STUDIES WITH PURPLE ADENINE MUTANTS IN *NEUROSPORA CRASSA*. VI: THE EFFECTS OF DIFFERENCES IN GENETIC BACKGROUND ON *ad-3A* × *ad-3B* CROSSES[1]

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IN previous studies (DE SERRES 1956) random ascospore analyses were made on various intercrosses of *ad-3A* and *ad-3B* mutants to obtain estimates on the map distance for the *ad-3A–ad-3B* region. Analysis of such crosses indicated a marked heterogeneity in the estimates of the map distance for this region in addition to marked differences in the patterns of chromosome interference. In one of the crosses that gave the closest linkage for the *ad-3A–ad-3B* region many more new adenine-independent progeny of parental genotype (with reference to outside markers) were recovered than expected, indicating marked negative chromosome interference, whereas the data from the other four crosses indicated positive chromosome interference.

Experiments described in this paper were performed to determine the significance of the differences in the frequency of recombination and interference in *ad-3A* × *ad-3B* crosses. Thirteen *ad-3A* mutants were crossed with various strains carrying markers at the closely linked *ad-3B*, *hist-2*, and *nic-2* loci. The data indicate that all major differences in the frequency of recombination and the patterns of chromosome interference can be attributed to differences in genetic background. The data can also be interpreted as indicating that the increase in recombination found in crosses between mutants induced in different wild-type strains is due to an increase in the frequency of reciprocal recombination and not to nonreciprocal recombination. Some aspects of these experiments were summarized in a previous paper (DE SERRES 1958a).

MATERIALS AND METHODS

Materials. Single mutant strains: ad-3A mutants A1 through A10 were derived from filtration-concentration experiments in which the St. Lawrence wild-type strain 74A was used. (I am indebted to Dr. MARY CASE of Yale University for mutants A4 through A10.) Mutants A2 and A5 were derived from untreated conidia, and the remainder from X-irradiated conidia. The original isolates of mutants A1 through A9 were used in these experiments; the isolate of A10 used was an adenine-requiring F_1 extracted from a cross of the original strain to a wild-type strain 74-OR8-1a. The cross was necessary to eliminate the methionine requirement since the original strain was recovered as a purple adenine, methionine double mutant. The *ad-3B* mutants B10 and B10-R2, a temperature-sensitive revertant of B10, have been described (DE SERRES 1958b). The isolates of *ad-3A* mutants 38701, 38709, and 68306 (derived from the experiments

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of BEADLE and TATUM 1945) are F_2 progeny from crosses to 74A. Selection was made among the progeny for those that (1) gave progeny of uniform morphology in crosses with 74A or 74-OR8-1a and (2) formed heterokaryons with mutants induced in 74A that have wild-type morphology and growth rate. Origin of the original isolates and strain numbers of these *ad-3A* and *ad-3B* mutants are given in Table 1.

Multiple mutant strains: All marked strains of *ad-3A* and *ad-3B* mutants used in the intercrosses (Table 2) were derived from crosses to strain 94-YU2-1a, a *hist-2* (C94) *nic-2* (43002) double mutant. The *hist-2 ad-3A* (A2) *ad-3B* (35203) *nic-2* strains are progeny from a cross of the quadruple mutant strain obtained as ascus 61.7 in the tetrad analysis (GILES, DE SERRES and BARBOUR 1957) of the cross A2 × 35203.

Media: All media used either for crosses or plating ascospores have been described (DE SERRES 1956). In these experiments, sorbose minimal medium used in overlayering contained 0.1% sucrose and sufficient sorbose to give a final concentration of 1.0% on each plate.

Methods. Setting up crosses: Since any differences in linkage in the *ad-3A–ad-3*B region could be caused by chromosome rearrangements, and because of the possible influence of other factors

TABLE 1

Code numbers, strain numbers, and mutagenic treatments used to obtain ad-3 *mutants used in crosses*

Locus	Code No.	Strain number	Wild-type strains	Mutagenic treatment
ad-3A	A1	74A-Y68-M13	74A	X-ray
	A2	74A-Y112-M13		None
	A3	74A-Y112-M15		X-ray
	A4	74A-Y152-M36		
	A5	74A-Y153-M6		None
	A6	74A-Y154-M22		X-ray
	A7	74A-Y154-M28		
	A8	74A-Y155-M6		
	A9	74A-Y155-M64		
	A10	74-OR7-17A		
	38701*	38701-OR2-5A	1A X 25a	UV
	38709*	38709-OR2-14A		
	68306*	68306-OR2-25A	Abb4A X 25a	
ad-3B	35203*	35203A	1A X 25a	UV
	B10	74A-Y112-M2	74A	X-ray
	B10-R2	74A-Y112-M2-Y121-R2		

* Data from BEADLE and TATUM (1945).

associated with differences in genetic background in the isolates of *ad-3A* mutants 38701, 38709, and 68306, the linkage relations of each of the *ad-3A* mutants were determined with markers at the *hist-2* and *nic-2* loci. In addition, since 10 of the *ad-3A* mutants have identical genetic backgrounds, the possibility of introducing additional variability into these comparisons was avoided by crossing the original isolates (rather than genetically marked derivatives) to various marked strains. Estimates of recombination in the *ad-3A–ad-3B* region were derived primarily from crosses of the type $A + ad\text{-}3A + + \times a\ hist\text{-}2 + ad\text{-}3B\ nic\text{-}2$, but some crosses were repeated with outside markers in repulsion ($a\ hist\text{-}2 + ad\text{-}3B + \times A + ad\text{-}3A + nic\text{-}2$). Estimates of recombination in the *hist-2–ad-3A* and *ad-3A–nic-2* regions were derived from crosses of the type $A + ad\text{-}3A + \times a\ hist\text{-}2 + nic\text{-}2$.

Analysis of crosses: Ascospores from crosses of these three types were analyzed by a modification of the overlayering technique developed for Neurospora by NEWMEYER (1954). The general procedure developed is as follows: aliquots of ascospores (in a 0.15% agar suspension of minimal medium, heat-shocked for 60–75 min at 60°C) from a given cross were mixed with differentially supplemented media and poured into Petri plates. After incubation at 4° C for 16 hr and at 35° C for 5 hr, an overlayer of sorbose minimal medium was added to each plate. Plates were then reincubated at 35°C for 24 hr and colony counts made after 48–72 hr incubation at room temperature.

Detection of pseudowild types: The studies of MITCHELL, PITTENGER and MITCHELL (1952) and PITTENGER (1954) showed that pseudowild type (PWT) progeny are recovered at low frequencies in various crosses of closely linked mutants in at least 5 of the 7 different linkage groups. In crosses of linkage group I mutants, however, only unisexual PWT's were recovered. Reasons for the failure to recover bisexual PWT's were unknown (PITTENGER 1954). Since the *hist-2* locus is closely linked to mating type (about 10 crossover units), the majority of PWT's formed in crosses of markers in the *ad-3* region should be bisexual. Thus it was anticipated that only a small proportion of the PWT's that actually occurred would be recovered. But since the majority of the recoverable PWT's should be phenotypically wild type (with reference to all markers), they are indistinguishable from adenine-independent progeny of parental genotype in all the crosses and have to be eliminated.

Early in these analyses, it was discovered that bisexual PWT's are recovered. Although most PWT's were of this type, they formed colonies of characteristic size, color, and morphology, and were readily distinguishable from normal wild-type colonies on this basis. A portion of the bisexual PWT's and probably all the unisexual PWT's formed colonies of normal size and morphology and were indistinguishable from colonies formed by normal wild-type ascopores. Adenine-independent progeny of parental and reciprocally recombinant genotypes recovered in the experiments were tested to eliminate PWT's, with one or a combination of the following methods being used. (1) Most bisexual PWT's were eliminated during colony counting on the basis of morphology (sparse growth), color (sometimes pigmented deep purple), and size (microcolony or slightly larger). When isolated to minimal medium, such colonies grow slowly and show varying degrees of purple pigmentation. (2) Isolates derived from colonies of normal size were tested by either of the following procedures.

Method 1: by crossing to the *hist-2 ad-3A ad-3B nic-2* tester strains: Crosses of such isolates to one or both of the tester strains were then analyzed to determine qualitatively whether the ratio of adenine-independent to adenine-dependent segregants gave the expected ratio of 1:1. Some of the adenine-independent segregants were then tested to determine whether the expected number were of the same genotype as the adenine-independent parent.

Method 2: by streaking dilute conidial suspensions on plates supplemented with 100 mg/l of histidine, 10 mg/l of niacin, and limiting adenine (1 mg/l): Under these conditions, adenine-requiring colonies have a distinctly abnormal morphology and are readily distinguishable from nonrequiring colonies. Colonies derived from isolates containing only adenine-independent nuclei are normal in morphology, whereas colonies derived from PWT isolates show various mixtures of the two colony types, ranging from almost equal numbers of normal and abnormal colonies to almost entirely abnormal colonies.

Calculation of data: Methods used in calculating the frequency of recombination in the *hist-2–*

TABLE 2

Strain numbers and genotypes of marked strains used in crosses

Strain number	Genotype
94-YU2-1a	a hist-2 (C94) nic-2 (43002)
74-OR17-4A	A ad-3A (A2) nic-2
38701-OR3-6A	A ad-3A (38701) nic-2
74-OR16-2a	a hist-2 ad-3B (B10)
35203-OR2-3a	a hist-2 ad-3B (35203)
94-YU3-6a	a hist-2 ad-3B (35203) nic-2
74-YU192-1a	a hist-2 ad-3A (A2) nic-2
74-OR13-2a	a hist-2 ad-3B (B10-R2) nic-2
74-OR10-15A	A hist-2 ad-3A (A2) ad-3B (35203) nic-2
74-OR10-10a	a hist-2 ad-3A (A2) ad-3B (35203) nic-2

ad-3A and *ad-3A–nic-2* regions in crosses of the type *ad-3A* × *hist-2 nic-2* have been described (DE SERRES 1958b). In crosses of the type *ad-3A* × *hist-2 ad-3B nic-2* or *ad-3A nic-2* × *hist-2 ad-3B*, the frequency of recombination in the *ad-3A–ad-3B* region was calculated by multiplying the total number of adenine-independent segregants by two (assuming an equal number of the double adenine mutants), dividing the product by the total number of viable ascospores, and multiplying the frequency thus obtained by 100. Confidence limits of 95% based on the statistics of a normal distribution are given for all estimates of the recombination percentage for the regions tested in the various crosses (BIRNBAUM 1954).

RESULTS

Linkage

Recombination frequency in the hist-2–ad-3A *and* ad-3A–nic-2 *regions:* Each of the 13 *ad-3A* mutants was crossed to the same double mutant strain, *a hist-2 nic-2*, and the progeny from each cross were analyzed by the general overlayering technique described. For this analysis, ascospores were plated on four different media to estimate the numbers of segregants of genotypes +++, H++, ++N, and H+N individually and collectively: (1) minimal medium and (2) minimal medium supplemented with histidine; (3) with niacin, and (4) with histidine and niacin. Total colonies obtained, or estimated, in aliquots of ascospore suspensions of the individual crosses in single or replicate experiments are shown in columns 2 through 5 of Table 3. Since the numbers of colonies obtained on plates supplemented with either histidine or niacin result both from single and double exchanges in Regions I and I + II and in II and I + II, recombination frequencies in each region can be calculated directly.

TABLE 3

Linkage relations of individual ad-3A *mutants in the* hist-2–ad-3A *and* ad-3A–nic-2 *regions*

CROSS:	a	hist-2	+	nic-2
X	A	+	ad-3A	+
	Region		I	II

Strain number	Number of colonies per plating series: M.*,†	M. + niacin	M. + histidine	M. + histidine + niacin	Recombination percentage: hist-2 - ad-3A	ad-3A - nic-2
A1	9	339	741	25,840	1.31 ± 0.14	2.87 ± 0.22
A2	6	489	775	25,880	1.89 ± 0.18	2.99 ± 0.22
A3	214	700	302	15,072	4.46 ± 0.36	2.00 ± 0.24
A4	3	307	473	15,833	1.94 ± 0.22	2.99 ± 0.28
A5	11	553	782	25,400	2.18 ± 0.18	3.08 ± 0.22
A6	3	271	387	13,824	1.96 ± 0.24	2.80 ± 0.28
A7	2	327	497	16,304	2.01 ± 0.22	3.05 ± 0.28
A8	6	350	490	17,032	2.05 ± 0.22	2.88 ± 0.26
A9	99	679	253	6,616	10.26 ± 0.74	3.82 ± 0.46
A10	11	605	1007	29,488	2.05 ± 0.16	3.41 ± 0.22
38701	3	355	477	16,344	2.17 ± 0.24	2.92 ± 0.26
38709	3	471	608	20,730	2.27 ± 0.20	2.93 ± 0.24
68306	2	386	594	20,784	1.86 ± 0.18	2.86 ± 0.24

* Minimal medium (M).
† Pseudowild-type colonies eliminated from the tabulation.

Evidence for anomalous linkage with mutants A3 and A9: Plating data and linkage calculations from the crosses of mutants A3 and A9 show a striking departure from those obtained with the remaining 11 strains. On minimal medium, the number of colonies obtained in both crosses is unexpectedly high. But although colonies that grow on minimal medium in these crosses should represent the number of double crossovers (after PWT's have been eliminated)

of genotype +++, in these two crosses phenotypically purple colonies presumably of genotype + A + were also recovered. These ranged from colonies that grew only slightly on minimal medium and were pigmented deep purple, to those that grew extensively and showed only a light purple pigment. Of the 214 colonies isolated from minimal medium from the cross of A3, only 7 were non-pigmented and adenine-independent and of genotype +++; in the cross of A9, none was of genotype +++. Linkages in regions I and II were calculated directly from the numbers of colonies shown in columns 3, 4, and 5 of Table 3. Estimates of recombination obtained in this manner are inconsistent with those obtained from crosses of the other mutants and suggest that these two mutant strains are associated with some type of chromosome rearrangement.

Similarity of the linkage relations of the remaining 11 strains: Data from analyses of the crosses of the remaining strains give quite similar estimates of linkage in both regions. If the linkage relations of these 11 mutants are considered independent estimates of the standard map distance for these two regions, and the data are pooled, average values of 1.96 and 3.00 crossover units are obtained, respectively, for the *hist-2–ad-3A* and *ad-3A–nic-2* regions. It is significant that all the mutants give remarkably similar linkage relations in both these regions, with the exceptions already noted. No evidence was obtained for any marked differences in the linkage relations of mutants 38701, 38709, and 68306 as compared with mutants A1 through A10, although both groups of mutants are of different origin (see Table 1).

Recombination frequency in the ad-3A–ad-3B *region:* Each of the *ad-3A* mutants was crossed to the triple mutant strain *a hist-2 ad-3B* (mutant 35203) *nic-2* to obtain individual estimates of linkage in the *ad-3A–ad-3B* region and to determine the genotype of the adenine-independent progeny. The crosses were analyzed by plating ascospore suspensions as described in MATERIALS AND METHODS: (1) medium supplemented with adenine, histidine, and niacin to estimate the total viable population and (2) medium supplemented with histidine and niacin to recover adenine-independent progeny of all possible genotypes. All adenine-independent progeny were isolated and tested on differentially supplemented media to determine genotypes. PWT's were then eliminated as described. The results of plating analyses on these crosses are presented in Table 4.

Additional evidence for anomalous linkage with mutants A3 and A9: The most striking departure from the general pattern was obtained with mutants A3 and A9. Both purple and non-purple colonies were obtained from the plates supplemented with histidine and niacin in the analysis of both of these crosses. In the cross of A3, 76 isolates were non-purple and adenine-independent; 31 were purple and showed partial requirements for adenine. Of the 954 isolates obtained from the cross of A9, 696 were non-purple and adenine-independent; 258 were purple and showed partial requirements for adenine. The purple isolates from the cross of A3 were not tested further, but tests on a sample of such isolates from the cross of A9 showed that they were distributed among the four genotypic classes, the majority being of the same genotype as the parental strain, A9. Since the origin and significance of the purple isolates have not been established, only

TABLE 4

Estimates of recombination in the ad-3A–ad-3B *region derived from crosses of* ad-3A *mutants with* ad-3B *mutant 35203*

Cross: X

						a	hist-2	+	ad-3B(35203)		nic-2		
						A	+	ad-3A		+		+	
								I		II		III	
				Region									
Strain number	Total viable ascospores	Percentage viability	Number of adenine-independent segregants*	Percentage recombination Region II		H+++	+++N	H++N		++++			
A1	16,433†	92.5	61	0.74 ± 0.14		61	0	0		0			
A2	16,783†	95.9	44	0.52 ± 0.12		44	0	0		0			
A3	11,883†	79.6	76(31)‡	1.28 ± 0.20		76	0	0		0			
A4	38,150	67.5	142	0.74 ± 0.09		141	0	0		1			
A5	41,075	78.0	125	0.61 ± 0.08		125	0	0		0			
A6	39,825	78.4	121	0.61 ± 0.08		119	0	0		2			
A7	41,425	76.5	113	0.56 ± 0.08		112	0	1		0			
A8	37,800	72.0	156	0.83 ± 0.10		156	0	0		0			
A9	39,200	20.6	696(258)‡	3.55 ± 0.20		139(1)	0(4)	2(3)		0(46)			
A10	38,050	73.3	138	0.72 ± 0.08		136	0	2		0			
38701	42,650	79.0	25	0.13 ± 0.04		21	0	3		1			
38709	45,850	86.3	55	0.25 ± 0.04		49	3	2		1			
68306	44,375	80.0	25	0.16 ± 0.04		25	0	2		2			

* Pseudowild-type isolates have been eliminated from the tabulation.

† Data from DE SERRES (1956).

‡ Numbers in parentheses represent phenotypically purple isolates with partial requirements for adenine. (A random sample of 195 of the 954 isolates from the cross of A9 were tested to determine genotypes.) Numbers of phenotypically purple progeny not used in estimating recombination percentage.

the numbers of the non-purple, adenine-independent isolates were used to estimate the linkage in the *ad-3A–ad-3B* region. In both the crosses, the majority of all the adenine-independent isolates are of the crossover type expected (H+++). Comparisons of the recombination percentages obtained in these two crosses with those from the remaining crosses show that they are extremely high. This may be accounted for, in part, by the lower viability of these crosses (A3 compared with A1 and A2, A9 compared with A4 through 68306). But since crosses of A9 show more marked ascospore abortion (probably owing to the presence of some additional gross chromosome rearrangement in this strain), ascospores containing adenine-dependent parental chromosomes may be less viable than those containing adenine-independent recombinant chromosomes. If such an assumption is made, and linkage in the *ad-3A–ad-3B* region is reestimated on the basis of the viability of the other crosses analyzed at the same time, a value of 0.95 crossover unit is obtained for the cross of A9, which is comparable to the estimates obtained from crosses of the other *ad-3A* mutants of identical origin.

Differences in linkage among the remaining 11 strains: In the remaining crosses, most all of the adenine-independent progeny recovered are of the expected crossover type (H+++). Progeny of parental genotype were recovered from the crosses of A4, A6, A7, A10, 38701, 38709, and 68306. In the cross of 38709, the progeny are distributed among all four classes, although most are of the crossover type expected. Data from these crosses indicate a marked similarity in the linkage of this series of alleles to the *ad-3B* locus. There is a striking difference, however, in the estimates of recombination obtained with the mutants derived from 74A as compared with mutants 38701, 38709, and 68306. The average distance for the first group of 8 mutants is 0.67 crossover units whereas the three mutants in the second group appear to be the most closely linked to the *ad-3B* locus, with an average distance of 0.16 crossover units.

Confirmation of the group differences from crosses of selected mutants with outside markers in repulsion: One mutant from each of the two *ad-3A* groups was crossed to *ad-3B* mutant 35203 to determine whether the marked differences

TABLE 5

Estimates of recombination in the ad-3A–ad-3B *region derived from crosses of* ad-3A *mutants with* ad-3B *mutant* 35203 *with markers in repulsion*

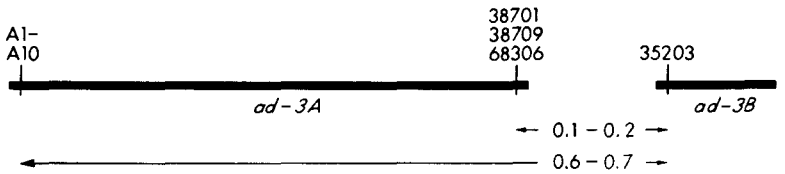
	a	*hist-2*	+	*ad-3B* (35203)	+
Cross ×					
	A	+	*ad-3A*	+	*nic-2*
Region		I	II		III

Mutants crossed	Total ascospores plated	Total viable ascospores	Number of adenine-independent segregants*	Percentage recombination Region II	Genotypes of adenine-independent segregants H++N	++++	H+++	+++N
38701 × 35203	377,400	231,660	116	0.10 ± 0.01	107	0	6	3
A2 × 35203	205,600	157,260	467	0.59 ± 0.04	454	0	5	8

* Pseudowild types eliminated from the tabulation.

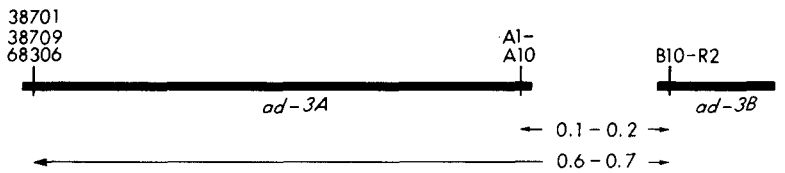
in linkage observed in the previous crosses were reproducible with outside markers in repulsion (Table 5). In the cross A2 × 35203, the frequency of recombination in the *ad-3A–ad-3B* region is 0.59%, in the cross 38701 × 35203, the frequency of recombination is 0.10%. Data from both crosses agree with those in Table 4 obtained with the same mutant combinations with outside markers in coupling. This shows that the differences in recombination frequency are reproducible and are not influenced by arrangement of the outside markers.

Combining both sets of data (Tables 4 and 5) from crosses of different *ad-3A* mutants with *ad-3B* mutant 35203 gives an order:



Mutants 38701, 38709, and 68306 are located near the distal end of the *ad-3A* locus closest to the *ad-3B* locus.

Crosses to a marked strain of ad-3B *mutant B10–R2:* A similar series of crosses of the 13 *ad-3A* mutants were made to a marked strain of *ad-3B* mutant B10–R2 (Table 6). Similar treatment of the data from these crossses gives an order:



This order is exactly the reverse of that obtained with *ad-3B* mutant 35203! On the basis of the data obtained with B10–R2, mutants 38701, 38709, and 68306 now appear to be located near the proximal end of the *ad-3A* locus furthest from the *ad-3B* locus.

Confirmation of data from crosses to B10: At this point in the analysis, the possibility was considered that, since B10–R2 is an X-ray-induced revertant derived from B10, the unexpected linkage relations of this mutant with the 38701, 38709, 68306 group was attributable to some structural rearrangement associated with the reverse-mutational event. If such a situation applies, however, the structural rearrangement should be associated with only B10–R2 and not the parental strain B10; thus the two *ad-3B* mutants should have different linkage relations with the 38701, 38709, 68306 group. One mutant was selected from each of the groups of *ad-3A* mutants, and each was crossed to mutant B10 with distal markers in repulsion (Table 7). In the cross A2 × B10, a distance of 0.15 map unit was obtained for the *ad-3A–ad-3B* region. In the cross 38701 × B10, a distance of 0.64 map unit was obtained for this same region. Since these estimates agree with those obtained with B10–R2 in similar crosses, we can see that the

TABLE 6

Estimates of recombination in the ad-3A–ad-3B *region derived from crosses of* ad-3A *mutants with* ad-3B *mutant B10-R2*

					a	hist-2	+	ad-3B	(B10-R2)	nic-2
				Cross:	X					
					A	+	ad-3A	+		+
				Region		I	II		III	
Strain number	Total ascospores plated	Total viable ascospores*	No. of adenine-independent segregants†	Percentage recombination Region II	Genotypes of adenine-independent segregants					
					H+++	+++N	H++N	++++		
A1	105,490	42,196	38	0.18 ± 0.04	35	0	0	3		
A2	178,470	71,388	68	0.19 ± 0.03	62	0	0	6		
A3	101,700	40,680	29(269)‡	0.14 ± 0.04	27(1)	0(4)	2(120)	0(144)		
A4	97,150	38,860	24	0.12 ± 0.04	20	1	3	0		
	196,300	78,520	59	0.15 ± 0.03	51	0	1	7		
A5	103,500	41,400	15	0.07 ± 0.03	14	0	1	0		
A6	103,440	41,376	51	0.25 ± 0.05	42	0	2	7		
A7	99,190	39,676	63	0.32 ± 0.05	57	0	1	5		
A8	100,045	40,018	47	0.23 ± 0.05	34	1	2	10		
A9	60,480	6,048	42(115)‡	1.39 ± 0.03	36(8)	1(1)	2(68)	3(38)		
A10	103,350	41,340	52	0.25 ± 0.05	40	0	4	8		
38701	245,700	98,280	349	0.71 ± 0.05	346	0	2	1		
38709	171,700	68,680	249	0.73 ± 0.06	240	0	1	8		
68306	255,000	102,000	388	0.76 ± 0.05	384	0	1	3		
B10-R2	183,000	73,200	1	---	0	1	0	0		

* Assuming 10% viability for A9 and 40% viability for the remainder.
† Pseudowild-type isolates eliminated from the tabulation.
‡ Numbers in parentheses represent phenotypically purple isolates with partial requirements for adenine.

results observed with mutant B10–R2 cannot be accounted for on the basis of some unique structural rearrangement.

Chromosome Interference

Although factors that influence interference relations in given chromosome regions have not been studied extensively, it seems reasonable to assume that the interference relations in a given chromosome region would be the same in crosses involving a series of allelic mutants, with identical genetic backgrounds, and the same marked strain. If, on the other hand, the comparison involved a series of alleles unrelated in origin to the same marked strain, introduction of unknown variables would make differences in interference relations in such a series of crosses not unexpected. In the data presented in Tables 3–7, too few progeny of

TABLE 7

Estimates of recombination in the ad-3A–ad-3B *region derived from crosses of* ad-3A *mutants with* ad-3B *mutant* B10 *with markers in repulsion*

	a	*hist-2*	+	*ad-3B* (B10)	+
Cross ×					
	A	+	*ad-3A*	+	*nic-2*
Region			I	II	III

Mutants crossed	Total ascospores plated	Total viable ascospores	Number of adenine-independent segregants*	Percentage recombination Region II	Genotypes of adenine-independent segregants H++N	++++	H+++	+++N
38701 × B10	261,600	162,475	521	0.64 ± 0.04	499	0	7	15
A2 × B10	283,600	159,250	122	0.15 ± 0.02	108	0	10	4

* Pseudowild types eliminated from the tabulation.

parental or reciprocally recombinant genotype were recovered from individual crosses to get reliable estimates of the interference relations obtained with individual mutants; so the data from crosses of mutants of identical or related genetic background were combined, and the interference relations of the two groups of *ad-3A* mutants were estimated. There are problems with this approach (see WEINSTEIN 1918), but the estimates of recombination in the different regions examined are sufficiently alike for mutants induced in the same wild-type strain that combining the data should not grossly distort the coincidence values. This approach seems to be the most reasonable way to determine whether there are differences in the coincidence values which are associated with the genetic origin of the *ad-3A* mutants studied. Examined in this way, the data from the various crosses show marked differences in the interference relations in the same chromosome regions when the crosses of A1 through A10 are compared with those of 38701, 38709, and 68306.

In the hist-2–ad-3A *and* ad-3A–nic-2 *regions:* For the purpose of these comparisons (Table 8), the map distances used are the average values obtained combining the data given in Table 3 and omitting the data from mutants A3 and A9.

TABLE 8

Chromosome interference calculations for crosses of the type ad-3A × hist-2 nic-2

Cross data combined	Total viable ascospores	Average recombination percentage Region I	Region II	Number of double recombinants Observed	Expected	Coefficient of coincidence (O/E)
A1, A2, A4, A5, A6, A7, A8, A10	169,601	1.91	3.04	51	49	1.04
38701 38709 68306	57,858	2.09	2.90	8	17.5	0.46

In the crosses of A1 through A10, 51 double recombinants were recovered where 49 were expected, giving a coefficient of coincidence of 1.04 and indicating no interference. Data from the crosses of 38701, 38709, and 68306, however, show that far fewer double crossovers were recovered than expected on the basis of no interference. Since the data give a coefficient of coincidence of 0.44, they indicate marked positive interference for the same regions. Thus, although both groups of mutants give essentially the same linkage relations in both regions, the interference relations are quite different.

In the hist-2–ad-3A, ad-3A–ad-3B, *and* ad-3B–nic-2 *regions:* Previous data (DE SERRES 1958b) from crosses of 11 *ad-3B* mutants with the *hist-2 nic-2* double mutant strain give an average value of 2.7 units for the *ad-3B–nic-2* region (Region III), and data from the present crosses give an average value of 2.0 units for the *hist-2–ad-3A* region (Region I). From these estimates, the coefficients of coincidence were calculated for double (I + II and II + III) and triple (I + II + III) exchange events in the crosses of both groups of *ad-3A* mutants to 35203 and B10–R2 in Table 9.

TABLE 9

Chromosome interference calculation for crosses of the type ad-3A × hist-2 ad-3B nic-2

						a	hist-2	+	ad-3B	nic-2		
					Cross:	×						
						A	+	ad-3A	+	+		
					Region		I		II	III		
						Coefficients of coincidence						
		Total	Average		I and II (++++)			II and III (H++N)			I, II, and III (+++N)	
	Crosses combined	viable ascospores	map distance ad-3A – ad-3B	Observed	Expected	O/E	Observed	Expected	O/E	Observed	Expected	O/E
ad-3A × hist-2 ad-3B (35203) nic-2												
	A1 through A10*	269,541	0.67	3	18.0	0.17	3	24.3	0.12	0	0.4	-
	38701, 38709, 68306	132,875	0.16	4	2.1	1.90	7	2.9	2.41	3	0.05	60.0
ad-3A × hist-2 ad-3B (B10-R2) nic-2												
	A1 through A10*	434,774	0.19	46	8.3	5.54	14	11.2	1.25	2	0.22	9.1
	38701, 38709, 68306	268,960	0.73	12	19.6	0.61	4	26.5	0.15	0	0.5	-

*Excluding crosses of A3 and A9.

Crosses with 35203: The data from the crosses of mutants A1 through A10 with 35203 show that fewer recombinants in regions I + II, II + III, and I + II + III were observed than expected, indicating marked positive interference. Data from the crosses of 38701, 38709, 68306, however, indicate negative interference in these same regions. The interference relations obtained in these two groups of crosses show a curious correlation with linkage relations in the *ad-3A–ad-3B* region; the closer the apparent linkage of the *ad-3* mutants the more recombinants recovered of genotypes expected to arise by multiple exchange events.

Crosses with B10–R2: In these crosses, the interference relations in the two groups of crosses are the reverse of those obtained in the crosses with 35203. The data from the crosses of A1 through A10 indicate negative interference in regions I + II, II + III, and I + II + III with coincidence values of 5.54, 1.25, and 9.1, respectively. In the crosses of 38701, 38709, and 68306, however, fewer recombinants were recovered than expected in either region, giving coincidence values of 0.61 and 0.15, respectively, indicating marked positive interference.

Although the interference relations of both groups of mutants is reversed in these crosses, the inverse correlation between the degree linkage in the *ad-3A–ad-3B* regions and the recovery of adenine-independent progeny of parental and reciprocal recombinant genotype is maintained.

DISCUSSION

The crosses of the 13 *ad-3A* mutants with other mutants in the immediately adjacent regions showed that although these strains showed essentially the same recombination frequency in the *his-2–ad-3A* and *ad-3A–nic-2* regions, markedly different recombination frequencies were obtained for the *ad-3A–ad-3B* region depending on which *ad-3B* mutant was used. In these latter crosses there were also marked differences in the patterns of chromosome interference. The data from 11 of the 13 *ad-3A* mutant crosses are compatible with the hypothesis that each mutant has resulted from a point mutation in the *ad-3A* locus; the data from the remaining two mutants, A3 and A9, require a different explanation.

Crossing data obtained with strains A3 and A9: Of all the *ad-3A* mutants studied, these two are perhaps the most interesting because of the unexpected recovery of segregants with partial requirements for adenine in all the crosses. These mutants seem to be unstable during meiosis when in combination with the wild-type *ad-3A* allele. Mutant A3 does not revert to wild type spontaneously or after an X-ray dose of 35,000 r (DE SERRES 1958b). The origin of such progeny with partial requirements for adenine is somewhat puzzling since, in the crosses with B10–R2, such progeny are of both parental genotypes; in the cross with 35203, the majority are of the same genotype as the *ad-3A* parent. It does seem possible that the segregants of genotype H++N are actually *ad-3B* mutants caused by mutation of B10–R2 to a more extreme mutant phenotype. But such a change would have to take place under rather rigid conditions since such segregants were recovered only from the crosses of A3 and A9.

GRIFFITHS (1971) has obtained evidence in more recent genetic analyses of A3 which shows that some of the data obtained in the present study can be explained on the basis of a paracentric inversion having one of its breakpoints to the left of *ad-3A* and the other one close to *al-2*. This hypothesis provides an explanation for the abnormal linkage relations of A3 in the cross with 94-YU2-1a (Table 3), but it does not provide an explanation for the occurrence of phenotypically purple isolates with partial requirements for adenine (Tables 4 and 6) in crosses with various *ad-3B* mutants.

Linkage relations of the remaining ad-3A *mutants:* Differences in the linkage relations obtained with the remaining strains are most readily interpreted on the basis of the correlation between the linkage in the *ad-3A–ad-3B* region and the origin of the mutants used. Mutants A1–A10, B10, and B10–R2 are all derived from wild-type strain 74A and have identical genetic backgrounds. Mutants 38701 and 38709 were derived from a cross of the Lindegren wild-type strains and mutant 68306 has one wild-type strain in common with them (Table 1). These latter four strains are not completely unrelated in origin to wild-type strain 74A, however, as can be seen by an examination of the pedigree of the various wild-type strains of Neurospora (BARRATT 1962). Nevertheless, an examination of the data in Tables 4–7 shows that the crosses giving low estimates for the *ad-3A–ad-3B* region (0.1–0.2 units) are between mutants induced in the same wild-type strain, whereas those crosses giving high estimates (0.6–0.7 units) are between mutants induced in different wild-type strains. Just what differences in the genetic background of these mutants might be responsible for this effect is not apparent from this analysis, especially since both groups of *ad-3A* mutants give the same linkage relations with more loosely linked markers in the crosses with the double mutant strain *a hist-2 nic-2*, which is unrelated in origin to either group of *ad-3A* mutants. Yet when A2, 38701, 35203, and B10 were crossed to this *a hist-2 nic-2* strain to obtain *hist-2 ad-3B* and *ad-3A nic-2* derivatives to make *ad-3A* × *ad-3B* crosses with distal markers in repulsion, the same sort of data were obtained (Tables 5 and 7) as in the previous crosses of these *ad-3* mutants; i.e., crosses between strains of identical or similar origin give a distance of 0.1–0.2 map unit for the *ad-3A–ad-3B* region, whereas crosses between unrelated strains gave a distance of about 0.5–0.6 map unit.

The results of these experiments are not unique, however, for MITCHELL (1956) reported quite similar results from crosses of the type *pyr-1* + + × + *pdx co*, using two different *pyr-1* alleles (*pyr-1a* and *pyr-1b*). Her data show that when the *pyr-1b* allele is used in such crosses, the map distances in the *pyr-1-co*, *pyr-1–pdx*, and *pdx–co* regions are much lower than those obtained in the same crosses using the *pyr-1a* allele.

The point of interest in the *ad-3A* × *ad-3B* crosses is that variation in the map distance in the *ad-3A–ad-3B* region is not correlated with variation in the map distance in the *hist-2–ad-3A* and *ad-3A–nic-2* regions. But it must be kept in mind that these observations were made on separate crosses, and the data obtained for each *ad-3A* mutant in each type of cross may not be directly comparable because of this.

Some light has been shed on this problem, moreover, by crosses with other markers to the left of the *ad-3A* locus, which were initiated to determine whether *ad-3A* mutants react differentially only to *ad-3B* mutants. Pilot experiments with a morphological mutant ragged (induced in 74A) indicate the same sort of relationship—low recombination between related strains, high recombination for unrelated strains.

Since the estimates of 0.6–0.7 map unit for the *ad-3A–ad-3B* region were obtained from crosses between strains unrelated in origin, these values may be considered overestimates of the map distance in this region owing to some synergistic effect associated with differences in genetic origin. The estimates of 0.1–0.2 crossover unit obtained from crosses of *ad-3* mutants closely related in origin may thus be considered the "standard" map distance for this region.

Associated with these differences in linkage were rather marked differences in chromosome interference. It is now clear that coincidence values below 1.0 were obtained in crosses between strains unrelated in origin, indicating marked positive interference, whereas coincidence values of above 1.0 were obtained in crosses between related strains indicating marked negative interference.

These results can be summarized as shown in Figure 1, where a contrast was made between crosses of *ad-3* mutants induced in the same wild-type strain *versus* crosses between mutants induced in different wild-type strains. For the purposes of this illustration, strain 68306 may be considered essentially identical with strains 38701 and 38709.

The origin of prototrophs in ad-3A × ad-3B *crosses:* The high negative interference observed in intercrosses of *ad-3A* and *ad-3B* mutants induced in the same wild-type strains (Tables 4–7) is similar to that observed by PRITCHARD (1955) in intercrosses of the *ad-8* mutants of Aspergillus. Although the class of prototrophs expected to arise by a single crossover was predominant, the two parental classes (double crossover) and the other recombinant class (triple crossover) occurred also at high frequency. Subsequent tetrad analyses of such crosses between closely linked markers (see review of WHITEHOUSE and HASTINGS 1965) showed that high negative interference is associated with nonreciprocal recombi-

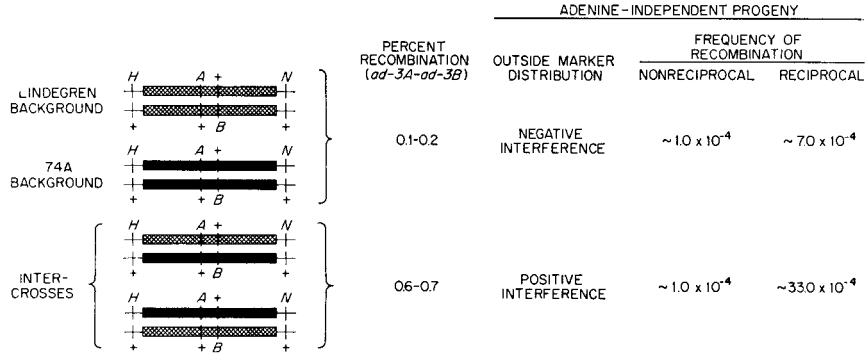


FIGURE 1.—Summary of results on genetic recombination and interference in intercrosses involving different strains of purple adenine mutants of *N. crassa*.

nation (gene conversion) in addition to reciprocal recombination. In a tetrad analysis of recombination at the *hi-1* locus, FOGEL and HURST (1967) showed that in asci where the *hi-1*$^+$ recombinants resulted from nonreciprocal recombination they were of all four genotypes with reference to outside markers, but in 97 out of 101 asci showing reciprocal recombination the *hi-1*$^+$ recombinants were of the genotype expected from a single crossover. The tetrad data from all of these studies indicate that the prototrophs in *ad-3A* × *ad-3B* crosses also result from both reciprocal and nonreciprocal recombination. Tetrad analysis on an *ad-3A* × *ad-3B* cross (GILES, DE SERRES and BARBOUR 1957) showed that in asci where the prototroph was of the genotype expected from a single crossover it resulted from reciprocal recombination since the *ad-3A ad-3B* double mutant was also recovered. Adenine-independent progeny of genotypes expected from multiple crossovers, however, most likely result from nonreciprocal recombination (gene conversion). Thus it should be possible to obtain a first approximation of the relative frequencies of these two types of recombination in each type of *ad-3A* × *ad-3B* cross by comparing the number of adenine-independent progeny of the genotype expected to arise by a single crossover with the number expected to arise by multiple crossovers.

The data from *ad-3A* × *ad-3B* crosses of mutants induced in the same wild-type strain are compared with the data from *ad-3A* × *ad-3B* crosses of mutants in-

TABLE 10

Estimates of the relative frequencies of reciprocal and nonreciprocal crossing over in intercrosses of ad-3A *and* ad-3B *mutants of* N. crassa

Cross type	Total viable ascospores	Adenine-independent progeny: Reciprocal recombination (single crossover genotype) Total no.	Reciprocal recombination Frequency (X 10^{-4})	Nonreciprocal recombination (multiple crossover genotypes) Total no.	Nonreciprocal recombination Frequency (X 10^{-4})
Between ad-3 mutants induced in different wild-type strains					
ad-3A(A1-A10*) X hist-2 ad-3B(35203)nic-2	269,541	894	33.2	6	0.2
ad-3A(A2)nic-2 X hist-2 ad-3B(35203)	157,260	454	28.9	13	0.8
ad-3A(38701, 38709, 68306) X hist-2 ad-3B(B10-R2)nic-2	268,475	970	36.1	16	0.6
ad-3A(38701)nic-2 X hist-2 ad-3B(B10)	162,475	499	30.7	22	1.4
TOTALS	858,236	2817	32.8	57	0.7
Between ad-3 mutants induced in the same wild-type strain					
ad-3A(38701, 38709, 68306) X hist-2 ad-3B(35203)nic-2	132,875	95	7.1	14	1.1
ad-3A(38701)nic-2 X hist-2 ad-3B(35203)	231,660	107	4.6	9	0.4
ad-3A(A1-A10*) X hist-2 ad-3B(B10-R2)nic-2	434,774	355	8.2	62	1.4
ad-3A(A2)nic-2 X hist-2 ad-3B(B10)	159,250	108	6.8	14	0.9
TOTALS	958,509	665	6.9	99	1.0

*Data from crosses of A3 and A9 were omitted.

duced in different wild-type strains in Table 10. The frequencies of reciprocal and non-reciprocal recombination in each group of four crosses were tested for homogeneity using the chi-square test (POTHOFF and WHITTINGHILL 1966). Homogeneity of the frequencies was rejected since this analysis indicated extra-binomial variation within each group ($\chi^2_{3df} = 18.5, 20.4, 26.6, 16.2$; $P < 0.01$). If this variation has an approximately normal distribution (COCHRAN 1943), then the group frequencies can be tested for equality by the analysis of variance. This test indicates that the frequency of reciprocal recombination in crosses between mutants induced in different strains is significantly greater ($F = 218$, $P < 0.001$) than the frequency of reciprocal recombination in crosses between mutants induced in the same strain. This same test indicated that the frequencies of nonreciprocal recombination in the two groups of crosses are not significantly different ($F = 0.34$, $P = 0.58$). Thus, this analysis shows that nonreciprocal recombination resulting from gene conversion occurs at approximately the same frequency in all crosses and the main difference in recombination frequencies in *ad-3A* × *ad-3B* crosses results from a difference in the frequency of reciprocal recombination (Figure 1).

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SUMMARY

(1) The linkage and interference relations of a series of 13 *ad-3A* mutants were studied with markers at the closely linked *ad-3B*, *hist-2*, and *nic-2* loci. (2) All the *ad-3A* mutants except A3 and A9 give essentially the same linkage relations in the *hist-2–ad-3A* and *ad-3A–nic-2* regions, giving an average map distance for each region of 1.96 and 3.00, respectively. (3) In *ad-3A* × *ad-3B* crosses between mutants derived from the same wild-type strain or mutants related in origin, the frequency of adenine-independent progeny indicates an average distance of 0.1–0.2 map unit for the *ad-3A–ad-3B* region. A large proportion of such progeny were of "exceptional" (parental or reciprocally recombinant) genotype, suggesting marked negative interference. (4) In *ad-3A* × *ad-3B* crosses between mutants unrelated in origin, the frequency of adenine-independent progeny indicates an average distance of 0.6–0.7 map unit for the *ad-3A–ad-3B* region. In these crosses only a small proportion of the progeny were of exceptional genotype, suggesting marked positive interference. (5) No consistent differences in linkage of *ad-3A* mutants of the same origin were observed that would permit an ordering of the markers within the *ad-3A* locus. (6) The marked extremes in linkage obtained with mutants A3 and A9 may be attributable to some type of chromosome rearrangement. In addition, both mutants seem unstable in *ad-3A* × *ad-3A*$^+$ crosses, since numerous segregants with partial requirements for adenine are recovered. (7) On the assumptions that the adenine-independent progeny of the genotype expected to arise from a single crossover result predominantly from

reciprocal recombination and that the adenine-independent progeny of the genotypes expected to arise from multiple crossovers result predominantly from nonreciprocal recombination (gene conversion), the relative frequencies of each type of recombination were determined in both types of *ad-3A* × *ad-3B* cross. This analysis shows that the differences in the frequency of recombination in the *ad-3A–ad-3B* region can be attributed to differences in the frequency of reciprocal recombination.

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