A MAP OF LINKAGE GROUP VI OF NEUROSPORA CRASSA

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THE linkage maps of *Neurospora crassa* compiled and prepared by BARRATT *et al.* (1954) provide a useful summary of our knowledge of this subject up to the present time. They also point up two serious shortcomings in the linkage data that have been gathered on this organism. At the time these maps were prepared, some 80 mutant loci had been demonstrated to lie in one or the other of the seven linkage groups. There was, however, very little reliable evidence as to which arm of a linkage group contained a particular locus. Indeed, in only one of the seven groups was there conclusive evidence that markers were known in both arms. This lack of knowledge is surprising in Neurospora, an organism in which information about the position of the centromere can be obtained in straightforward genetic tests. A second major flaw in our mapping data is that in most cases in which we know genes to be closely linked, we do not have reliable information about their order of arrangement on the chromosome.

When a new mutant is found to be in a particular linkage group, one way of determining which chromosome arm contains this locus is to examine a cross of this mutant to a known linked mutant. Asci in which the mutant of known location has recombined with its centromere are studied to determine whether there has been coincident recombination between the two mutant loci. This method will be discussed in greater detail in a later section. The point to be made here is that such tests have seldom been carried out in the past because they require asci with 2nd division segregation for the mutant of known location. The known mutants which have been employed in linkage studies have nearly always been very close to the centromere, and thus the test for chromosome arm location has required a rare meiotic event.

The placing of mutant loci on the maps of BARRATT *et al.* is based on centromere distances (2nd division segregation frequencies). In many cases the number of asci studied has been small and the standard error large, so that the range of possible location overlaps with that of a neighboring marker, and the order is not known with certainty. The conviction is implicit, however, that with enough data on 2nd division segregation frequency the markers could be accurately placed along the linkage map. This conviction rests on the assumption that a given mutant will have the same frequency of 2nd division segregation in any cross. Experimental data do not substantiate this assumption. BARRATT (1954) himself has pointed out a number of cases in each of which a given mutant has shown distinctly different frequencies of 2nd division segregation in two different crosses, and he has suggested that these variations in crossover frequency result from some difference between two original wild stocks from which many of our current Neurospora stocks were derived. Studies

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TABLE 1

Frequencies of 2nd division segregation for asco

Cross	1st division segregations	Alternating 2nd division segregations	Symmetrical 2nd division segregations	% 2nd division segregation
A (I)	5020	280	276	10.0%
B (1)	4157	300	297	12.6%
C (9)	89	11	7	16.8%
D (8)	338	40	39	18.9%
E (5)	793	86	106	19.5%
F (6)	553	53	88	20.3%
G (II)	4026	523	536	20.8%
H (3)	481	62	90	24.0%
J (4)	206	28	37	24.0%
K (2)	1066	199	194	26.9%
L (7)	218	49	50	31.2%
M (III)	513	133	131	34.0%
N	283	85	80	36.8%
O	183	60	74	42.3%
P	120	58	53	48.1%
Q (IV)	329	235	229	58.4%

Counts of asci showing the different patterns in crosses segregating for the mutant *asco*. An Arabic number in parentheses after the letter designating a cross signifies that the data are from the cross bearing that number in table 3. Roman numerals indicate the crosses represented by the same symbols in figure 1.

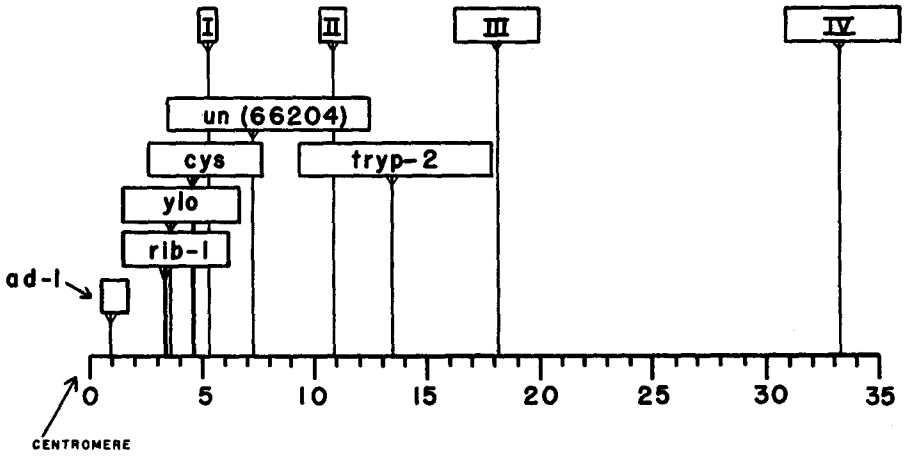

FIGURE 1.—A map of linkage group VI mutants based on 2nd division segregation frequencies. The vertical lines show the map distances from the centromere as calculated from the 2nd division segregation frequencies, and the horizontal bars indicate the limits of statistical fluctuation (plus or minus twice the standard error) of these figures. The loci shown in the lower part of the map (those with the mutant symbols in the horizontal bars) are copied directly from the map of group VI prepared by BARRATT *et al.* (1954). The four points designated by roman numerals are measures of 2nd division segregation frequency for the ascospore mutant, *asco*, in four different crosses. All map distances are corrected for interference by the mapping function curve on page 16 of BARRATT *et al.* (1954).

now in progress with an ascospore mutant (STADLER 1956) and with other mutants lead to the conclusion that there are a large number of separately heritable factors affecting crossover frequency which occur generally in stocks now in use. Until more is known of the nature and distribution of these factors, the assignment of absolute crossover values for regions of chromosome maps would seem to be of limited significance. Counts on a large number of crosses involving the ascospore mutant have given 2nd division segregation frequencies for this locus as low as 10% and as high as 58% and all levels between (table 1). In any given cross, the frequency is constant. Figure 1 demonstrates how this mutant might be placed in any of several quite different locations depending on what cross was analysed. It becomes clear then, that, as in other organisms, the order of neighboring loci can only be determined with confidence if they are studied simultaneously in the same cross. This requires three point crosses (involving three linked mutants or two mutants and the centromere).

The preparation of the linkage map reported in this study has involved two steps: 1) location of all mutants with respect to a given marker and to the centromere; 2) arrangement of neighboring loci by means of three and four point crosses.

LOCATION OF MUTANT LOCI WITH RESPECT TO A GIVEN MARKER AND TO THE CENTROMERE

The method used in the present study has been to cross the linked mutant of unknown location to the given marker and to select those asci in which there has been a crossover between the given marker and the centromere. The extent of recombination between the two mutants has then been determined for this group of asci. (In some crosses, asci with no crossover between the given marker and the centromere have also been analysed.) The method requires that the given marker be close enough to the centromere to insure that there will seldom be more than a single crossover in this region, yet far enough to allow recovery of a reasonable number of crossover asci. The requirement is eminently satisfied by a gene controlling an ascospore character which is closely linked to the centromere, because asci with crossing over between this locus and the centromere can be recognized by their spore patterns, without the labor of dissection.

Ascospore mutant 37402

It was pointed out to the author by MRS. M. B. MITCHELL that GOOD (1951), in a study of lysine-requiring mutants of Neurospora, had observed that one of them, 37402, caused delayed ascospore maturation. On the basis of this information, lysine-requiring mutant 37402 was crossed to wild type and the cross was examined nine days after fertilization. Each ascus which contained any mature, black spores was seen to have two pairs of these and two pairs of colorless ones (figure 2). When such a cross was aged, an occasional ascus was found with a fifth spore maturing, which, when grown, always required lysine and carried the ascospore mutant character. Attempts to separate these two phenotypic expressions by crossing over have failed.

37402 (hereafter referred to as *asco*) is really an ascospore lethal mutant. Most of the spores carrying it never mature. Attempts to bring them to maturity in high

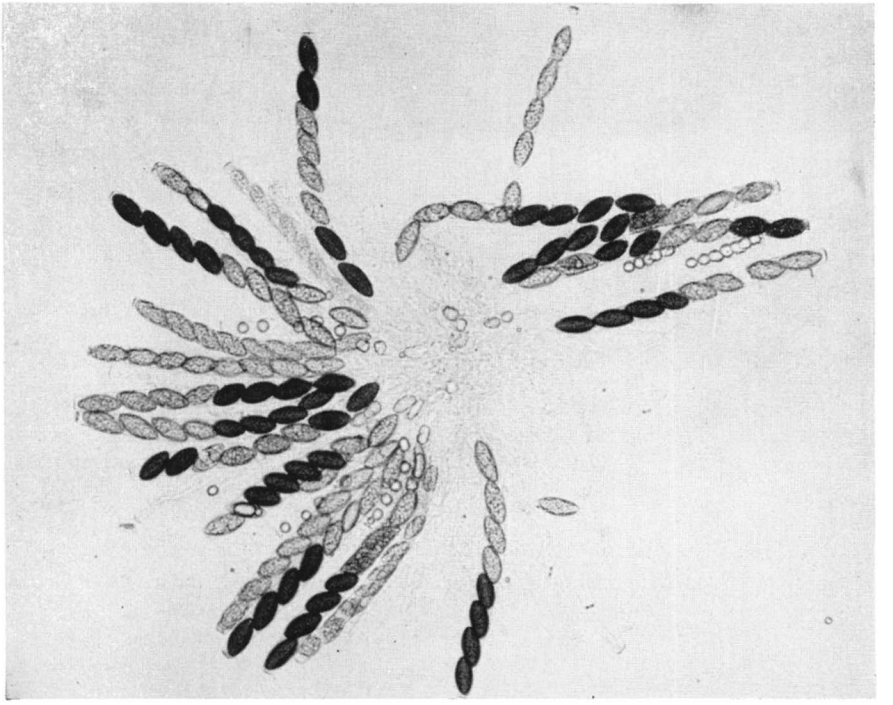

FIGURE 2.—Photomicrograph of asci from a cross segregating for the ascospore mutant, 37402. The mature asci include nine showing 1st division segregation for the ascospore character, one with the alternating pattern of 2nd division segregation and four with symmetrical 2nd division segregation patterns.

lysine medium or other supplemented media have been unsuccessful. This means that in each ascus of a cross segregating for *asco* only the two spore pairs carrying the wild allele can be grown and tested for other markers.

Segregation patterns of *asco* were first studied in a cross to wild strain Abbott 4A. Counts of 2nd division segregation frequency for *asco* were made on 5576 asci of this cross and consistently gave a value of about 10% (cross I of table 1), indicating the locus of *asco* was about five crossover units from the centromere (in this cross). Crosses were then made between *asco* and biochemical mutants close to the centromeres of the various linkage groups. Random spores of these crosses were germinated on minimal medium. Nearly all germinating spores in such a cross carry the wild allele of *asco*, and, if the other mutant involved is not linked to *asco*, half of the germinating spores should exhibit the restricted growth characteristic of a biochemical mutant on unsupplemented medium. If the second mutant is linked to *asco*, more than 50% of the germinating spores will be mutant. In the cross 37402a × 51602A (riboflavin-requiring mutant at 35°C, wild at 25°C), 92.8% of the germinating spores carried the riboflavin requirement, indicating that the ascospore mutant was located in linkage group VI (group B of HOULAHAN *et al.* 1949).

Eight mutants known to be in linkage group VI were crossed individually to *asco*.

TABLE 2

Mutants of linkage group VI

Mutant symbol	Isolation number	Phenotype	Published centromere distance
tryp-2	75001	requires tryptophane	13.5 map units
rib-1	51602	requires riboflavin at 35°C	3.4 m.u.
ad-1	3254	requires adenine	0.9 m.u.
ylo	Y30539y	yellow conidia	3.6 m.u.
cys-1	84605	requires cysteine (or methionine)	4.6 m.u.
un	66204	no growth at 35°C	7.3 m.u.
cyt	C117	slow growth; altered cytochrome system	16.4 m.u. from *rib-1*
cys-2	38401 80702	requires cysteine (or methionine)	0.8 m.u. from *cys-1*

The information on the first six mutants listed is from BARRATT *et al.* (1954) and references there cited. The information on *cyt* is from MITCHELL *et al.* (1953). The linkage information on *cys-2* is calculated from PITTENGER (1954).

(Biochemical and genetic information about these mutants is summarized in table 2.) The crosses were analysed to place the other mutant loci with respect to *asco* and to the centromere. The *asco* locus was arbitrarily placed in the left arm.

Expected types of asci

Figure 3 illustrates the most probable meiotic events which would result in the various ascus types in a cross of *asco* to a linked mutant. The assumption is made that the *asco* locus is close enough to the centromere to insure that there will not be more than a single crossover in this region. This means that all asci with a 1st division segregation pattern for the spore character have had no crossing over between that locus and the centromere, and those with a 2nd division segregation pattern have had one and only one crossover in that region. In some crosses it is possible to detect double crossovers between *asco* and the centromere, and these have been observed to occur with a low frequency. However, they are so rare that they do not seriously affect the analysis.

Figure 3a shows the expected ascus types in a cross between *asco* and a linked mutant in the opposite arm at a position such that there is usually either no crossover or a single crossover between this locus and the centromere. In this cross we can detect crossing over in "region I" (*asco* to centromere) and "region II" (centromere to linked mutant). Examination of the viable (*asco*$^+$) spore pairs from asci showing 1st division segregation patterns for *asco* (no crossover in region I) will reveal no recombination (both pairs mutant) in those cases in which there has been no crossover in region II. Single recombination (one pair mutant, one pair wild) will be observed if there has been a crossover in region II. Asci with the 2nd division segregation patterns for *asco* (single crossover in region I) will show single recombination between the mutant loci if there has been no crossover in region II or if there has been a crossover in this region making a 3-strand relationship with the one in region I. A 2-strand double crossover between the two regions will result

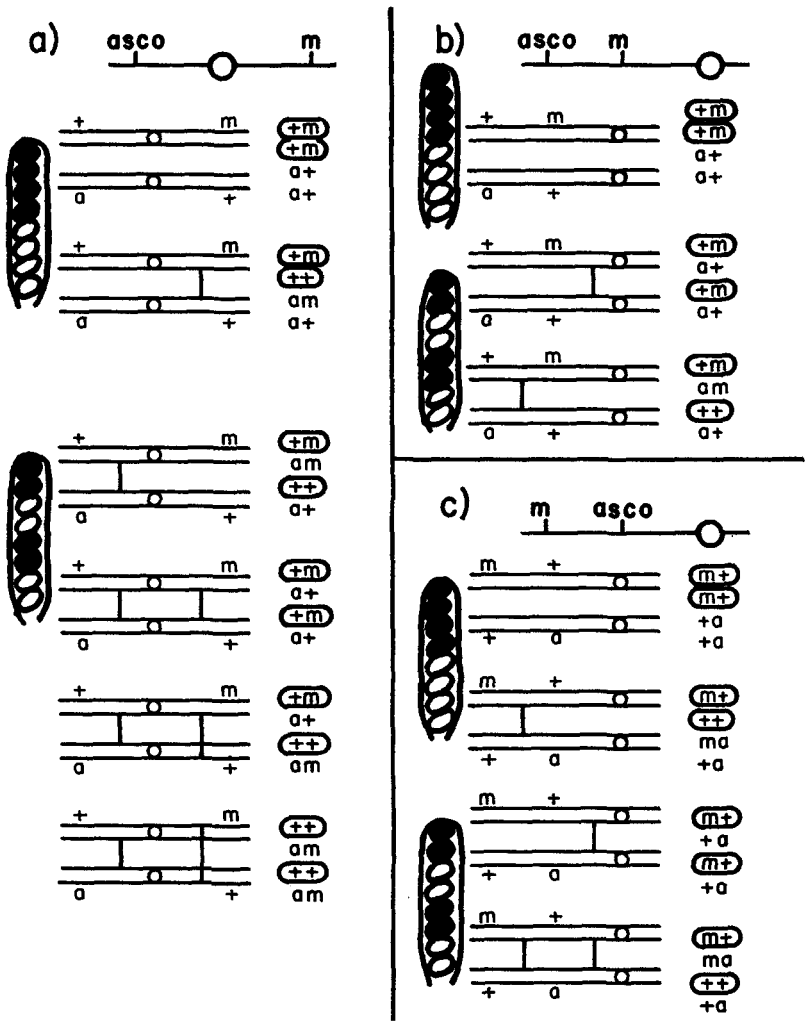

FIGURE 3.—Patterns of segregation resulting from simple crossing over events in a cross between *asco* and a linked mutant. Only one pattern is shown for each situation; patterns which might be achieved by reversing the two spore pairs in either half of an ascus or by reversing the whole ascus are grouped together in the analysis. Ovals are drawn around the genotypes of spore pairs which, since they carry the wild allele of *asco*, can be checked for the presence of the other segregating mutant.

in a non-recombination ascus, and a 4-strand double crossover yields a double recombination ascus (both pairs wild). It is clear that among the asci showing 2nd division segregation for *asco* in such a cross, we would expect the majority to have single recombination between the mutant loci, with the less frequent non-recombination and double recombination types occurring about equally often (assuming no chromatid interference across the centromere). Such a situation is illustrated in the results (table 3) in the cross between *asco* and *tryp-2*.

TABLE 3

Crosses of asco *to linked mutants*

Mutant crossed to *asco*		Map distances		Classification for linked mutant of viable (*asco*$^+$) spore pairs of:						Position of mutant locus
		asco to centromere	*asco* to linked mutant	asci showing 1st division segregation for *asco*			asci showing 2nd division segregation for *asco*			
				m m	*m* +	+ +	*m m*	*m* +	+ +	
rib-1	(1)	6.3 m.u.	7.2 m.u.	101	2	1*	4†	395	1	opposite arm from *asco*
	(T)	8.5–26.0					7‡	474	8	
tryp-2	(2)	13.5	27.5	68	32	1§	12	105	9	opposite arm from *asco*
ad-1	(3)	12.0	11.0				3	78	0	near centromere, arm un
	(T)	12.0–17.5					1	92	1	determined
ylo	(4)	12.0		26	0	0	11	22	0	in same arm, proximal to *asco*
cys-1	(5)	9.7	3.1				27	18	0	in same arm, proximal to *asco*
cys-2	(6)	10.2	7.3				28	32	0	in same arm, proximal to
	(7)	15.6					22	11	0	*asco*
un	(8)	9.5	1.7	36	0	0	24	1	0	near *asco*, probably proxi-
	(T)	8.5–17.0					48	5	0	mal to it
cyt	(9)	8.4	6.5	16	1	0	16	0	0	in same arm, distal to *asco*

Map distances listed for the interval between *asco* and the centromere are the frequencies of 2nd division segregation for *asco* (table 1) divided by two. Map distances between *asco* and the linked mutants were determined by counts of wild growers among germinating random spores on minimal medium.

Lines labeled (T) contain data totalled from several crosses involving *asco* and the mutant under study. Values given in these lines for the map distance from *asco* to the centromere represent the range among the crosses involved.

* This ascus probably resulted from a four-strand double crossover between *asco* and the centromere.

† Three of these asci probably resulted from meiotic nuclear passing with no crossing over in the marked regions.

‡ One of these asci may have resulted from meiotic nuclear passing with no crossing over in the marked regions.

§ This ascus resulted from a four-strand double crossover either between *asco* and the centromere or between the centromere and *tryp-2*.

The expected ascus types in a cross between *asco* and a linked mutant located between *asco* and the centromere are shown in figure 3b. The segment from *asco* to the linked mutant now becomes region I, and from the linked mutant to the centromere is region II. Asci showing 1st division segregation for the ascospore character have no crossing over in either marked region and thus no recombination. Asci with 2nd division segregation for *asco* have a single crossover which can be in either marked region. If it is in region I, they will show single recombination; if the crossover is in region II, there will be no recombination. The percentage of single recombination asci in this group can be anything from 0 to 100 depending upon the position of the linked mutant along the interval from *asco* to the centromere. This type of cross is well illustrated in the results (table 3) in the cross between *asco* and *ylo*.

In the cross to a linked mutant located in the same chromosome arm as *asco* and distal to it, the expected ascus types would be those shown in figure 3c. Single recombination and non-recombination asci may both occur, whether there is 1st or 2nd division segregation for *asco*. However, if there is crossing over interference between the two regions, the frequency of single recombination asci may be expected to be lower among those showing 2nd division segregation for *asco*.

RESULTS

The results of the crosses of the eight linked mutants to *asco* are given in table 3. The analysis of the crosses to one mutant, *rib-1*, will be described in some detail. The *rib-1* locus is clearly very close to the centromere, but an extensive analysis is required if this cross is to tell which arm contains the locus. In the first cross listed, 400 asci with the 2nd division segregation pattern for *asco* were tested, and 395 had a single recombination. Of the remaining five, four had no recombination, but in three of these it could be shown, by means of other segregating markers, that the 2nd division segregation pattern probably resulted from nuclear passing at meiosis rather than crossing over. The method used for this test is very similar to that described by HOWE (1954), and the experiment will be described in greater detail elsewhere. It is sufficient here to state that the evidence is convincing that among 397 asci with crossing over between *asco* and the centromere, all except two had single recombination between the mutant loci. These two include one double recombination and one non-recombination ascus. In the other crosses studied between *asco* and *rib-1*, crossing over was more frequent in the region between the centromere and the *rib-1* locus. There were seven non-recombination and eight double recombination asci among 489 showing 2nd division segregation patterns. One of the seven non-recombination asci may have resulted from nuclear passing with no crossing over (though here the test was less efficient). This gives a total of seven or eight non-recombination asci and nine double recombination asci among those in which *asco* segregated in the 2nd division in all crosses to *rib-1*. If *rib-1* were in the opposite arm from *asco*, the non-recombination asci would be those in which a 2-strand double crossover took place across the centromere, while the double recombination asci would result from the 4-strand double crossovers. If, on the other hand, *rib-1* were in the same arm as *asco*, then the double recombination asci could only result from a triple crossover (a 4-strand double between *asco* and *rib-1*, and a single between *rib-1* and the centromere); non-recombination asci would result every time there was a crossover between *rib-1* and the centromere with no crossover (or a 2-strand double) between *asco* and *rib-1*. It is highly improbable that these two events would be equally frequent. The evidence is thus compelling that *rib-1* is in the opposite arm from *asco*.

In the cross *asco* × *tryp-2* no test was made for nuclear passing in meiosis. The proportion of double crossover asci was so high that the results could only be obscured by aberrant meiotic events occurring much more frequently than has been observed in other crosses.

There was no evidence of meiotic nuclear passing among the four non-recombination asci in the cross to *ad-1*. The data are not sufficient to determine which arm

contains the *ad-1* locus. If it is in the same arm as *asco*, the double recombination ascus must represent a triple crossover. If *ad-1* is in the opposite arm, then the one double recombination ascus and the four non-recombination asci result from equally probable events, if there is no chromatid interference across the centromere (HOWE 1954; STADLER 1955).

In the cross of *asco* to *cyt*, the analysis of asci tells us that *cyt* is in the left arm but gives no convincing evidence as to whether it is proximal or distal to *asco*. The correct location, as between these two alternatives, is indicated by the distance between the mutant loci as measured by random spore analysis. If *cyt* were 6.5 map units proximal to *asco* (which itself is only 8.4 units from the centromere in this cross), the majority of the 2nd division segregation asci would have a recombination between the mutants. The random spore distance between the mutant loci was also employed in determining the position of *cys-1* and of *cys-2*. In both of these cases the analysis of asci demonstrated that the mutant locus was either between *asco* and the centromere or a long distance beyond it in the same arm.

The cross *asco* × *cyt* required a special method. Both *asco* and *cyt* are relatively infertile. *Asco* can be made to act as a protoperithecial parent only with difficulty, and *cyt* has never been made to serve in this capacity. All attempts to get a pure cross of *asco* and *cyt* failed. The cross was finally made by inoculating both *asco* and a strain of *ad-1* of like mating type in a tube of unsupplemented mating medium. This gave a heterocaryotic growth which produced protoperithecia and was successfully fertilized with *cyt*. When the cross matured, only those perithecia containing asci segregating for *asco* were selected for study. The technique of mixing in a third strain to achieve a cross between parents of poor fertility has been used before (BEADLE and COONRADT 1944; LEIN and LEIN 1952). The advantage of the present method is that by using an ascospore marker in one of the two parent strains of like mating type, it is possible to select the progeny of the desired cross without laborious dissection and growth tests.

At this point it may be worthwhile to make a tentative map (fig. 4) of linkage group VI based on the results of the crosses of the other markers to *asco*. No measure of absolute units is suggested, and even the order of arrangement of the mutant loci is not yet established, but will be determined by the three-point crosses in the following section. The positions on this map of the markers between *asco* and the centromere are derived from the frequencies of recombination in asci showing 2nd division segregation for *asco*. *Rib-1* and *tryp-2* are placed according to their 2nd division segregation frequencies in asci showing 1st division segregation for *asco*. *Ad-1* is placed at the centromere pending further evidence.

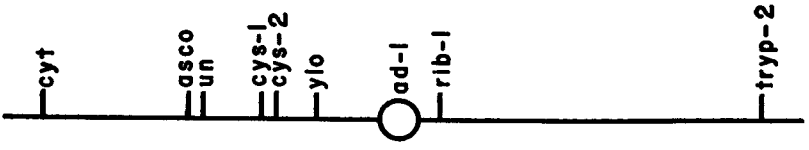

FIGURE 4.—Described in text.

DETERMINATION OF ORDER OF NEIGHBORING LOCI

Crosses involving two mutant loci and the centromere

If two mutant loci are known to be in the same chromosome arm, their order of arrangement with respect to the centromere can frequently be determined by a study of asci, in which there has been recombination between the mutant loci. There are two marked regions in such a cross: region I, bounded by the two mutant loci, and region II, extending from the proximal mutant locus to the centromere. Recombination asci are those with crossing over in region I. If region II is short enough so that there is usually no coincident crossover in this region, the majority of the recombination asci will show 1st division segregation for the proximal mutant and 2nd division segregation for the distal one. A number of the mutants in linkage group VI have been placed by this type of analysis, summarized in table 4.

There has been no direct test of the relative positions of *un* and *cys-1*. A cross between them gave few viable complete asci, and no double mutant progeny were recovered. The cross was discarded. It has been tentatively assumed that *cys-1* is

TABLE 4

Analyses of crosses involving two linked mutants and the centromere

Cross	Non-recombination asci		Single recombination asci		Order of loci
	1st division segregation for both mutants	2nd division segregation for both mutants	2nd division segregation for one mutant	2nd division segregation for both mutants	
1. *un* × *cys-2*	35	8	4 { MI seg. for *cys-2* MII seg. for *un*	0	*un*—*cys-2*—cent.
2. *cys-2* × *ylo*	67	2	3 { MI for *ylo* MII for *cys-2*	0	*cys-2*—*ylo*—cent.
3. *cys-1* × *ylo*	37	3	3 { MI for *ylo* MII for *cys-1*	0	*cys-1*—*ylo*—cent.
4. *ylo* × *ad-1*	106	0	14 { MI for *ad-1* MII for *ylo*	0	*ylo*—*ad-1*, cent.
5. *rib-1* × *tryp-2*	129	4	62 { MI for *rib-1* MII for *tryp-2* 2 { MI for *tryp-2* MII for *rib-1*	1	cent.—*rib-1*—*tryp-2*
6. *rib-1* × *tryp-2*	8	2	14 { MI for *rib-1* MII for *tryp-2*	0	
7. *rib-1* × *tryp-2*	18	1	21 { MI for *rib-1* MII for *tryp-2*	0	

No double recombination asci were observed in any of these crosses.

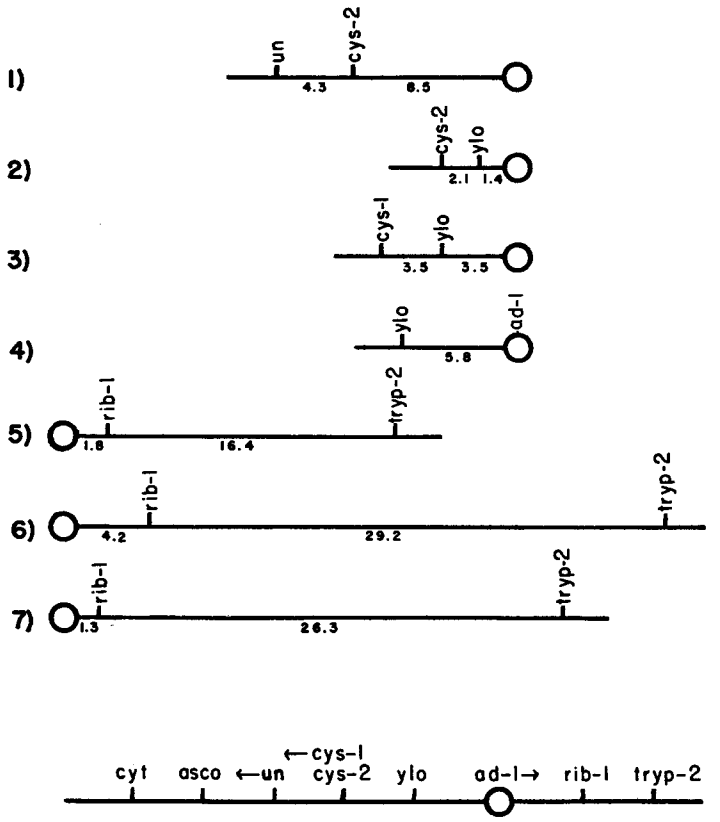

FIGURE 5.—Above: Maps of the results of the seven crosses listed in table 4. Below: A map illustrating what has been determined about the relative positions of the nine mutants and the centromere up to this point. An arrow emanating from a mutant symbol means the position of this locus with respect to the next neighboring mutant has not been established with certainty.

to the right of *un*, as *cys-1* has consistently shown closer linkage to *cys-2* than has *un*. A cross to be described in a later section reveals *cys-1* to be to the right of *cys-2*, thus obviating the study of the relative positions of *un* and *cys-1*.

The cross *ylo* × *ad-1* does not reveal which chromosome arm contains the *ad-1* locus, as this mutant failed to separate from its centromere in any of the 120 asci. The cross demonstrates only that *ad-1* is to the right of *ylo*.

The three *rib-1* × *tryp-2* crosses were also segregating for *asco*, and thus only the two spore pairs carrying the plus allele of *asco* in each ascus could be classified for *rib-1* and *tryp-2*. The observations reported here were confined to those asci showing 1st division segregation for *asco* (as these gave more information about the centromere relations of the other mutants). The data therefore represent a selected group of asci. However, if there is no interference across the centromere (HOWE 1954; STADLER 1955), this type of selection should not alter the apparent linkage relationship of *rib-1*, *tryp-2*, and the centromere.

The data listed in table 4 are shown as partial chromosome maps in figure 5.

TABLE 5

Analyses of crosses involving three linked mutants

Cross	Germination conditions	Phenotype selected	Phenotype for unselected marked	Total germinated spores
un × *asco cys-2*	methionine at 35°C	*un*$^+$ *asco*$^+$	17 *cys-2*, 3+, 6 pseudowilds (+)	1611
ad-1 × *cys-2 rib-1*	methionine at 35°C	*ad-1*$^+$ *rib-1*$^+$	18 *cys-2*, 5+	1920

These results demonstrate again (as was shown earlier for the frequencies of 2nd division segregation for *asco*) that a given chromosome region may show markedly different rates of crossing over in two different crosses. At the bottom of figure 5 is a map summarizing what has thus far been determined about the relative positions of the mutant loci.

Crosses involving three mutant loci

If the relative positions of two closely linked mutants, *a* and *b*, are unknown, but they are both known to be on the same side of another nearby mutant, *c*, then it is possible to determine the relative positions of the loci by a study of the cross *a c* × *b*. Random spores are germinated on selective medium that allows growth of only those which are a^+, b^+ (but does not select between *c* and c^+). Growing colonies are then picked up and classified for *c*. If the *b* locus lies between *a* and *c*, most of the growers will carry the mutant *c*, while if *a* is between the other two mutant loci, most of the growing colonies will carry the wild allele of *c*.

This method was used in the crosses recorded in table 5. The first cross demonstrates that *un* is to the right of *asco*, and the second shows that the *rib-1* locus is to the right of *ad-1*.

Those colonies which express the wild phenotype for all three segregating mutants in these crosses could be pseudowilds (MITCHELL *et al.* 1952) rather than products of crossing over in the marked region. Such cultures were regularly tested for presence of the parental mutant genes by crossing to wild type or by growing and testing macroconidia which failed to grow on minimal medium (PITTENGER 1954). The inclusion of undetected pseudowilds in the data could obscure the results of an experiment of this type.

The position of the ad-1 locus

Experiments described above have demonstrated that the *ylo* locus is in the left arm, *rib-1* is in the right arm, and *ad-1* lies between them. But there has been no decisive evidence as to the position of *ad-1* with respect to the centromere. In order to settle this point, *rib-1* was crossed to *ylo ad-1*, and ordered asci were analysed in the hope of finding one segregating in the 2nd division for *ad-1*. The expectation was that in such an ascus *ad-1* would almost certainly recombine with the mutant which was in the opposite arm while not recombining with the mutant in the same arm.

Among 146 asci studied, 143 had no recombination in the marked region—parental ditypes with 1st division segregation for all three mutants. Two asci segregated in the 2nd division for *ylo*, and there was one ascus with 2nd division segregation for *rib-1*. *Ad-1* segregated in the 1st division in every ascus.

Studies of 2nd division segregation frequencies for *asco* in crosses incubated at different temperatures have revealed that crossing over in the region between *asco* and the centromere is two to three times more frequent among mature asci reared at 18°C than among those grown at the customary 25°C. There is some indication that the effect is most pronounced in the region of the chromosome near the centromere. The effect appears to be just as marked if the cross is at 18° only four days, during a "sensitive period" (fertilization on the 5th day, 18° from the 9th to the 13th day). It is not yet known whether the observations result from a direct effect of the altered temperature on the meiotic apparatus. In any case, it provides a useful method of increasing the yield of recombinant progeny.

The same strains of *rib-1* and *ylo ad-1* were again crossed and were put for a part or all of the incubation period at 18°. Analysis of 64 asci revealed two in which *ad-1* segregated in the 2nd division. Both asci were of the following constitution:

1st spore pair:	*ylo*	*ad-1*	*rib-1*
2nd spore pair:	+	+	*rib-1*
3rd spore pair:	*ylo*	*ad-1*	+
4th spore pair:	+	+	+

This reveals that the *ad-1* locus is in the left arm.

The relationship between cys-1 and cys-2

Crosses involving *cys-1* and *cys-2* and the neighboring markers *ylo* and *un* are reported in table 6. Classification of the cysteine-independent progeny for the unselected markers reveals that, in every case, the most numerous class is that which would result from a single crossover between the *cys* loci if the order of arrangement on the chromosome were *un—cys-2—cys-1—ylo*. Further evidence that *cys-2* and *cys-1* are at separate loci arranged in this order is gained from a study of whole asci of the cross *un cys-2* × *cys-1 ylo*. Dissected asci were germinated on minimal agar plates at 25° and examined after 15–20 hours. Of 43 asci, 42 had all spore pairs showing the restricted growth characteristic of cysteine requirement. The other ascus contained one wild growing pair. The four spore pairs of this ascus were picked up on complete agar slants and classified for all segregating mutants with the following result:

1st spore pair:	+	+	*cys-1*	*ylo*	a
2nd spore pair:	*un*	*cys-2*	*cys-1*	*ylo*	—
3rd spore pair:	+	+	+	+	A
4th spore pair:	*un*	*cys-2*	+	+	A

The genotypes are those which would be expected if the loci were arranged as shown, and a single crossover took place between *cys-2* and *cys-1*, involving the 2nd and 3rd spore pairs. The presumed double mutant (*cys-2 cys-1*) was identified

TABLE 6

Crosses between the two cys *mutants*

Cross	Germination conditions	Phenotype selected	Phenotype for unselected markers	Total germinated spores
cys-2 × *cys-1 ylo*	minimal medium	*cys*$^+$	14 +, 8 *ylo*	2420 (approx.)
cys-2 × *cys-1 ylo*	minimal medium	*cys*$^+$	8 +, 2 *ylo*	1425 (approx.)
cys-1 × *cys-2 ylo*	minimal medium	*cys*$^+$	4 +*, 11 *ylo*	6054
un cys-2 × *cys-1 ylo*	minimal at 25°C	*cys*$^+$	16 +, 2 *un*, 1 *ylo*	1330 (approx.)

* These four cultures tested negative for pseudowild.

only by its vegetative growth habit. In liquid methionine medium at 25° it grew distinctly less than either parent mutant. Perhaps the best test for identification of a double mutant between two closely linked mutants of similar phenotypes is made by studying the backcrosses to both parent mutants (MITCHELL 1955). Unfortunately this test could not be made, as the presumed double mutant was completely sterile.

The above evidence indicates that the *cys-2* and *cys-1* loci are separable by crossing over. However, some of the cysteine-independent progeny in the crosses between the two *cys* mutants could not result from a single crossover. In the first cross listed in table 6, random spore analysis reveals *ylo* to be 7.9 map units from the *cys* loci. To account for the phenotypes of the cysteine-independent progeny by crossing over, we must assume that among the isolates with exchange between the *cys* loci, 8 of 22 also crossed over in this short neighboring region. This unexpectedly high frequency of *ylo* among the cysteine-independent progeny is reminescent of the behavior of two pyridoxine mutants of Neurospora reported by MITCHELL (1955). In a cross between the pyridoxine mutants, she demonstrated that pyridoxine-independent progeny could result from a process which did not show the characteristics of classical crossing over.

It is not clear whether *cys-1* and *cys-2* are involved in separate steps in the synthesis of cysteine. A study of the nutritional requirements of cysteine mutants of Neurospora (HOROWITZ 1950) suggests the following pathway of cysteine synthesis: . . . . sulfate . . . . sulfite . . . . thiosulfate . . . . (sulfide) . . . . cysteine. Both *cys-1* and *cys-2* can utilize thiosulfate (or subsequent products) for growth but cannot utilize sulfite or sulfate. However, the two mutants do show some differences in growth response. The response to thiosulfate of *cys-1* is much weaker than is that of *cys-2*. *Cys-1* strains on cysteine grow poorly at 25° but well at 35°; the growth at 25° is enhanced by the addition of tyrosine (HOROWITZ and SHEN 1952). *Cys-2* grows well on cysteine at either temperature and shows no response to tyrosine. FLING (unpublished) has found that some reisolates of *cys-1* show no response to tyrosine, and it is not yet known what part modifiers may play in the growth characteristics.

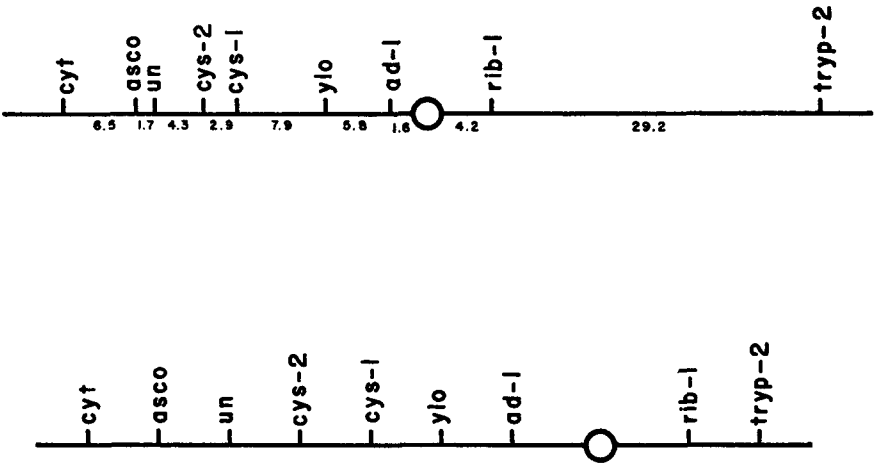

FIGURE 6.—Described in text.

The arrangement of the nine mutants with respect to each other and to the centromere is shown in figure 6. In the upper map the lengths of the intervals between neighboring loci are determined by the highest recombination frequencies observed for these intervals in the crosses reported in this paper. The author places no significance on the absolute numbers involved, and even the relative lengths of the intervals should be considered as only the crudest kind of guides for further studies. With our present limited knowledge of the factors influencing crossing over frequency in Neurospora, the less presumptious map at the bottom of figure 6 is perhaps the most appropriate representation of the findings reported here.

SUMMARY

An ascospore mutant of *Neurospora crassa* has been described and shown to be in linkage group VI. Crosses of the ascospore mutant to eight linked mutants have been analyzed in order to determine the positions of these loci relative to the ascospore mutant locus and to the centromere.

Three and four point crosses have been studied to establish the order of arrangement of neighboring mutant loci.

Some factors influencing crossing over frequency in Neurospora have been discussed, and it has been concluded that absolute distances on crossover maps can have only limited significance in our present state of knowledge.

An effect of temperature of incubation on frequency of recombinants recovered from a cross has been described and has been utilized in determining the position of a mutant locus closely linked to the centromere.

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