NEW MARKERS AND MULTIPLE POINT LINKAGE DATA IN NEUROSPORA[1]

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LINKAGE maps of *Neurospora crassa* based on compiled tetrad data were published by BARRATT, NEWMEYER, PERKINS and GARNJOBST in 1954. Improved and extended maps based on new data have since been provided by MITCHELL and MITCHELL (1954) for group IV, and by STADLER (1956a) for group VI. The purpose of the present work has been to augment and improve the maps in order to provide suitably marked chromosomes for investigations related to crossing over.

It has become increasingly clear since 1954 that the intrinsic variability of crossover values for the same interval, in crosses of different parentage, may be so great that gene order cannot be established reliably by combining two-point data from heterogeneous sources (see especially STADLER 1956a). For this reason, the data presented here have been collected predominantly from multiple point crosses.

These data will be presented in a series of four papers. The present paper describes new markers that have been mapped in all seven groups, and presents segregation data for genes in groups I, II, VI and VII. Linkage data for markers in groups III, IV, and V are presented in the accompanying papers (PERKINS and ISHITANI 1959; MALING 1959b; STRICKLAND, PERKINS, and VEATCH 1959).

Fifty-three previously unmapped mutants have been localized in the seven established linkage groups. Among them are at least 25 new loci, which are mapped in relation to previously known genes. The remainder are recurrences. Several linkage groups have been extended by the addition of new terminal markers, and the sequences of a number of previously mapped loci have been clarified.

The data were obtained almost entirely from segregants collected by nonselective methods, as random segregants rather than as tetrads. Techniques are described that have made it easier to detect linkage and to obtain segregation data of the type desired.

MATERIALS AND METHODS

Wild type and mutant strains

ST. LAWRENCE wild type strains 74A and 73a, or derivatives from them, were used as standard parents for inbreeding and testing. Cytological studies of 74A

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TABLE 1

Mutants previously unassigned to loci or linkage groups

Isolation number	Locus symbol, name, and linkage group	Origin: treatment and strain	Characteristics and remarks
B4	*ro-1* : ropy-1* (IV)	U.V.; 74A	Cable-like aggregations of hyphae grow up tube from the agar.
STL4	*lys-4* : lysine-4 (I)	spontaneous; 31873	
STL6	*un*(STL6) : unknown* (I)	U.V.; 39113	Suboptimal response to methionine. Fluffyish morphology, late conidiation.
B6	*bis* : biscuit* (V)	U.V.; 74A	Colonial. Flat at first, then sending up densely conidiating growth.
B9	*ad-3* : adenine-3 (I)	U.V.; 74A	Accumulates purple pigment.
B12	*bis* : biscuit* (V)	U.V.; 74A	See B6.
B15	*ro-1* : ropy-1* (IV)	U.V.; 74A	See B4.
M16	*os* : osmotic (I)	U.V.; B53, B123	See B135.
B18	*vel* : velvet* (III)	U.V.; 74A	A soft, conidiating colonial.
B20	*ro-2* : ropy-2* (III)	U.V.; 74A	Phenotypically indistinguishable from *ro-1* (B4).
B30	*bis* : biscuit* (V)	U.V.; 74A	See B6.
B40	*bn* : button* (VII)	U.V.; 74A	Nonconidiating restricted colonial growth.
B53	*rg* : ragged* (I)	U.V.; 74A	Poorly conidiating colonial growth. Small, densely conidiating, pigmented, *cr rg* colonies are useful for plating and replication (MALING 1959a).
B54	*com* : compact* (III)	U.V.; 74A	Small colonies.
B56	*bal* : balloon* (II)	U.V.; 74A	Restricted growth on agar as a hemispheric colony. A good protoperithecial parent.
B57	*mat* : mat* (IV)	U.V.; 74A	Colonial. Prefers sucrose to glycerol.
B66	*wl* : woolly* (V)	U.V.; 74A	Conidiation variable. Probably associated with an aberration.
B74	*cr* : crisp (I)	U.V.; 74A	See B123.
B102	*al* : albino (I)	U.V.; 74A	
B105p	*ad-3* : adenine-3 (I)	U.V.; 74A	Accumulates purple pigment.
B106	*sk* : skin* (VII)	U.V.; 74A	Leathery nonconidiating rapid growth hugs agar surface.
B110	*fr* : frost* (I)	U.V.; 74A	Delicate branching on surface, and delicate aerial growth. Nonconidiating.
B118	*pl* : plug* (V)	U.V.; 74A	Characteristic dense hyphae fill diameter of 10 mm tube.
B122	*cr* : crisp (I)	U.V.; 74A	See B123. Suspected aberration.
B123	*cr* : crisp (I)	U.V.; 74A	Early conidiation, uniform over agar surface.
B128	*st* : sticky* (I)	U.V.; 74A	Difference from wild type subtle but can be scored.
B132	*sp* : spray* (V)	U.V.; 74A	Characteristic surface growth followed by growth upward and outward.

Isolation number	Locus symbol, name, and linkage group	Origin: treatment and strain	Characteristics and remarks
B135	*os* : osmotic (I)	U.V.; 74A	Sensitive to high osmotic pressure. Can be scored by appearance. Conidia rare except in combination with crisp.
B137	*del* : delicate* (VI)	U.V.; 74A	Growth less profuse than wild type.
B148	*col-4* : colonial-4 (IV)	U.V.; 74A	Dense balls of conidia high in slant.
B149	*slo* : slow* (I)	U.V.; 74A	Normal morphology achieved days later than wild type. Not tested for allelism with *cyt*(C115).
M155-5	*fl* : fluffy (II)	H263R4A×H263R3a	
B180	*cr* : crisp (I)	U.V.; 74A	Least flat and restricted of the crisp alleles.
B187	*rg* : ragged* (I)	U.V.; 74A	See B53.
JH216	*al* : albino (I)	N-mustard	
B230s	*so* : soft* (I)	X-ray; 74A	Resembles peach. Dense, delicately pigmented growth fills lower part of slant.
B233t	*ti* : tiny* (I)	X-ray; 74A	Small dense colony, with gnarled, short hyphae.
B234	*sk* : skin* (VII)	X-ray; 74A	Probable allele of B106, which it resembles. (Intercrosses are not fertile.)
P346	*fl* : fluffy (II)	51504A×Pa	
Y602	*al* : albino (I)	N-mustard; 1A×25a	
P605	*fl* : fluffy (II)	Spontaneous; 74A background	
Y2170	*al* : albino (I)	N-mustard; 1A×25a	
Y2171	*al* : albino (I)	N-mustard; 1A×25a	
4894	*me-7* : methionine-7* (VII)	X-ray; LA×La	Uses homocysteine, not cysteine.. One unit from centromere (Buss 1944).
JH9698	*al* : albino (I)	N-mustard	
27947	*arg-5* : arginine-5* (II)	X-ray; 1A×19a	Uses ornithine, citrulline, or arginine.
30820	*arg-11* : arginine-11* (VII)	X-ray; 1A×19a	Requires citrulline or arginine plus a purine plus a pyrimidine.
Y31881	*nic-3* : nicotinic-3* (VII)	N-mustard; Y8743	Uses 3-hydroxyanthranilic acid.
37501	*leu*(37501) : leucine* (IV)	U.V.; 1A×25a	More stable than possible allele 8839. (D. Regnery, unpub.).
45201	*val* : valine* (V)	U.V.; Abb4×25a	No isoleucine requirement. First two digits of isolation number possibly in error.
al^{C}	*al* : albino (I)	X-ray; B53, B123	
al^{M}	*al* : albino (I)	U.V.; B53, B123	
fl^{P}	*fl* : fluffy (II)	Spontaneous; 74A background	

* Asterisks designate previously unmapped loci. Mutants are arranged in numerical order of their isolation numbers, without regard to letter prefixes.

by 73a crosses revealed no gross meiotic abnormalities (ST. LAWRENCE, personal communication). These strains were not highly inbred, however, and were not genetically homogeneous. (See CASE 1957 for differences in heterocaryon compatibility.) Other wild type strains were derived from 74A × 73a by a series of sib- and backcrosses comprising eight generations. Various of these derivative wild types were used in place of the original strains in crosses analyzed since 1956. Mutant genes that originated in other genetic backgrounds or were obtained following irradiation of ST. LAWRENCE strains were routinely inbred to the standard wild type strains to minimize extraneous genetic differences and to improve fertility. Three backcross generations were often necessary in order to achieve reasonable fertility and uniformity of appearance and growth among segregants, but this degree of inbreeding was not always achieved before markers were used to obtain data on crossing over.

Mutants which have not previously been mapped are listed in Table 1. Most of those used in the present study are characterized by visible differences from wild type in morphology or pigmentation. The 33 mutants prefixed B were isolated by DR. V. W. WOODWARD in the course of experiments employing the filtration technique of WOODWARD, DEZEEUW and SRB (1954). Mutants prefixed JH were isolated by DR. W. D. MCELROY and made available to us by DR. F. T. HAXO. Mutants prefixed STL and P originated in experiments of DR. P. ST. LAWRENCE and of the author, respectively. Mutants prefixed Y were isolated by TATUM, BARRATT, FRIES, and BONNER (1950). Isolation numbers without any prefix indicate that mutants originated from experiments of BEADLE and TATUM (1945). Mutants al^{c}, M16 and al^{M} were isolated by DR. R. W. COLBURN and MRS. BARBARA MALING.

Linkage detection

Linkage-tester stocks containing one readily scored marker in each of the seven linkage groups were used to localize a number of the unmapped mutants. In stocks LT1A and LT2a, the markers are: I. *al-2* : albino-2 (15300); II. *fl* : fluffy; III. *sc* : scumbo (5801); IV. *pan-1* : pantothenic-1 (5531); V. *inos* : inositol (37401); VI. *ylo* : yellow (Y30539y); VII. *nt* : nicotinic-tryptophan (C86). The multiple tester stock is most conveniently used as fertilizing parent. *al-2* is epistatic to *ylo*, and *sc* to *fl*. Scoring for linkage in VI and II is therefore limited to one half the progeny. One hundred fifty ascospores have been isolated routinely for preliminary tests. Linkage has been confirmed by a 2- or 3-point cross using markers appropriate for localizing the mutant within its own group. The correct linkage group was indicated for 13 out of 15 unknown morphological mutants that have been crossed to the multiple tester stock. Of the 15, only balloon and ragged appeared to be unlinked to all the test markers, and required other exploratory crosses before being located.

In a number of instances phenotypic resemblance to markers at known loci

(such as albino, crisp, and ropy) was used as a basis for testing unknown mutants directly for allelism or for linkage in a specific group. Linkage of several mutants with mating type was indicated during the course of backcrossing. In other series of tests to detect linkage, unknowns were crossed to *al* (4637T) (I and II), to *fr cr aur* or *fr aur* (I), or to *asco tryp-2* (VI).

Procedure for crossing

All crosses were carried out at 25°C on synthetic crossing medium (WESTERGAARD and MITCHELL 1947). Fertilization by nonconidiating strains was accomplished by rubbing a mass of mycelium firmly over the surface of the protoperithecial parent by means of a stiff needle or spatula, so as to distribute fragments as widely as possible. Although crosses may eject ascospores within a week, ascospores were not isolated until at least 18 days after fertilization, because selection due to differential ripening sometimes occurs when spores are isolated earlier.

nic-1 ascospores mature poorly unless the synthetic crossing medium is supplemented, even though the protoperithecial parent in a heterozygous cross is *nic*$^+$ (ST. LAWRENCE, personal communication). No supplement was used in most of our earlier crosses of this type involving *nic-1* or other nutritional markers. This may be responsible for the deficiency of *nic*$^-$ and *thi*$^-$ segregants in crosses such as 9 and 12, Table 3.

Isolation of ascospores

Total isolation: A loop of sterile water was used to collect ascospores from the wall of the cross tube and to spread them over a four percent agar surface so that they were optimally spaced for isolation. Isolation was accomplished under 60× magnification using a flattened and sharpened platinum-iridium blade which was flamed between transfers. A small piece of agar bearing a single ascospore was cut out with a single motion of the blade and carried directly to a 75 mm slant without intermediate transfer to a separate agar block.

Selective isolation: Two alleles are frequently distinguishable from one another by the growth and appearance of hyphae from ascospores directly following germination on appropriate media (LEIN, MITCHELL and HOULAHAN 1948; WAGNER and MITCHELL 1955, Figure 29). In such cases it is possible to select and isolate a specific class or classes of segregants to tubes for further testing, thus saving the effort that would be required for total isolation. The few crosses where spores were isolated selectively are so designated in the tables; total isolation was employed for nearly all the crosses reported.

Scoring and handling of markers

When both morphological and nutritional markers are segregating in a cross, scoring of segregants is often more satisfactory on agar media in 75 mm tubes than on liquid test media.

Growth factors were generally used at the following concentrations: vitamins:

10 μg per ml (except inositol, 30 μg per ml); amino acids, purines and pyrimidines: 0.1 mg or 0.2 mg per ml (except arginine, 0.5 mg L-arginine HCl per ml).

For optimal growth arginine mutants generally require the addition of arginine even to complete medium. 0.1 mg/ml L-arginine HCl was used for crossing (NEWMEYER 1957).

For optimal growth arginine-11 (30820) (SRB 1950) requires arginine (or citrulline) plus *both* a purine *and* a pyrimidine (NEWMEYER, personal communication). 0.3 mg/ml L-arginine, 0.2 mg/ml adenylic acid, and 0.1 mg/ml uridine have proved adequate for good germination and growth.

button *(bn)* germinates and survives better on minimal medium than on complete. bn^- segregants can be scored for nutritional traits by inoculating on plates (25 or more tests per plate), but false negative readings may result unless inocula are checked microscopically for viability.

Ascospores from crosses segregating histidine or homoserine requirements were isolated to specifically supplemented minimal medium in order to avoid inhibition by constituents of complete medium (LEIN, MITCHELL and HOULAHAN 1948; HAAS, MITCHELL, AMES and MITCHELL 1952; TEAS, HOROWITZ and FLING 1948).

nitrate *(nit)* was scored visibly using a pH indicator (FINCHAM, personal communication). When one percent KNO_3 and 20 μg/ml brom-cresol purple are included in the medium, nit^- turns the medium yellow after growth is complete, whereas nit^+ turns it purple.

Growth of osmotic *(os)* segregants from germinating ascospores is inhibited markedly by glycerol but not by one percent sucrose (ST. LAWRENCE, personal communication). osmotic ordinarily can be scored by appearance, but can be checked using medium to which four percent NaCl has been added. The mutant cannot grow under these conditions.

skin *(sk)* ascospores mature somewhat more slowly than sk^+, but good allele ratios were obtained at three weeks or longer after fertilization.

Germination of ascospores was good only below 30°C in the case of tiny, and probably of *amyc* as well.

Either indole (10 μg per ml) or DL-tryptophan plus DL-phenylalanine (0.2 mg of each per ml) was used for tryptophan mutants.

Scoring for mating type was accomplished in Petri dishes using wild types *A* and *a* as protoperithecial tester parents. The testers were inoculated into separate plates containing 12–15 ml of synthetic cross medium. After six days the isolates to be tested were inoculated at marked spots on plates containing each mating type. Twenty-four or more isolates can be tested on a single pair of plates. Negative tests were not trusted without confirmation against the other mating type.

PRESENTATION OF DATA

Newly mapped mutants in all seven groups have been listed in Table 1. Linkage data with markers in groups I, II, VI and VII are presented in Tables 2 and 3, and summarized in Figure 1. The format of the tables was adapted from EMERSON,

TABLE 2

Two and three point crosses with markers in groups I, II, VI, and VII

Zygote genotype and recombination percent	Parental combinations		Recombinations: Singles region 1		Singles region 2		Doubles regions 1 and 2		Total and percent germination	Marker isolation numbers
+ *b* *c* / *a* + +	+*bc*	*a*++	+++	*abc*	+*b*+	*a*+*c*	++*c*	*ab*+	...	(a) (b) (c)
+ + *arg-1* / *fr* *leu-3* + 25.3 22.9	48	49	14	17	12	15	4 7 C=1.1 (>0.57)		166 83%	B110 47313 B369
+ *leu-3* *cr* / *fr* + + 31.0 31.0	12	..	8	..	8	..	1 .. C=0.4		29 (*fr*+ only) 60%	B110 47313 *cr*^{L}
+ *rg* + / *fr* + *aur* 26.3 36.8	9	7	4	4	5	7	0 2 C=0.5		38 38%	B110 B187 34508
+ *ad-3* + / *fr* + *aur* 27.0 24.3	9	12	4	3	2	4	0 3 C=1.2 (>0.26)		37 92%	B110 B105p 34508
+ *cr* *aur* / *fr* + + 40.0 48.0	10	..	3	..	5	..	7 .. C=1.5 (>0.58)		25 (*fr*+ only) 92%	B110 *cr*^{L} 34508
+ + + / *fr* *cr* *aur* 50.0 58.3	3	2	3	2	5	2	7 0 C=1.0		24 100%	B110 *cr*^{L} 34508
+ + + / *fr* *cr* *aur* 43.0 31.4	24	10	14	11	8	7	5 7 C=1.0		86 86%	B110 *cr*^{L} 34508
+ *un* + / *fr* + *aur* 34.6 6.4	25	23	15	10	2	1	2 0 C=1.2 (>0.14)		78 78%	B110 STL6 34508
+ *al-2*(*lys-3*)*os* / *fr* + (+)+ 39.2 24.7	25	13	24	11	8	13	3 0 C=0.3		97 97%	B110 15300 (4545) E11200
+ *arg-1* + / *leu-3* + *cr* 15.8 3.9	36	25	6	6	3	0	0	0	76^{N} 62%	47313 46004 *cr*^{L}
+ *a* / *amyc* *A* 5.5	..	17	..	1	..	..	..	..	18 (*amyc*$^{-}$ only)	K422 sex
A + *arg-1* / *a* *ad-5* + 6.2 0	19	26	2	1	0	0	0	0	48^{N} 96%	sex 71104 46004
A *arg-1* *ti* / *a* + + 14.3 0	18	..	..	3	..	0	0	..	21 (*ti*$^{-}$ only) 76%	sex 46004 B233t

TABLE 2—Continued

Two and three point crosses with markers in groups I, II, VI, and VII

Zygote genotype and recombination percent	Parental combinations		Recombination: Singles region 1		Singles region 2		Doubles regions 1 and 2		Total and percent germination	Marker isolation numbers
(+) *A arg-1* + / (*leu-3*) *a* + *cr* (1.8, 5.5)	49	52	0	2	2	4	0	0	109^{N} 56%	(47313) sex 46004 cr^{L}
A ti / *a* + (7.1)	13	..	..	1	..	..	..	..	14 (*ti*⁻ only) 83%	sex B233t
A aur + / *a* + *so* (26.8, 31.4)	20	14	7	5	7	8	5 1 C=1.1 (>0.39)		67 96%	sex 34508 B230s
(*A*) + *arg-1* *cr* / (*a*) *un* + + (2.3, 11.6)	57	54^{a}	2	1^{a}	11	4^{a}	0	0	129^{MA} 64%	(sex) 55701t 46004 cr^{L}
+ *arg-1* *cr* / *ad-5* + + (0, 2.9)	14	20	0	0	0	1	0	0	35^{N} 70%	71104 46004 cr^{L}
+ *rg* *cr* / *ad-5* + + (10.2, 13.8)	50	80	11	3	9	11	2 1 C=1.3 (>0.26)		167 82%	71104 B53 B123
(a) + *ti* (+) / (A) *arg-1* + (*cr*) (1.6)	61	..	..	1^{b}	..	..	..	..	62 (*ti*⁻ only) 88%	(sex) 46004 B233t (cr^{L})
(a) + *lys-4* + / (A) *rg* + *cr* (6.6, 9.0)	104	36	8	3^{A}	7^{a}	8	0	0	166 58%	(sex) B53 15069 B123
(A) + + + / (a) *rg* *lys-4* *cr* (1.3, 1.3)	43	35	1^{A}	0	0	1^{a}	0	0	80 80%	(sex) B53 15069 B123
+ + (*arg-6*) *al-2* / *rg* *cr* (+) + (14.3, 33.3)	5	6	1	2	4	3^{e}	0	0	21 52%	B53 B123 (29997) 15300
+ + *al-2* (*hs*) / *rg* *cr* + (+) (16.7, 38.1)	10	12	1	3	9	4^{g}	0 3 C=1.1 (>0.23)		42 70%	B53 B123 15300 (51504)
+ + *al* / *rg* *cr* + (9.7, 45.1)	41	43	4	8	33	41	2 3 C=0.7		175 79%	B53 B123 4637T
+ + *aur* (*hs*) / *rg* *cr* + (+) (5.3, 44.7)	12	8	0	1	10	6^{g}	0 1 C=1.1 (>0.03)		38 63%	B53 B123 34508 (51504)

+ *cr* +	21	25	1	1	10	10	0	0	68	B187
rg + *aur*										B123
2.9 29.4									68%	34508
+ + *(arg-6) aur*	15	4	0	0	7	10[f]	0	0	36	B53
rg *cr* (+) +										B123
0 47.2									90%	(29997)
										34508
+ *st* +	43	27	1[A]	3[1]	7[A]	5[a]	0	0	86	35203
ad-3B + *thi-1*										B128
4.6 14.0									86%	56501
+ *thi-1* +	44	45	15	10	11	10	2	1	138	35203
ad-3B + *al-2*							C=0.6			56501
20.3 17.4									69%	15300
+ + +	34	28	7	2	3	7	0	0	81	*cr*[L]
cr *thi-1* *me-6*										56501
11.1 12.4									81%	35809
+ + *me-6*	49	42	2	3	7	8	1	0	112	B149
slo *thi-1* +							C=1.2			56501
5.4 14.3							(>0.03)		81%	35809
+ *thi-1* *nit*	33	30	0	2	13	13	0	0	91	B149
slo + +										56501
2.2 28.6									91%	34547
(a) + *me-6*	19	19	2[a]	5[A]	..	..	..	..	45	(sex)
(A) *slo* +										B149
15.5									90%	35809
+ + *al-2*	25	15	8	4	11	9	0	1	73	B149
slo *me-6* +							C=0.3			35809
17.8 28.8									73%	15300
+ *nit* *aur*	18	11	9	10	3	5	1	4	61	B149
slo + +							C=1.0			34547
39.4 21.3									79%	34508
+ + *nit*	83	75	8	10	8	4	0	0	188	56501
thi-1 *me-6* +										35809
9.6 6.4									95%	34547
+ + *al-2*	19	13	3	3	5	8	1	0	52	56501
thi-1 *me-6* +							C=0.5			35809
13.5 26.9									87%	15300
+ *mac* *aur*	34	30	2	3	6	8	1	0	84	56501
thi-1 + +							C=0.9			65108
7.1 17.9									84%	34508
(+) + *me-1*	..	..	13	..	..	..	..	..	54	(56501)
(thi-1) *me-6* +										35809
48.0									90%	38706
+ *un* *aur*	..	35	2	..	..	3	0	..	40	34547
nit + +									(*un*[+]	STL6
5.0 7.5									only)	34508
									>80%	
(a) + *al-2*	..	243	..	1[A]	..	..	..	..	244[s]	(sex)
(A) *arg-6* +									(*arg*⁻	29997
0.4									only)	15300

TABLE 2—Continued

Two and three point crosses with markers in groups I, II, VI, and VII

Zygote genotype and recombination percent	Parental combinations	Recombination: Singles region 1	Recombination: Singles region 2	Recombination: Doubles regions 1 and 2	Total and percent germination	Marker isolation numbers
(*a*) + *aur* (*A*) *arg-6* + 0.4	.. 260	.. 1[A]	.. ..	.. ..	261[s] (*arg*⁻ only) 34508	(sex) 29997
+ + *hs* *arg-6* *aur* + 0.4	.. ..	5 ..	3 ..	.. ..	8[s] (*arg*⁺*hs*⁺ only) 51504	29997 34508
(+ + *nit*) + *aur* (+ *nic-1*) (*cr thi-1* +) *al-2* + (*lys-3* +) 4.2	.. ..	1[d] ..	.. ..	.. ..	48 80%	(*cr*[L]) (56501) (34547) 15300 34508 (4545) (3416)
(*a*) + *hs* (*A*) *al-2* + 2.5	77 ..	.. 2[h]	.. ..	.. ..	79[s] (*hs*⁻ only) 51504	(sex) 15300
+ *hs* *al*[M] + 0	.. 76	.. 0	.. ..	.. ..	76[B] (*al*⁻ only) 75%	*al*[M] 51504
(*a*) + *hs* (*A*) *aur* + 0.4	277 ..	.. 1[a]	.. ..	.. ..	278[s] (*hs*⁻ only) 51504	(sex) 34508
+ *os* + *aur* + *so* 26.9 7.7	35 41	27 17	4 5	1 0 C=0.4	130 87%	34508 B135 B230s
+ *os* *so* *nic-1* + + 28.2 6.0	30 48	22 10	5 1	1 0 C=0.5	117 78%	3416 B135 B230s
+ + + (+) *bal* *arg-5* *pe* (*fl*) 11.1 33.3	5 ..	1 ..	3 ..	0 ..	9 (*bal*⁺ only) 50%	B56 27947 Y8743m (*fl*[L])
+ *arg-5* (*pe fl*) *bal* + (+ +) 1.2	35 47	0 1[c]	.. ..	.. ..	83 83%	B56 27947 (Y8743m) (*fl*[L])
bal-centromere 0	29–1st division segregation asci	0–2nd division segregation asci			29 tetrads 100%	B56 centromere
(*bal*) + + + (+) *arg-5* *pe* *fl* 28.6 31.4	.. 15	.. 9	.. 10	.. 1 C=0.3	35 (*bal*⁺ only) 83%	(B56) 27947 Y8743m *fl*[L]

+ *pe* *fl* *arg-5* + + 5.8 28.8	18	16	1	2	7	8	0	0	52 76%	27947 Y8743m *fl*^{L}
+ + + *pe* *arom* *fl* 2.9 23.0	45	58	4	0	14	18	0	0	139 91%	Y8743m Y7655 *fl*^{L}
+ *tryp-3* *ac* + 11.9	22	15	3	2	..	..	..	..	42 70%	Y2492 C83
+ *asco* (*tryp-2*) *del* + (+) 1.5	..	64	1	..	..	..	..	..	65^{H} (*asco*$^{+}$ only) 65%	B137 37402 (45302)
+ *bn* + *thi-3* + *arg-10* 0 23.9	36	34	0	0	13	9	0	0	92 88%	18558 B40 B368
+ *bn* *arg-10* *thi-3* + + 3.4 21.4	32	35	3	0	11	8	0	0	89 89%	18558 B40 B317
+ + + *bn* *arg-10* *nt* 26.8 7.3	28	..	10	..	2	..	1 C=1.2 (>0.03)	..	41 (*bn*$^{+}$ only) 88%	B40 B370 65001
+ *arg-11* + *bn* + *nt* 10.8 10.8	82	19	11	3	13	1	0	0	129^{k} 66%	B40 30820 65001
+ *arg-10* + *arg-11* + *nt* 1.6 1.6	97	84	1	2	1	2	0	0	187 94%	30820 B317 65001
+ *arg-10* + *arg-11* + *nt* 1.1 5.3	89	..	..	1	..	5	0	..	95 (*nt*$^{+}$ only) 95%	30820 B317 65001
+ + *sk* *arg-10* *nt* + 6.2 17.2	17	32	3	1	5	6	0	0	64 45%	B370 65001 B106
+ *nt* *sk* *arg-10* + + 5.9 17.6	13	..	..	1	..	3	0	..	17 (*sk*$^{-}$ only) 82%	B370 C86 B106
+ + *sk* *arg-10* *nt* + 12.3 6.8	24	35	4	5	5	0	0	0	73 73%	B317 65001 B234

Numbers of progeny are given in the body of the table. Progeny genotypes are not designated explicitly, but the genotype of each class can be determined from the order of presentation. The left-hand number of each pair of complementary classes represents the genotype that contains the plus allele of the left-most marker scored (or mating type A). Alleles shown in parentheses in the genotype column were not scored or recorded, nor were classes of segregants where dashes replace numbers. Regions are numbered from left to right, and isolation numbers in the last column are listed in the same order. Crosses are tabulated in a sequence corresponding to the position of their left-most markers in the linkage group. Where gene order is uncertain, one arbitrarily chosen order has been used consistently. C=coincidence. When C exceeds one, the minimum coincidence is also given from which the observed value does not deviate significantly (2.5 percent one-sided level).

[a] Mating type *a*.
[A] Mating type *A*.
[B] Data of MR. EDWARD G. BARRY.
[b] *A*---+ by progeny test.
[c] ---- by progeny test.
[d] Phenotypically ++—+++—+.
[e] Two *arg*$^{-}$, *one arg*$^{+}$.
[f] Nine *arg*$^{-}$, one *arg*$^{+}$.
[g] *All hs*$^{-}$.
[H] Data of MR. JERRY L. HOWARD.
[h] One *A*, one *a*.
[i] One *A*, two *a*.
[k] Plus 23 *bn*$^{-}$ not scored.
[MA] Data of MRS. M. K. ALLEN.
[N] Data of D. NEWMEYER.
[s] Germinated ascospores isolated selectively from minimal plates.

TABLE 3

Four- five- and six-point crosses. Conventions as in Table 2. All markers are in Linkage Group I. Coincidence values are based on pairs of crossovers both in double and in triple recombination classes

Zygote genotype recombination percent and marker isolation numbers	Parental combinations	Recombinations: Singles Region 1	Singles Region 2	Singles Region 3	Singles Region 4	Singles Region 5	Doubles (Regions in parentheses)	Triples	Total and percent germination
+ *leu-3* *ad-3B* *al-2* *lys-3* *fr* + + + + 39.5 4.6 34.9 20.9 B110, 47313, 35203, 15300, 4545	13 ..	8 ..	1 ..	7 ..	2 ..	.. ..	5 .. (1&3)	4 .. (1&4)	2 .. (3&4) C=1.0	1 .. (2,3,4)	43 (*fr*⁺ only) 85%
+ + *cr* *thi-1* *fr* *leu-3* + + 26.0 11.7 7.8 B110, 47313, cr^L, 56501	47 ..	15 ..	7 ..	3 ..	.. ..	.. ..	2 .. (1&2) C=0.9	3 .. (1&3)	.. ..	0 ..	77 (*fr*⁺ only) 80%
+ *A* + + *fr* *a* *cr* *aur* 16.7 4.2 29.2 B110, —, B180, 34508	14 ..	2 ..	1 ..	5 ..	.. ..	.. ..	2 .. (1&3)	.. ..	.. ..	0 ..	24 (*fr*⁺ only) 90%
+ *A* *slo* + *fr* *a* + *aur* 36.4 13.6 20.4 B110, —, B149, 34508	6 9	4 10	4 1	4 4	.. ..	.. ..	1 0 (1&2) C=0.5	1 0 (1&3)	.. ..	0 0	44 88%
A + *rg* *cr* *thi-1* *al-2* *a* *arg-1* + + + + 10.6 2.1 2.1 17.0 19.2 —, 46004, B53, B123, 56501, 15300	15 10	1 2	0 1	1 0	3 4	4 4	1 0 (1&4)	1 0 (1&5)	.. ..	0 0	47^N 95%
+ *rg* *cr* *thi-1* *al-2* *arg-1* + + + + 2.0 2.0 7.8 23.5 46004, B53, B123, 56501, 15300	23 11	0 0	0 1	1 3	7 4	.. ..	1 0 (1&4)	.. ..	.. ..	0 0	51^N 64%
+ *ad-3B* + *thi-1* *al-2* *rg* + *cr* + + 0 8.9 7.1 17.9 B53, 35203, B123, 56501, 15300	20 18	0 0	3 1	2 2	5 4	.. ..	0 1 (2&4)	.. ..	.. ..	0 0	56 70%
+ + + + *ad-3A* *nit* *aur* *os* 16.9 12.1 24.2 35203, 34547, 34508, B135	37 36	6 6	4 4	9 10	.. ..	.. ..	1 0 (1&2) C=1.6 (>0.43)	2 1 (2&3) C=1.7 (>0.60)	2 3 (1&3)	2 1	124 83%

(A) + *cr* *thi-1* *al-2* *(a) lys-4* + + + 2.4 4.7 29.4 (–), STL4, B123, 56501, 15300	20	34	2[A]	0	4	0	8	17	..	..	..	..	.. ..	.. ..	.. ..	0	0	85 42%
(a) + *un* + + *(A)* *cr* + *thi-1* *al-2* 1.0 6.9 23.8 (–), B123, 44409t, 56501, 15300	32	38	0	1[A]	4	2	12	11	..	..	..	..	1 0 (2&3) C=0.6	.. ..	.. ..	0	0	101 67%
+ + + + + + *cr* *thi-1* *nit* *aur* *nic-1* *os* 10.1 23.4 14.8 18.0 6.2 *cr*^{L}, 56501, 34547, 34508, 3416, B135	28	22	4	4	9	15	8	6	4	8	2	4	0 1 (1&3) 2 1 (2&4)	3 0 (1&4) 2 0 (3&4) C=0.6	1 1 (2&3) C=0.5 0 2 (4&5) C=1.4 (>0.17)	0 1 (1,2,4) C=0.3 for 1&2		128 85%
+ + + + + + *cr* *thi-1* *nit* *aur* *nic-1* *os* 10.4 28.8 6.4 4.0 25.6 *cr*^{L}, 56501, 34547, 34508, 3416, E11200	32	12	0	9	18	10	0	4	1	3	20	3	3 1 (1&5) 3 1 (2&5)	1 2 (2&3) C=1.3 (>0.27) 1 0 (3&5)	1 0 (2&4)	0	0	125 83%
+ + *nit* *aur* *cr* *me-6* + + 13.4 3.0 29.8 *cr*^{L}, 35809, 34547, 34508	19	22	3	1	0	2	9	6	..	..	..	..	3 2 (1&3)	.. ..	.. ..	0	0	67 96%
+ + + + *cr* *nit* *aur* *os* 42.9 19.0 27.0 B122, 34547, 34508, B135	13	9	9	7	3	1	4	4	..	..	..	..	3 1 (1&2) C=1.2 (>0.43)	1 1 (2&3) C=1.2 (>0.33)	2 3 (1&3)	0	2	63 93%
+ *nic-1* *os* *so* *al* + + + 5.1 11.5 3.8 4637T, 3416, B135, B230s	30	34	2	1	5	2	0	2	..	..	..	..	1 0 (1&2) C=2.2 (>0.05)	1 0 (2&3) C=2.9 (>0.08)	.. ..	0	0	78 79%
+ + + *so* *aur* *nic-1* *os* + 11.3 9.7 1.6 34508, 3416, B135, B230s	55	44	7	4	6	3	2	0	..	..	..	..	1 2 (1&2) C=2.2 (>0.44,	.. ..	.. ..	0	0	124 88%

[A] Mating type *A*. [N] Data of D. NEWMEYER.

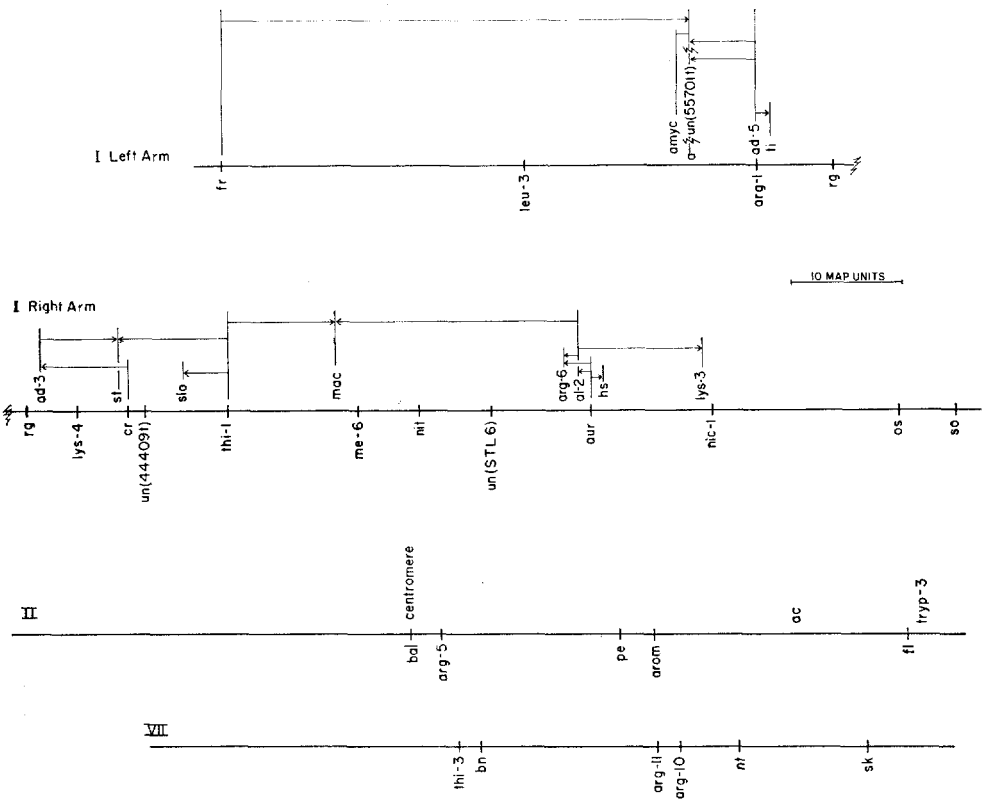

FIGURE 1.—Partial maps of groups I, II and VII summarizing the data from Tables 2 and 3. The sequence of loci written below the heavy line is based directly on 3-point data for all the genes involved. Above the heavy line, only the relationships indicated by arrows are based on 3-point data. Two opposing arrows indicate that the marker was situated medially in the 3-point cross; each unopposed arrow originates from a medial marker and leads to a marker that was not medial. Interval lengths are imprecise owing to the variability of crossing over. The map is based solely on data from the present paper, except that the position shown for genes above the line may reflect the results of other workers in cases where our own data would require order to be decided arbitrarily or to be based only on quantitative 2-point data from different sources. See text for sequences established by other workers. The centromere is located near *rg* in I, near *bal* in II and left of *thi-3* in VII.

BEADLE and FRASER 1935. Groups I and VI correspond to groups A and B of HOULAHAN, BEADLE and CALHOUN 1949.

These crosses were selected from a much larger number on the basis of several criteria. Two-point crosses have generally not been reported unless they involved markers for which adequate 3-point data were lacking. Crosses with low germination or poor allele ratios ordinarily were included only if they provide information that is not available from other crosses less likely to have been distorted by selective survival. The author is indebted to several persons for permission to use unpublished data, as indicated in footnotes to the tables.

Linkage of amycelial to sex was first established by DR. K. C. ATWOOD. Linkage of osmotic in group I was first shown by MRS. MARY R. EMERSON, and of *un* (STL6) by DR. P. ST. LAWRENCE. Location of *arg-5* in II was suggested by DR. R. W. BARRATT on the basis of its linkage to *al*(4736T) (SRB 1946). Linkage of *arg-11* in group VII was discovered by DR. D. NEWMEYER. *nic-3* (Y31881) and *me-7*(4894) were first shown to be linked in VII by MRS. M. K. ALLEN; their location was aided by unpublished data of MRS. MIRIAM BONNER.

Evidence for allelism or close linkage was obtained from intercrosses between mutants that appeared to be recurrences. The numbers of mutant progeny among which no wild type recombinants occurred were: B187 × *rg*(B53) : 68. STL4 × *lys-4*(15069) : 556. B74 × cr^L : 15. B122 × cr^L : 16. B123 × cr^L : 181. B74 × *cr* (B123) : 14. B122 × *cr*(B123) : 13. B180 × *cr*(B123) : 115. B102 × *aur*(34508) : 53. B102 × *al-2*(15300) : 23. JH216 × *al-2*(15300) : 23 (plus one wild type). JH9698 × *al-2*(15300) : 19. B102 × al^s : 22 (probable *al-2* allele, SANSOME *et al.* 1945). al^c × *al*(B102) : 24. Y602 × *al*(B102) : 27. Y2170 × *al*(B102) : 23. Y2171 × *al*(B102) : 27. al^M × *hs*(51504) : 76 (data of E. G. BARRY). B135 × *os* (E11200) : 124 (including data of P. ST. LAWRENCE). M16 × *os*(E11200) : 113 (data of ST. LAWRENCE). M155–5 × fl^L (LINDEGREN's) : 136. P346 × fl^L : 124. P605 × fl^L : 124. fl^p × fl^L : 168. B148 × *col-4*(70007) : 138. B12 × *bis*(B6) : 189. B30 × *bis*(B6) : 123.

Relation to the data of other investigators: The maps presented in Figure 1 are based solely on the data from the present paper, and therefore include only the markers used in our crosses. For the convenience of the reader, all known markers in groups I and VII are listed in Tables 4 and 5, together with references to the work of other investigators that bears on their status as markers.

A number of gene sequences that were not included in the 1954 compilation of BARRATT *et al.* have been established or confirmed by other workers. In linkage Group I these are: *leu-3 sex phen* centromere *ad-3B cr* (BARRATT and OGATA 1954); *sex hist* (K12) 0.7 *hist* (K26) *arg-3 al-2* (MATHIESON and CATCHESIDE 1955, most likely order); *sex arg-1 arg-3* (NEWMEYER 1957, most likely order); *sex* 4.4 *ad-5* 2.2 centromere 6.1 *vis* (3717) (HOWE 1956); centromere 0.5 *hist-2* 2.0 *ad-3A* 0.1 *ad-3B* 3.0 *nic-2* (DE SERRES 1957); *ad-3 T al-2* (HOROWITZ and FLING 1956); and *al-2 lys-3 nic-1 os* (ST. LAWRENCE 1956). In linkage Group VII: centromere *sfo thi-3 nt* (REISNER, BARRATT and NEWMEYER 1953); and *bn arg-10 nt* (NEWMEYER 1957). The data in the present paper are consistent with these results.

There remain a number of uncertainties regarding gene order, such as the sequence of *amyc* and *un*(55701t) with respect to mating-type; *ad-5* and *ti* with respect to *arg-1; st* with respect to *cr*; and *bal* and *arg-5* with respect to centromere and *pe*. (The seriation shown for *ti* and *arg-1* depends on a single isolate.)

Markers and sequences in linkage groups III, IV, and V are considered in the three accompanying papers of this series. Our contributions to groups II and VI have been insufficient to justify separate tabulations for them. Information not included in BARRATT *et al.* 1954 will be found in the following publications:

STADLER 1956a, b; TOWE 1958 (Group VI map; *asco*); PITTENGER 1954 (*cys-1*, *cys-2*, VI); CASE 1957, 1958; CASE and GILES 1958a, b (*ylo ad-1 pan-2 tryp-2*, VI); HOULAHAN, BEADLE and CALHOUN 1949 (*phen*(38602), VI).

DISCUSSION

New markers and more reliable maps are obviously useful as tools for cytogenetic work. The data reported here and in the accompanying papers on groups III, IV and V may also be of interest for the information they provide on interference, the distribution of loci within the chromosome complement, and the variability of crossing over. Results from all four papers in the series will be discussed together here in relation to these subjects.

Interference: The present results from random segregants indicate that Neurospora does not differ essentially from higher organisms such as Drosophila with respect to chiasma interference. A close similarity to Drosophila is also apparent in tetrad data (PERKINS 1958). On the other hand, Neurospora does appear to differ from the homothallic ascomycete *Aspergillus nidulans*, where no evidence of positive chiasma interference has been obtained either with random isolates (KÄFER 1958) or with tetrads (STRICKLAND 1958).

An excess of double crossovers ("negative interference") has been reported in Aspergillus for very short intervals where selective techniques of analysis are normally employed (PRITCHARD 1958). A similar excess of double crossovers was also reported in nonselective experiments with longer intervals by CALEF (1957), who pointed out, however, that the numbers obtained nonselectively were too small to be conclusive. Computation of fiducial limits for double crossovers shows that the numerical coincidence value of 3.0, obtained by CALEF in

TABLE 4

Loci of linkage group I

Symbol and name*	Standard mutant	References†
a : *sex* or mating type	....	B
ad-3A : adenine-3A	38701	B, DE SERRES 1956, 1958; GILES *et al.*, 1957; DE SERRES *et al.*, 1958
ad-3B : adenine-3B	35203	B, DE SERRES 1956, 1958; GILES *et al.*, 1957; BARRATT and OGATA 1954; DE SERRES *et al.*, 1958
ad-5 : adenine-5	71104	B, HOWE 1956
al-1 : albino-1 (see aurescent)		
al-2 : albino-2	15300	B, PITTENGER 1954; ST. LAWRENCE 1956
amyc : amycelial	K422	ATWOOD 1949
arg-1 : arginine-1	46004	B, NEWMEYER 1957
arg-3 : arginine-3	30300	B, NEWMEYER 1957; MATHIESON *et al.*, 1955

arg-6 : arginine-6	29997	B
aur : aurescent	34508	B, PITTENGER 1954
bl : black colonial	C113	SCHAEFFER 1953 (Lost)
can : canavanine resistant	46003R	B
cr : crisp	cr^{L}‡	B, BARRATT and OGATA 1954; MALING 1959a
cyt(C115) : cytochrome	C115	MITCHELL *et al.*, 1953
dir : dirty	‡	B (Lost? Possibly = *os*)
fr : frost	B110	
gap : gap	‡	B (Lost?)
hist-2 : histidine-2	C94	B, DE SERRES 1956; GILES *et al.*, 1957
hist-3 : histidine-3	C140	B
hist(K12) : histidine	K12	MATHIESON *et al.*, 1955
hist(K26) : histidine	K26	MATHIESON *et al.*, 1955
hs : homoserine	51504	B
leu-3 : leucine-3	47313	B, BARRATT and OGATA 1954
lys-3 : lysine-3	4545	B
lys(28815)		B (Allelic *lys-3*(4545), ST. LAWRENCE, personal communication)
lys-4 : lysine-4	15069	B
mac : methionine-adenine-cystine	65108	DUBES 1953 (Possible allele *me-6*)
me-6 : methionine-6	35809	B (Assigned locus number on basis of cross 40, Table 2, showing nonallelism with *me-1*)
mt : mating type (see *a*)		
m-1(vis) : modifier-1 of *vis*(3717)	....	HOWE 1956
m-2(vis) : modifier-2 of *vis*(3717)	....	HOWE 1956
nd : natural death	....	B (Lost?)
nic-1 : nicotinic-1 (= *q*)	3416	B, ST. LAWRENCE 1956
nic-2 : nicotinic-2	43002	B, DE SERRES 1956; GILES *et al.*, 1957
nit : nitrate nonutilizer	34547	B
n-nit : nitrate nonutilizer (see *nit*)		
os : osmotic	E11200	EMERSON *et al.*, 1958; ST. LAWRENCE 1956
pa : pale	‡	B (Lost?)
phen : phenylalanine	H6196	BARRATT and OGATA 1954
q : quinolinic acid (see *nic-1*)		
rg : ragged	B53	MALING 1959a
sex : sex (see *a*)		
slo : slow	B149	(No allelism test with *cyt*(C115)
sn : snowflake	C136	MITCHELL 1958
so : soft	B230s	
st : sticky	B128	
su-1-me : suppressor-1 of methionine	R1	B, FISCHER 1957
suc : succinic	35402	B
T : tyrosinase thermostability	....	HOROWITZ *et al.*, 1953, 1956
thi-1 : thiamine-1	56501	B, EBERHART 1956
ti : tiny	B233t	
un(46403): unknown requirement		B (Probably *suc* DUBES 1953)
un(STL6) : unknown requirement	STL6	
un(44409) : unknown requirement	44409t	B
un(55701) : unknown requirement	55701t	B
vis(3717) : visible	3717	B, HOWE 1956

* Mutants believed to be lost, mutants of doubtful value as markers, and mutants that are probably alleles at already established loci are indented.

* B: Documented in BARRATT *et al.*, 1954 compilation. Additional references are cited only if they provide new information on linkage, scoring, or gene structure.

‡ The standard mutant is LINDEGREN's. (No isolation number.)

TABLE 5

Loci of linkage group VII

Symbol and name	Standard mutant	References*
arg-10 : arginine-10	B317	B, NEWMEYER 1957
arg-11 : arginine-11	30820	SRB 1950
bn : button	B40	NEWMEYER 1957
me-7 : methionine-7	4894	BUSS 1944; M. ALLEN unpub.
nic-3 : nicotinic-3	Y31881	BONNER *et al.*, 1949; M. ALLEN unpub.
nt : nicotinic-tryptophan	65001	B, REISNER *et al.*, 1953; NEWMEYER 1957
sfo : sulfonamide requiring	E15172	B, REISNER *et al.*, 1953
sk : skin	B106	
thi-3 : thiamine-3	18558	B, REISNER *et al.*, 1953; EBERHART 1956

* B: Documented in BARRATT *et al.*, 1954 compilation. Additional references are cited only if they provide new information on linkage, scoring, or gene structure.

his most reliable nonselective analysis (intervals 1 and 2, Table 1, 1957), is not significantly greater than a minimum coincidence of 0.4.

The present data from Neurospora, which were obtained nonselectively, provide no indication of negative interference. On the contrary, interference is typically positive. Coincidence values are given in the tables wherever one or more double crossovers occurred in adjacent intervals. In a few crosses (e.g., 1, 4, 5, 8 in Table 2) the numerical coincidence value exceeds one. MOLINA's (1942) and STEVENS' (1942) tables were used in all such cases to test the significance of the excess doubles observed, and to compute the minimum coincidence values from which observed numbers of doubles were deviates at the 2½ percent one-sided level (equivalent to a conventional five percent level). In no case is the observed excess of doubles statistically significant. Only six double crossovers were observed within intervals less than 15 units long, in groups IV and V. Although coincidence exceeds one in these cases, the double crossovers observed do not constitute statistically significant evidence of negative interference.

The data presented here give no information as to whether exchanges might occur as doubles or clusters within very short regions (see FREESE 1957; PRITCHARD 1958) but such a model seems unlikely from a consideration of various tetrad data (WEINSTEIN 1957).

Distribution of loci: A total of approximately 125 loci have now been mapped in nine of the 14 chromosome arms of Neurospora, but only in Groups I and VI are markers known that are located at appreciable distances from the centromere in both chromosome arms. The remaining five arms are still effectively devoid of markers. Such a distribution is unexpected on the basis of cytological observations, which indicate that all seven centromeres are definitely nonterminal in location (MCCLINTOCK 1945; SINGLETON 1953).

Group I, the mating type group, now contains about 40 known loci. It has been extended in both directions by the mapping of frost on the left and of soft and osmotic on the right. This reinforces its status as the best marked group of the complement. Group I also exceeds any other group in map length (over 125

units), even though cytologically it is located in the second shortest chromosome of the complement, chromosome 6 (ST. LAWRENCE 1952).

Genes in linkage group II (fluffy, *arom*, etc.) are located in the long arm of chromosome 1, which is cytologically the longest chromosome (ST. LAWRENCE 1952; MCCLINTOCK 1945). Known genes in linkage group IV (*pdx-1*, *pan-1*, etc.) are probably all located in the short (nucleolus organizer) arm of chromosome 2, which is the second longest chromosome (ST. LAWRENCE 1952). The short arm of chromosome 1 and the long arm of chromosome 2, although they are devoid of genetic markers, are nevertheless each longer than the best mapped chromosome, 6, in its entirety (see SINGLETON 1953).

Groups III, V, and VII have not yet been assigned to specific chromosomes, but regardless of whether chromosomes 3, 4, 5, or 7 are involved, the same question is posed. Why have loci not been identified in both arms?

It may be that the strikingly uneven distribution of genes is merely a chance result that reflects the location of markers that happen to have been used for testing linkage. The long-standing availability of mating-type and albino for linkage tests in I has no doubt contributed substantially to locating markers throughout this linkage group. In other groups, testing has frequently been carried out with markers far removed from the centromere, e.g., *fl* in II, *pan-1* in IV, *inos* in V, and *nt* in VII. Linkage of genes in the opposite arms might have gone undetected in such tests.

It is also possible that the "empty" arms may be genetically inert, or that mutations occurring in them may be of such a nature that they are not recovered as typical markers. It should be possible to obtain evidence distinguishing the main alternatives. Suitable markers close to the centromere in each group are now available for linkage tests that should enable any gene loci in the five unmapped arms to be identified. Table 6 lists the genes that seem to be most suitably located for extending the various linkage groups beyond their present limits.

TABLE 6

Useful markers for the centromere and for extremes of each linkage group

Centromere markers	I	II	III	IV	V	VI	VII
Visible	*cr*	*bal* (B56)	*sc* (5801)	(*col-4*) (70007)	(*sp*) (B132)	*ylo* (Y30539y)	*bn* (B40)
Nutritional	*hist-2* (C94)	*arg-5* (27947)	*thi-4* (85902)	*pyr-1* (H263)	*lys-1* (33933)	*ad-1* (3254)	*me-7* (4894)

Distal markers	I-L	I-R	II-R	III-R	IV-R	V-R	VI-L	VI-R	VII-R
Visible	*fr* (B110)	*os* (E11200)	*fl*	*vel* (B18)	*mat* (B57)	*pl* (B118)	*del* (B137)	—	*sk* (B106)
Nutritional	(*leu-3*) (47313)	(*nic-1*) (3416)	*tryp-3* (C83)	*tyr* (Y6994)	*pyr-2* (38502)	*asp* (S1007)	*asco* (37402)	*tryp-2* (75001)	(*nt*) (65001)

Markers with symbols in parentheses are located ten or more units from the centromere or from the most distal marker. *rg*(B53) is closer to centromere than *cr*, and *so*(B230s) and *cyt*(C117) are more distal than *os* and *asco* respectively, but *cr*, *os*, and *asco* are probably preferable for technical reasons.

Recurrences: Eight previously unmapped albino mutants of various origins are reported in Table 1. These are all apparently located within the same region of group I as the eight albino mutants previously mapped by HUNGATE (1945). Visible mutants have also been recovered recurrently at a number of other loci: crisp (4 recurrences), fluffy (4), biscuit (3), osmotic (2), ragged (2), ropy-1 (2), skin (2), and colonial-4 (1).

Variability of crossing over: Striking differences in recombination frequencies were observed for identical intervals in a number of the crosses reported here, in spite of the fact that markers had been more or less inbred to standard strains in an effort to decrease heterogeneity. The differences in crossing over between crosses of different parentage are great enough to have led to spurious conclusions regarding gene order if sequences had been determined only on the basis of two-point data combined from different crosses. This source of error can be avoided by using multiple point crosses, which enable order to be decided on the basis of data from a single cross. Three-point data have been used wherever possible to indicate gene order in the present study, and they have done so unambiguously in most cases, even though the number of segregants has been small.

A detailed statistical treatment of the crossing over variations observed here does not seem justified in view of the fact that the experiments were not designed to obtain information on variability, and no effort was made to analyze large numbers of progeny from crosses that showed unusually high or low recombination frequencies. Consequently, sampling error often cannot be eliminated as a possible explanation. Selection may well be responsible for several deviant recombination values, in crosses where low germination or poor allele ratios were observed. (This is probably true, for example, of crosses 21, Table 2; 12, Table 3; and 24, group V.) The remaining variations in crossing over, that cannot be explained in these ways, are probably genetic rather than environmental in origin. Crosses were carried out under carefully controlled standard conditions, and similar recombination values have been obtained consistently when crosses between the same two parents were repeated. Reproducibility of results has also been observed by MALING (1959a), in crosses repeated on a large scale.

STADLER (1956a, c) and TOWE (1958) have used tetrads to obtain extensive information regarding the genetic control of crossing over in Neurospora. Exchange frequencies between their group VI markers remained constant in repeated crosses between the same two parents, but crossing over varied widely in crosses of different parentage. In their experience, crossing over generally increased (and never decreased) in successive backcross generations.

In the present work, group I markers were most inbred, group IV probably least, before being used to obtain linkage data. The group I crossover values reported here are consistently greater than those collected from heterogeneous sources by BARRATT *et al.* (1954). On the other hand, the group IV values reported here by MALING are somewhat smaller than those in BARRATT *et al.*, and only about one half as great as those of MITCHELL and MITCHELL 1954.

It is not surprising that crossover frequencies are heterogeneous in Neurospora,

where mutants were originally obtained starting with a variety of different wild type strains, where exposure to X-rays and other mutagens has often been high, and where stocks have commonly been maintained as vegetative clones for long periods of time through repeated transfers. With respect to recombination variability, as with interference, Neurospora resembles Drosophila and maize more nearly than it resembles *Aspergillus nidulans*. The striking homogeneity of crossover values in Aspergillus may be related to the fact that all genetic work has employed strains tracing back to a single nucleus (see KÄFER 1958).

SUMMARY

Fifty-three previously unmapped mutants of *Neurospora crassa* are described, of which at least 25 are at new loci.

Data on random segregants obtained by nonselective methods from 75 multiple-point crosses have placed 14 of the previously unmapped loci in linkage groups I, II, VI, and VII, and have clarified the sequence of previously known genes. These results, taken together with new data in a series of three accompanying papers on linkage groups III, IV, and V, bring the total number of mapped loci in Neurospora to over 130, and the number of loci in group I to at least 40. All of the mapped loci fall in only nine out of the 14 chromosome arms. (A few genes near the centromeres are possible exceptions.) Coincidence values range from zero to not significantly greater than one for neighboring regions of increasing length. Gene sequences have been established by 3-point tests rather than by combining data from 2-point crosses, in order to avoid errors due to variations in crossing over frequency.

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