Kitani, Y.

Inter- and Intra-locus linkage analysis in *Sordaria fimicola*

Research in *Sordaria fimicola* has been discontinued since Kihara Institute for Biological Research closed in 1984. There is little prospect of completing work on four projects that were then in progress. It therefore seems appropriate to publish the substantial information already acquired.

The projects concerned: 1) Confirmation of three grey and two hyaline mutants as grey locus (g-locus) alleles; 2) determination of the conversion frequencies for these three alleles; 3) centromere distances and linkage relationships of ascospore color mutants; and 4) linkage relationships of morphological mutants to the indigo-locus (i-locus).

The three grey alleles were spontanious mutants, and the two hyaline mutants were induced with ICR 170. All were isolated and maintained by Kitani. The morphological mutants tested in the analysis of linkage relationship to the i-locus were of two groups: Dwarf or restricted growth mutants or spore shape mutants in the first group of 34 are listed in the Sordaria Stock List (Neurospora Newsletter 29:122-128, 1982). Mutants in a second group of 43 have been induced by Kitani with nitug/mt rosoguanidine. All the color mutants tested in the linkage analysis are listed in the Sordaria Stock List.

Determinations of both allelic relationship and conversion frequency (including conversion spectrum) of the five ascospore color mants were done under the microscope. The constitution of conversion asci was confirmed by testing progeny obtained by micro-manipulation.

For the ascospore color mutant linkage analysis, ascus types were mostly determined under the microscope, and asci were dissected only when necessary to resolve an uncertainty. For the testing of morphological mutants to the i-locus, all the asci were dissected by micromanipulation, and the phenotypes of progeny were determined after cultivation.

All other procedures were as in previous reports (El-Ani, Olive and Kitani 1961 Am. J. Bot. 48:716-723, Kitani 1978 Genetics 89:467-497, Kitani 1978 Jpn. J. Genet. 53:301-308, Kitani 1982 Jpn. J. Genet. 57:467-481, Kitani and Olive 1967 Genetics 57:767-782, Kitani and Olive 1969 Genetics 62:23-66, Kitani and Olive 1970 Proc. Natl. Acad. Sci. 66:1290-1297 and Kitani, Olive and El-Ani 1962 Am. J. Bot. 49:697-706).

Results

1. Confirmation of five alleles in the g-locus. Lack of recombinant asci was confirmed in cross of g1 x h11, g1 x h12, h5 x g8, h5 x g9 and h5 x g10.

2. Determination of conversion frequencies and conversion spectra of the new g-locus alleles. The results are shown in Table 1. The conversion frequencies of the five new alleles appeared virtually the same as previously determined for other g-locus alleles, about 2 x 10^-3. Conversion spectra of the three new grey alleles followed the grey alleles -- a relatively small proportion of the Aberrant 4:4 type and large proportion of ascus types having excess wild type spores. Conversion spectra of the two hyaline alleles also followed the general tendency of hyaline alleles -- large proportions were of the Aberrant 4:4 and the 5:3 types.

3. Centromere distances and linkage relationships of ascospore color mutants. Centromere distances are useful to know and are readily determined for ascospore mutants. First- (MI) and second- (MII) division segregation frequencies are shown in Table 2. Asci were scored microscopically without isolating progeny unless confirmation was necessary or stocks were desired. As has been generally true of ascospore color mutants in S. fimicola, all tested mutants appeared far from the respective centromeres. Only when the proportion of MII asci is below 67% is it useful to express the centromere distance in Morgan units; otherwise, the distance is shown as >30 in the table. The centromere distance for the g-locus is based on a very large number of asci from this and previous work (see El-Ani, Olive and Kitani 1961 Am. J. Bot. 48:716-723).

Linkage relationships of the 12 ascospore color mutants are shown in Fig. 1. The three numbers given in each box of the figure represent the numbers of asci that were parental ditype (PD), tetratype (T) and nonparental ditype (NPD). Ratios of PD to NPD indicate whether linkage exists: when the ratio is near 1:1, the genes are unlinked, and when PDs are significantly in excess of NPDs, the genes are linked. When linked, the frequency of the T type is proportional to the distance between genes.

The number of tetratype asci is not given in a box of the figure when a 1:1 ratio for PD:NPD indicated absence of linkage.

Linkage maps of two linkage groups have been published previously (El-Ani, Olive and Kitani 1961 Am. J. Bot. 48:716-723). Figure 2 shows current maps for all seven linkage groups.

4. Linkage relationship of morphological mutants to the i-locus. Efforts were made to find flanking markers that would improve the efficiency of conversion analysis at the i-locus (Kitani 1982 Jpn. J. Genet. 57:467-481). As shown in Table 3, all the morphological mutants deposited with FGSC showed no linkage to the i-locus. In this table, r-8, dw-4 and a-14 appeared to be located very close to their respective centromeres.

Three colony morphology mutants out of 43 newly induced ones were linked to the i-locus, as shown in Table 4. Morphological mutants r-22 and dw-18 might well be useful as flanking markers. The other 28 mutants were unlinked, as shown in Table 5. Table 6 gives data for the 12 mutants known to be located near one of the seven centromeres. These mutants would be useless as flanking markers even if linked with the i-locus which is located far from the centromere.

5. Alteration of some allelic symbols in the i-locus. Some mutants deposited with FGSC under different names were later found to be alleles at the i-locus (Kitani 1982 Jpn. J. Genet. 57:467-481). Table 7 shows the revised names.

Table 1. Frequency and spectrum of gene conversion of new alleles in the g-locus

Ascus type	g1	h2	h3	h4	h5	g6	g7	g8*	g9*	g10*	h11*	h12*
6+,2m	76	5	1	0	14	60	37	55	39	57	9	6
5+,2m	95	27	21	8	28	88	67	73	59	71	21	24
Ab 4:4	9	30	48	24	25	15	**23**	**19**	**12**	17	28	33
3+,5m	22	30	59	37	11	31	**27**	**18**	**20**	14	36	46
2+,6m	10	4	10	5	1	5	4	7	13	8	13	7
Conversion frequency at 10^-3	2.03	2.09	2.32	2.28	2.24	2.34	2.17	2.09	2.16	2.12	2.44	2.21

Table 2. Centromere distances of the ascospore color mutants in Sorderia fimicola

	MI	(%)	MII	(%)	Total	Centromere distance
g		(33)		(67)	--	>50
m	3922	(47.0)	4428	(53.0)	8350	26.5
t	1496	(38.1)	2434	(61.9)	3930	30.1
i	1111	(38.3)	1792	(61.7)	2903	30.1
y	1542	(47.6)	1699	(52.4)	3241	26.2
am	1116	(42.9)	1483	(57.1)	2599	28.6
ha	1086	(46.4)	1251	(53.6)	2336	26.8
su	1320	(44.6)	1641	(55.4)	2961	27.7
buff	808	(32.4)	1688	(67.6)	2496	>30
mo	876	(32.8)	**1793**	**(67.2)**	2669	>30
sky	308	(34.2)	**592**	**(65.8)**	900	>30
br-3	438	(37.8)	**721**	**(62.2)**	1159	>30
br-5	861	(35.4)	1568	(64.6)	2429	>30
br-6	777	(35.8)	1359	(64.2)	2172	>30
br-7	1064	(40.1)	1590	(59.9)	2654	>30

Table 3. Tests for linkage of morphological mutants to the i-locus

Gene	Cross	MI/MII		PD : T : NPD
r-1	r-1 x i1	5/4		2 : 6 : 1
r-2a	i1 r-2a x +	4/5		**0 : 7** : 2
r-3	r-3 x blu3	10/29		**6 :32** : 4
r-4	r-4 x blu3	3/11		4 : 8 : 3
r-5	r-5 x i1	4/8		1 : 9 : 2
r-6	r-6 x i1	2/4		0 : 6 : 1
r-7	r-7 x i1	4/6		1 : 6 : 3
r-8	r-8 x i1	36/0	iMI	0 : 12 : 0
			iMII	11 : 0 : 13
r-12	r-12 x i1	8/23		7 : 20 : 4
r-13	r-13 x i1	2/3		1 : 4 : 1
r-14	r-14 x i1	24/39		12 : 43 :10
r-15	r-15 x i1	1/6		**1 : 5** : 1
r-17	r-17 x i1	10/20		**5 :24** : 2
r-18	i1 r-18 x +	6/5		3 : 7 : 1
			iMI	3 : 19 : 4
dw-3	dw-3 x blu3	74/167		41 : 134:32
dw-5	dw-5 x i1	3/6		2 : 4 : 3
dw-7	dw-7 x il	37/0	iMI	0 : 12 : 0
			iMII	13 : 0 : 12

Gene	Cross	MI/MII		PD : T : NPD
halo	x i1	5/28		7 : 23 : 3
dm-3	x i1	4/1		0 : 4 : 1
ba	x i1	25/21		6 : 38 : 4
lo	x i1	2/5		0 : 5 : 2
ro	x i1	6/6		2 : 9 : 1
hym-5	x i1	19/3		3 : 13 : 6
hm-4	x i1	1/10		2 : 7 : 2
dm-4	x i1	41/0	iMI	0 : 24 : 0
			iMII	11 : 0 : 5
api-1	x blu3	31/57		19 : 58 :10
api-2	x i1	11/20		5 : 23 : 3
a-4	x blu3	17/41		7 : 27 :11
a-6	x blu3	3/9		3 : 6 : 3
a-7	x blu3	4/7		1 : 8 : 2
a-8	x blu3	8/16		5 : 16 : 3
a-11	x blu3	2/11		3 : 7 : 3
a-13	x blu3	1/12		4 : 7 : 2
a-14	x blu3	45/0	iMI	22 : 0 : 12
			iMII	0 : 11 : 0

	g	m	t	i	y	am	ha	su	buff	sky	br-3	br-5
g					X	DL	X	X	A	A	X	X
m						DL	X	X	X	X	X	X
t				X	DL	X	DL	X	X	X	X	DL
i			177 375 164		X	X	X	X	X	X	X	L
y	307 532 220		177 396 78	142 324 122		DL	A	X	X	X	X	L
am	278 521 106	93 154 43	174 414 162	235 562 199	143 256 49		X	A?	X	X	L	X
ha	239 419 197	43 93 40	179 386 81	43 154 32	*	128 --- 109		X	DL	X	X	X
su	126 240 101	99 209 69	151 369 128	63 --- 59	68 99 38	*?	163 --- 151		X	X	L	X
buff	*	213 410 144	76 154 60	176 335 161	232 446 131	174 457 113	282 545 135	216 394 130		A	X	X
sky	*	246 460 209	242 408 168	1694 ---- 1397	70 298 63	183 436 162	239 426 218	303 738 279	*		X	X
br-3	117 218 94	85 185 83	165 329 135	178 370 168	180 393 144	383 402 11	153 345 111	1003 211 0	121 236 118	191 415 120		A?
br-5	181 374 138	153 345 102	186 466 99	213 322 55	118 195 18	225 543 206	79 86 37	188 469 126	204 499 198	188 495 115	*?	

Figure 1. Numbers of Parental ditype (upper left), Tetratype (middle), and Nonparental ditype (lower right) asci, and linkage relationships of ascospore color mutants.
A : allelic L : linked DL : distantly linked? X : unlinked
* : recombination rare *? : allelic or epistasis of mutant over wild type

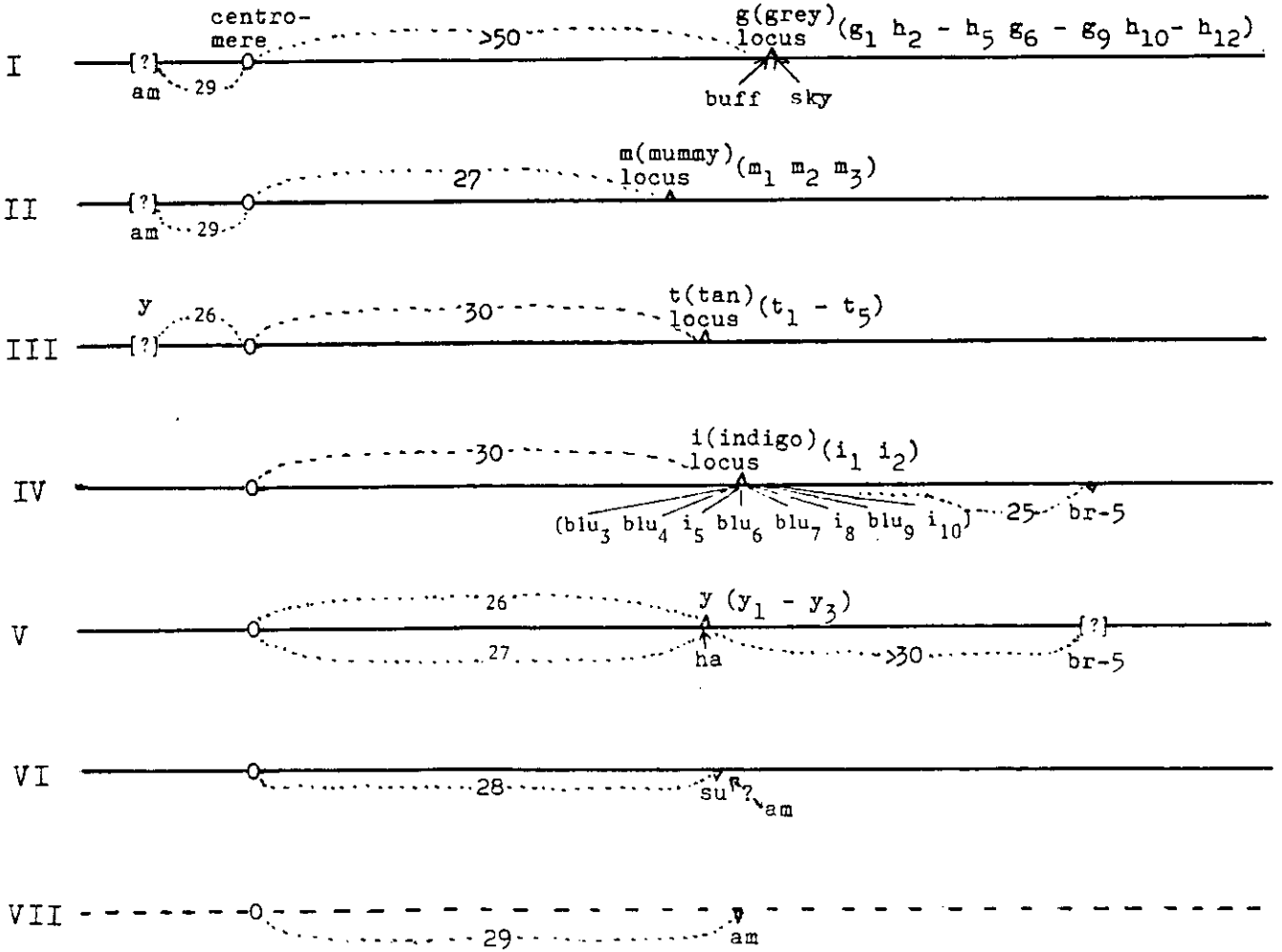

Figure 2. Seven chromosome linkage map of Sordaria fimicola

Table 4. Linkage of colony morphology mutants to the i-locus

Gene	PD	:	T	:	NPD	MI/MII
r-22	25		64		2	15 30
r-36	34		96		14	-
dw-18	36		113		9	-

Table 5. Nonlinkage of colony morphology mutants to the i-locus

Gene	PD	:	T	:	NPD	Total
r-21	0		6		2	8
r-23	0		7		1	8
dw-8	1		3		3	7
dw-9	0		5		3	8
dw-10	1		5		2	8
r-26	6		14		3	23
r-27	**1**		**5**		**1**	**7**
r-30	**5**		**14**		**3**	**22**
r-32	**1**		**6**		**1**	**8**
r-33	**1**		**12**		**3**	**16**
r-34	**6**		**24**		**11**	**41**
dw-11	**1**		**6**		**1**	**8**
dw-12	1		6		1	8
dw-14	1		6		1	8
dw-15	1		4		2	7
dw-16	3		13		1	17
dw-17	8		27		3	38
r-38	2		9		5	16
r-39	1		4		3	8
r-40	2		8		0	10
r-42	0		6		1	7
r-43	6		17		3	26
dw-20	3		10		3	16
dw-21	1		11		3	15
r-44	8		24		8	40
r-45	5		6		4	15
dw-22	2		5		1	8
dw-23	**2**		**5**		**1**	**8**

Table 6. Ascus type distribution of colony morphology mutants close to a centromere

Gene	PD (i = MII)	:	T (i = MII)	:	NPD (i = MII)	P (i = MI)	:	T (i = MI)	:	NPD (i = MI)
r-19	0		8		0	---				
r-20	0		8		0	---				
r-24	0		8		0	---				
r-25	0		8		0	---				
r-28	0		8		0	---				
r-29	0		8		0	---				
r-31	0		8		0	6	0			9
dw-13	0		8		0	---				
r-35	0		8		0	---				
r-37	0		8		0	---				
r-41	0		7		0	---				
dw-19	0		8		0	---				

Table 7. Revised symbols for i-locus allele

present name	Former name and FGSC no.	
i1	i1	(2811)
i2	i2	(2812)
i5	i5	(2815)
i8	gr	(2840)
i10	ny	(2841)
blu3	i3	(2813)
blu4	blu4	(2814)
blu6	bg	(2839)
blu7	blu7	(2817)
blu9	cy	(2836)

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