, and the *rib* parent happened to carry the low-crossover allele at each segregating locus, but this seems improbable.

Localization of effects on crossing-over: In order to get more precise information about the distribution of crossing-over effects, crosses were studied which were segregating not only for *asco*, but also for three linked markers: *cys-2* (cysteine-requiring), *ad-1* (adenine-requiring) and the visible mutant *ylo* (yellow conidia). The order of the loci is *asco*—*cys*—*ylo*—*ad*—centromere. In

TABLE 2

The effect of inbreeding on crossing-over as revealed by the frequency of second division segregation for asco *and by the frequency of recombinants among random spores*

Strain crossed to *asco a*	Total asci	Second division segregation asci	Frequency of second division segregation	Standard error	Percent of prototrophs among germinated random spores	Number of random spores scored
rib 1A	2088	264	.126	.007	7.7	223
Progeny of *asco a* × *rib 1A*:						
rib 11A	309	36	.117	.018	...	...
rib 12A	194	33	.170	.027	...	...
rib 13A	165	30	.182	.030	...	...
rib 14A	535	104	.194	.017	...	...
rib 15A	266	58	.218	.025	...	...
rib 16A	359	84	.234	.022	...	...
rib 17A	765	181	.237	.015	12.6	247
rib 18A	777	264	.340	.017	17.9	235
Progeny of *asco a* × *rib 17A*:						
rib 171A	254	76	.299	.029	...	...
rib 172A	188	59	.314	.034	...	...
rib 173A	448	165	.368	.023	...	...
rib 174A	158	59	.373	.038	...	...
Progeny of *asco a* × *rib 18A*:						
rib 181A	195	71	.364	.034	...	...
rib 182A	171	70	.409	.038	...	...
rib 183A	317	134	.423	.028	26.2	202
rib 184A	231	111	.481	.033	22.7	216

an ascus with a second division pattern of segregation for *asco* it is possible to determine which of the four included intervals was the site of the crossover by classifying the two *asco*+ spore pairs for *cys*, *ylo* and *ad* (Figure 1).

Second division segregation for *asco* occurred in 26.8 percent of the asci (1299 second division segregations in 4847 total asci) in the cross *asco a* × *cys ylo ad A*. Fourteen progeny from this cross with the genotype *cys ylo ad A* were crossed back to the *asco* parent, and the frequencies of segregation patterns were recorded for these crosses. None of the 14 backcrosses had a lower frequency of second division segregation than the parent cross, and in nine of the crosses the frequency was significantly higher. The backcross with the highest frequency of second division segregation (37 percent; 654 second division segregations in 1769 total asci) was chosen for further comparison with the parent cross. From each of these two crosses 89 asci in which *asco* segregated in the second division were dissected, and the *asco*+ spore pairs were grown and classified for the other segregating markers.

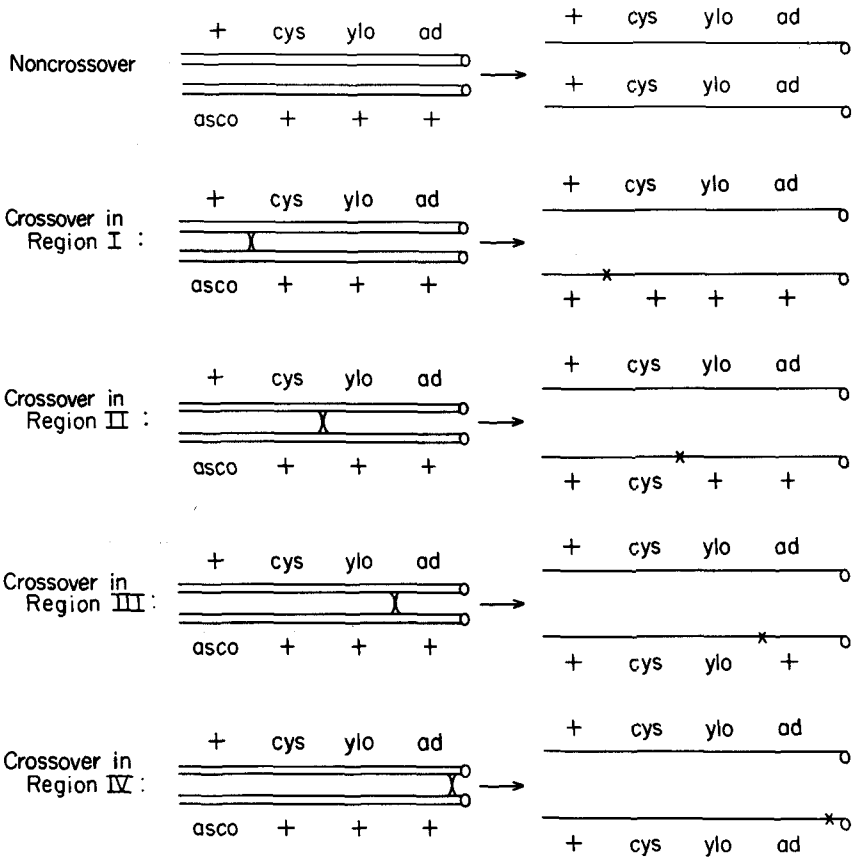

FIGURE 1.—Genotypes of the viable spore pairs resulting from crossing-over in the marked regions of the cross *asco* × *cys–ylo–ad*.

The numbers of crossovers in each of the four marked regions are shown in Table 3. Some three-strand double crossovers and some triple crossovers are detectable, and these are included in the table. Three-strand doubles in which one of the exchanges is in region IV or in which one of the exchanges does not involve either of the *asco*+ spore pairs will be scored as single crossovers. Two-strand and four-strand double crossovers result in first division segregation for *asco*, and the analysis of asci with first division patterns (Table 4) reveals that these events sometimes occur, in the backcross at least.

The segregation pattern produced by crossing-over in a region adjacent to a centromere can be mimicked by nuclear passing in the second meiotic division. In the seven asci classified as crossovers in region IV a check of the segregation pattern of an independent centromere marker (mating type) gave no indication of nuclear passing.

The crossover maps for the two crosses (Table 5) are based on all detected crossing-over in asci with segregation in either the first or second division. The

TABLE 3

Analysis of crossing-over in asci with second division segregation for asco. *Region I:* asco–cys; *region II:* cys–ylo; *region III:* ylo–ad; *region IV:* ad–*centromere*

	Single crossovers				Multiple crossovers	
	Region I	Region II	Region III	Region IV	Number of asci	Type
Parent cross (89 asci)	39	22	24	3	1	I, II, IV triple
Backcross (89 asci)	33	22	27	2	5	III, III, IV triple I, III double I, II double I, II double II, III double

TABLE 4

Analysis of crossing-over in asci with first division segregation for asco

	Noncrossovers	Double crossovers	
		Number	Type
Parent cross (37 asci)	37	0	
Backcross (49 asci)	46	3	All two-strand doubles in regions II and III

TABLE 5

Crossing-over maps for the parent cross and the backcross

	Crossover map units				
	Region I *asco–cys*	Region II *cys–ylo*	Region III *ylo–ad*	Region IV *ad*–centromere	Total *asco*–centromere
Parent cross	6.02	3.46	3.61	0.60	13.69
Backcross	7.48	7.13	8.37	0.62	23.60

amount of increase of crossing-over with inbreeding does not appear to be uniform throughout the regions marked in these crosses. The rate is more than doubled in the *cys–ylo–ad* region, while there is little increase, if any, in the adjacent regions.

DISCUSSION

The experiments reported here indicate that crossing-over in Neurospora is suppressed by heterozygosity. The source of the differences between the parent strains could be the inherent difference between the original strains collected from nature, or it could be the by-product of the extensive radiation employed

to produce mutants (BARRATT 1954; PERKINS 1959; FROST 1961). It is not known what degree of heterozygosity is involved. Chromosomal aberrations, when heterozygous, are known to suppress crossing-over in the surrounding region. Patterns of spore abortion characteristic of heterozygous aberrations were not observed in these crosses; spore abortion would be expected to change some of the 4:4 asci (4 black spores:4 colorless spores) into 2:6 and 0:8. There are many 0:8 asci (all spores colorless) in all crosses segregating for *asco* which have been examined. However if these asci resulted from heterozygous aberrations which were also responsibile for the limitation of crossing-over, their frequency should diminish with inbreeding. There is no evidence of such a trend in the frequency of 0:8 asci, and appreciable numbers of 2:6 asci were not observed in any of the crosses.

There is some evidence in these results that crossing-over interference is relaxed by inbreeding. The crude approximations permitted by the data indicate that the coincidence of crossing-over in region I,II with that in region III,IV is about 0.2 in the parent cross and very near unity in the backcross. A positive correlation of interference with heterozygosity was also observed by DE SERRES (1958), but he found that recombination between two closely-linked adenine loci in Neurospora was *increased* with diversity of origin of the parent strains.

Although no experiment was performed to locate the genetic factors influencing crossing-over frequency in the *asco*–centromere region, it seems probable that they are not confined to this region. The *asco* locus has shown linkage to the centromere in all crosses studied to date, so at least one of the asci in Table 1a should be a parental ditype for the whole region from *asco* to the centromere. However, none of the asci are parental ditypes for crossing-over factors. Also, nearly all of the *rib* progeny which were backcrossed to *asco* (Table 2) probably carried an *asco*–centromere–*rib* region identical to that of their *rib* parent, so that any heterozygosity in this region in the parent cross would have persisted in the backcross. However, nearly all the backcrosses showed more crossing-over than the parent cross.

Factors which influence the frequency of crossing-over in Drosophila are especially effective in the regions around centromeres, and it has been proposed that the centromere plays a key role in the crossing-over event (MATHER 1938). FINCHAM (1951) observed that two related species of Neurospora had markedly different frequencies of recombination in the centromere region of the mating-type chromosome. RIFAAT (1959) reported that the effect of incubation temperature on recombination frequency was most pronounced in a marked region including the centromere of linkage group I in *Neurospora crassa*. In the present study it is noteworthy that the very small interval adjacent to the centromere (region IV) did not show fluctuations of recombination frequency. This was observed for the effect of inbreeding, as reported here, and also when recombination was increased by changing the temperature of incubation (TOWE and STADLER, in preparation).

The inviability of the *asco* spores has limited the types of crosses which could be performed and the amount of information about segregation which could be gained in the present study. However, the use of the frequency of second division

segregation patterns as an assay for crossing-over has an important advantage over methods based on counts of parental and recombinant types in the progeny. We would like a direct measure of the crossing-over in meiosis. The spore classification method would give this information only if it revealed the various genotypes in the proportions present at the conclusion of meiosis. This method would be accurate if all mutant combinations matured at the same rate as wild type, if all types were equally viable under the culture conditions employed, and if all types remained viable for the same length of time after maturation. Our experience with various nutritional and morphological mutants is that all three of these assumptions may be invalidated. Even the analyses based on complete asci may be spurious if the occurrence of certain recombinant types can influence the rate of maturation or if certain genotypes are relatively inviable. The segregation-count method escapes these pitfalls when only a single gene pair is segregating (as was true in the first experiment reported here, but not in the subsequent ones). There are two mutant spore pairs and two wild-type pairs in every ascus whether it is a crossover or a noncrossover type.

SUMMARY

Crosses segregating for an ascospore mutant have been employed to study the effects of heritable factors on crossing-over frequency in Neurospora. The frequency of recombination in the interval studied here bears an inverse relationship to the degree of genetic difference between the parents in the cross. Although these heterozygous factors have not been located, it has been shown that there must be two or more separable factors contributing to the effect. Inbreeding results in an increase in crossing-over and a decrease in crossing-over interference. The change in crossing-over frequency is not uniform throughout the marked region. The greatest effect takes place in a segment linked, but not adjacent, to the centromere.

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