LINKAGE MAPS IN CHLAMYDOMONAS REINHARDI[1]

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SEVERAL examples of linkage in *Chlamydomonas reinhardi* have been reported (EVERSOLE 1956; EBERSOLD 1956; EBERSOLD and LEVINE 1959). From studies using linked loci it has been shown that crossing-over occurs at the four-strand stage and that chiasma interference is positive at least in the one chromosome arm where data are conclusive (EBERSOLD and LEVINE 1959). No information has been available, however, regarding the number of linkage groups. The purpose of the present work has been to determine the number of linkage groups and to construct genetic maps to be used as a basis for further genetic investigations.

The mutant loci in 45 independently isolated mutant strains, the mating-type locus, and the centromeres have been mapped in 11 linkage groups. Six of these groups are clearly independent. Data are still insufficient to exclude the possibility that two or more of the remaining groups may represent genes in the same group.

MATERIALS AND METHODS

Wild-type cultures of *C. reinhardi* (strain 137c) were obtained from the late G. M. SMITH. The mutant strains used in this investigation were obtained following ultraviolet irradiation or alloxan treatment (*thi-4a*, *4b*, and *8*) of wild-type cells. The majority of these mutant strains, which are listed in Table 1, were isolated by R. A. EVERSOLE (1956) and are prefixed EV. Unfortunately, several of the mutants described by him were lost before the present work began, and only those strains listed in Table 1 are still extant. All of the paralyzed-flagella mutants, prefixed L, were isolated and made available to us by R. A. LEWIN. All remaining were isolated by LEVINE or EBERSOLD and are prefixed H and E, respectively.

The methods used for maintenance of cultures, mating, and tetrad analysis were identical to those described previously (EBERSOLD and LEVINE 1959).

For growth of nutritional mutants, minimal medium (LEVINE and EBERSOLD 1958) was supplemented as follows: for the arginine mutants, 10 μg/ml L-arginine HCl; for the thiamine mutants, 1.0 μg/ml thiamine HCl; for the acetate mutants, 2.0 mg/ml sodium acetate; for the nicotinic mutants, 0.75 μg/ml nicotinamide; and for the para-aminobenzoic mutants, 0.5 μg/ml *p*-aminobenzoic

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TABLE 1
Index of mutant strains

Isolation number	Locus symbol, name, and linkage group	Remarks and references
EV1a	*ac-1:* acetate-1 (VII)	EVERSOLE 1956. Colonies almost white.
H8	*ac-12:* acetate-12 (II)	
EV60	*ac-12(ac-12a):* acetate-12a (II)	Obtained as an unknown from R. A. EVERSOLE. Allelic or closely linked to *ac-12.*
EV164c	*ac-12(ac-12b):* acetate-12b (II)	EVERSOLE 1956. Originally designated *ac-164c* Allelic or closely linked to *ac-12* and *ac-12a.*
H21	*ac-14:* acetate-14 (I)	
H12	*ac-14(ac-14a):* acetate-14a (I)	Allelic or closely linked to *ac-14.*
H59	*ac-14(ac-14b):* acetate-14b (I)	Allelic or closely linked to *ac-14* and *ac-14a.*
H9	*ac-15:* acetate-15 (IX)	
EV16	*ac-16:* acetate-16 (X)	Obtained as an unknown from R. A. EVERSOLE.
H11	*ac-17:* acetate-17 (III)	
H15	*ac-21:* acetate-21 (XI)	LEVINE 1960. Colonies small and light colored.
H13	*ac-28:* acetate-28 (III)	
H14	*ac-29:* acetate-29 (VI)	Colonies yellow.
EV31	*ac-31:* acetate-31 (V)	Obtained as an unknown from R. A. EVERSOLE. Colonies yellow.
H10	*ac-31(ac-31a):* acetate-31a (V)	Colonies yellow. Allelic or closely linked to *ac-31.*
EV51	*ac-51:* acetate-51 (IX)	Obtained as an unknown from R. A. EVERSOLE.
H70	*ac-76:* acetate-76 (I)	
EV157b	*ac-157:* acetate-157 (VIII)	EVERSOLE 1956.
E23	*arg-1:* arginine-1 (I)	EBERSOLD 1956. Uses arginine, citrulline, or ornithine.
EV89	*arg-2:* arginine-2 (I)	EVERSOLE 1956. Uses arginine only.
EV1	*nic-1:* nicotinic-1	EVERSOLE 1956. Linkage group not known. Not linked to any of the other *nic* loci.
EV37	*nic-2:* nicotinic-2 (II)	EVERSOLE 1956.
EV43	*nic-2(nic-2a):* nicotinic-2a (II)	EVERSOLE 1956. Originally designated *nic-3.* Allelic or closely linked to *nic-2.*
EV160a	*nic-7:* nicotinic-7 (VI)	EVERSOLE 1956.
EV169b	*nic-7(nic-7a):* nicotinic-7a (VI)	EVERSOLE 1956. Originally designated *nic-8.* Allelic or closely linked to *nic-7.*
EV10	*nic-11:* nicotinic-11 (IV)	Obtained as an unknown from R. A. EVERSOLE.
H6	(VI)	Requires nicotinamide.
H7	(VI)	Requires nicotinamide.
EV30	*pab-1:* para-aminobenzoic-1 (III)	EVERSOLE 1956.
EV209e	*pab-1(pab-1a):* para-aminobenzoic-1a (III)	EVERSOLE 1956. Originally designated *pab-3.* Allelic or closely linked to *pab-1.*
EV208h	*pab-2:* para-aminobenzoic-a (I)	EVERSOLE 1956.
L1	*pf-1:* paralyzed-1 (V)	
L2	*pf-2:* paralyzed-2 (XI)	
L12	*pf-12:* paralyzed-12 (II)	
L13	*pf-13:* paralyzed-13 (IX)	
L14	*pf-14:* paralyzed-14 (VI)	
L15	*pf-15:* paralyzed-15 (III)	
L16	*pf-16:* paralyzed-16 (IX)	
L17	*pf-17:* paralyzed-17 (VII)	
L18	*pf-18:* paralyzed-18 (II)	

TABLE 1—Continued
Index of mutant strains

Isolation number	Locus symbol, name, and linkage group	Remarks and references
L19	*pf-19:* paralyzed-19 (X)	
L20	*pf-20:* paralyzed-20 (IV)	
EV5	*thi-1:* thiamine-1 (VIII)	EVERSOLE 1956. Uses thiamine only.
EV169c	*thi-2:* thiamine-2 (III)	EVERSOLE 1956. Requires thiazole plus pyrimidine.
EV187g	*thi-3:* thiamine-3 (I)	EVERSOLE 1956. Uses thiazole.
EV193c	*thi-4:* thiamine-4 (IV)	EVERSOLE 1956. Uses thiazole.
H1	*thi-4(thi-4a):* thiamine-4a (IV)	Uses thiazole. Allelic or closely linked to *thi-4.*
H2	*thi-4(thi-4b):* thiamine-4b (IV)	Uses thiazole. Allelic or closely linked to *thi-4* and *thi-4a.*
H3	*thi-8:* thiamine-8 (V)	Uses pyrimidine.
H5	*thi-10:* thiamine-10 (VI)	Requirement not determined.
	mt(mt$^+$ or *mt*$^-$*):* mating type (VI)	

acid. Since all of the vitamin mutants grew to some extent on minimal medium, the various test media used for scoring were supplemented with growth-factor analogs at concentrations which selectively inhibited the growth of mutant but not of wild-type cells. For selective inhibition of the para-aminobenzoic mutants, 1 μg/ml sulfanilamide was added to the test media; for the nicotinic mutants, 7.5 μg/ml 3-acetyl pyridine was used. The analogs used for selective inhibition of the thiamine mutants depended upon the specific requirement of each mutant strain (see Table 1). For *thi-1* 5.0 μg/ml oxythiamine was added, and for *thi-3* 0.01 μg/ml oxythiamine plus 1.0 μg/ml pyrithiamine was added, and for *thi-8* 50 μg/ml oxythiamine was added to the test media. Selective inhibition of *thi-2*, *thi-4*, *thi-4a*, and *thi-4b* was not obtained with various concentrations of the two analogs or with either analog alone. It has been found recently that certain lots of oxythiamine are ineffective as inhibitors of growth of the thiamine mutants. The concentrations listed above were those used with oxythiamine obtained from the California Corporation for Biochemical Research.

All paralyzed-flagella mutants were scored unequivocally by simply placing a small drop of water on a colony and observing whether the cells become motile.

Scoring for mating type was accomplished in the following manner: Cells of unknown sex were suspended in 0.3 ml water in each of two tubes. After illumination for two hours a drop of *mt*$^+$ cells was added to one tube and a drop of *mt*$^-$ cells to the other. Both suspensions were then plated on minimal agar medium. After 24 hours the plates were examined for the presence of zygotes. This procedure is more time consuming than the method described by SAGER (1955). However it has the advantage that colonies which might consist of cells of both mating types can be detected, especially when only a very few cells of one mating type are present.

EXPERIMENTAL RESULTS

The crosses listed in Tables 2, 3, 4, and 5 were selected from approximately

TABLE 2

Three-point and four-point crosses

Cross number	Zygote genotype and recombination percent	Linkage groups	Observed tetrad numbers Parental ditype:Non-parental ditype:Tetratype for gene pairs					
			AB	AC	BC	AD	BD	CD
1.	A *arg-1* / + — 5.2 — B *arg-2* / + — 15.4 — C *pab-2* / + — 29.9 — D *thi-3* / +	I	From EBERSOLD and LEVINE (1959)					
2.	+ / *ac-14* — 3.1 — + / *arg-2* — 17.3 — *pab-2* / +	I	46: 0: 3	30: 1:18	33: 1:15	. . . . .	. . . . .	. . . . .
3.	+ / *ac-31* — 3.7 — *thi-8* / + — 33.6 — + / *pf-1*	V	88: 1:10 106: 0:10 107: 0: 4 86: 0: 5	29: 1:69 34: 0:82 29: 0:83 21: 0:70	38: 0:61 43: 0:73 32: 0:79 25: 1:65	. . . . .	. . . . .	. . . . .
4.	c* *arg-2* / + — 18.2 — *pab-2* / + — 15.1 — c + / *ac-31* — 12.2	I, V	88: 0:38	30:36:60	19:23:84	. . . . .	. . . . .	. . . . .
5.	c *arg-1* / + — 11.1 — c + / *ac-12b* — 15.0 — *nic-2* / + — 35.9 — c *pab-1* / + — 5.9	I, II, III	43:47:66	30:21:105	54:10:92	60:49:47	54:45:57	25:18:113
6.	c *arg-1* / + — 10.9 — *nic-2* / + — c *pab-1* / + — 6.5 — c + / *ac-31* — 14.7	I, II, III, V	7: 5:36	23:10:15	6: 4:38	14:14:20	8: 6:34	18:12:18
7.	*arg-1* / + — *nic-2* / + — *pab-1* / + — + / *ac-16*	I, II, III, X	8: 5:20	8:14:11	5: 5: 23	6:12:15	3: 6:24	6:10:17

TABLE 2—Continued

Three-point and four-point crosses

Cross number	Zygote genotype and recombination percent	Linkage groups	Observed tetrad numbers Parental ditype:Non-parental ditype:Tetratype for gene pairs AB	AC	BC	AD	BD	CD
8.	c *pab-1* / + — 6.5 — c / *ac-31* — 14.2 — + / *pf-1* — 20.4	III, V	41:36:43	31:34:55	37: 0:83	. . . . .	. . . . .	. . . . .
9.	*nic-11* c / + — 1.1 — + / *thi-4* — 6.8 — c + / *pf-17* — 2.2	IV, VII	128: 0:24	76:66:10	71:55:26	. . . . .	. . . . .	. . . . .
10.	*nic-11* c / + — 2.5 — c *mt*+ / *mt*− — 23.6 — c + / *pf-17* — 1.2	IV, VI, VII	29:37:58	65:50: 9	34:33:57	. . . . .	. . . . .	. . . . .
11.	c + / *thi-4* — 5.6 — c *nic-7* / + — 24.6 — c + / *pf-17* — 5.6	IV, VI, VII	34:28:68	47:56:27	36:26:68	. . . . .	. . . . .	. . . . .
12.	*nic-11* c / + — 1.2 — c *pf-17* / + — 3.7 — c + / *thi-1* — 18.6	IV, VII, VIII	59:64:13	47:37:52	47:34:55	. . . . .	. . . . .	. . . . .
12a.	*ac-31* c / + — 13.3 — *pf-1* / + — 22.1 — c + / *thi-1* — 15.8	V, VIII	28: 0:70	28:25:45	20:24:54	. . . . .	. . . . .	. . . . .

* c=centromere.

TABLE 3

Two-point crosses showing linkage

Cross number	Cross	Linkage group	Observed number of tetrads PD:NPD:T	Recombination percent
13	*arg-2* × *ac-76*	I	77: 0:12	6.7
13a	*arg-1* × *ac-76*	I	85: 0: 3	1.7
14	*ac-14* × *ac-14a*	I	48: 0: 0	0
15	*ac-14* × *ac-14b*	I	53: 0: 0	0
16	*ac-12a* × *nic-2*	II	36: 7:88	33.5
17	*ac-12* × *pf-12*	II	74: 0:22	11.5
18	*ac-12* × *pf-18*	II	66: 1:45	20.9
19	*pf-12* × *pf-18*	II	84: 0:17	8.4
20	*pf-12* × *nic-2*	II	54: 0:45	22.7
21	*pf-18* × *nic-2*	II	93: 0:18	8.1
22	*ac-12* × *ac-12a*	II	83: 0: 0	0
23	*ac-12a* × *ac-12b*	II	41: 0: 0	0
24	*nic-2* × *nic-2a*	II	600: 0: 0	0
25	*pab-1* × *pf-15*	III	37: 0:27	21.1
26	*pab-1* × *thi-2*	III	36: 2:47	30.0
27	*ac-17* × *pf-15*	III	41: 1:63	31.0
28	*ac-28* × *pf-15*	III	88: 0: 1	0.6
29	*thi-2* × *pf-15*	III	Does not show linkage. Data lost.	
30	*pab-1* × *pab-1a*	III	400: 0: 0	0
31	*thi-4* × *pf-20*	IV	37: 0:55	29.9
32	*thi-4a* × *pf-20*	IV	39: 0:54	29.0
33	*nic-11* × *pf-20*	IV	25: 0:63	35.8
34	*thi-4* × *thi-4a*	IV	117: 0: 0	0
35	*thi-4a* × *thi-4b*	IV	113: 0: 0	0
36	*ac-31a* × *ac-31*	V	98: 0: 0	0
37	*nic-7* × wild type	VI	>100. No recombination with mating type.	
38	*nic-7a* × wild type	VI	>100. No recombination with mating type.	
38a	*nic-7* × *pf-14*	VI	27: 1:70	36.7
39	*pf-17* × *ac-1*	VII	101: 0:10	4.5
40	*thi-1* × *ac-157*	VIII	65: 1:38	19.2
41	*ac-51* × *pf-16*	IX	65: 0:41	19.3
42	*pf-16* × *pf-13*	IX	26: 1:70	37.1
43	*ac-15* × *pf-13*	IX	69: 0:30	15.2
44	*ac-15* × *pf-16*	IX	48: 0:55	26.7
45	*ac-15* × *ac-51*	IX	25:10:66 (P=0.012)	42.7
46	*pf-13* × *ac-51*	IX	6: 7:66	Does not show linkage.
47	*ac-16* × *pf-19*	X	203: 0: 3	0.7
48	*ac-21* × *pf-2*	XI	25: 0:26	25.5

250 crosses from which more than 20,000 tetrads were analyzed. The data presented are only those necessary for establishing linkage or nonlinkage and mapping centromeres. The results from more than 100 two-point crosses involving mutant strains listed in Table 1 have been omitted. The data from these crosses confirm the conclusions drawn below, but do not provide necessary additional information. From each of the crosses listed, at least 90 percent of the

TABLE 4

Two-point cross data used for determining second division segregation frequencies and demonstrating nonlinkage

Cross number	Cross	Linkage groups	Observed number of tetrads PD:NPD:T	Tetratype frequency	Second division segregation frequency		Recombination percent locus A-cent.
	Locus A Locus B				Locus A*	Locus B†	
49	*thi-2* × *ac-12b*	III, II	18:22:51	0.560	0.473	0.300	23.6
50	*thi-2* × *thi-4*	III, IV	23:17:44	0.524	0.487	0.136	24.3
51	*pf-15* × *pf-17*	III, VII	22:23:46	0.535	0.519	0.074	25.9
52	*ac-17* × *pf-17*	III, VII	54:53: 9	0.078	0.004	0.074	< 0.1
53	*pf-14* × *pab-1*	VI, III	32:39:34	0.324	0.242	0.129	12.1
54	*pf-14* × *ac-31*	VI, V	28:23:33	0.393	0.189	0.284	9.4
55	*ac-15* × *pf-17*	IX, VII	42:30:36	0.333	0.291	0.074	14.6
56	*pf-16* × *pf-17*	IX, VII	30:33:32	0.337	0.296	0.074	14.8
57	*pf-16* × *pab-1*	IX, III	34:33:24	0.264	0.167	0.129	8.4
58	*pf-16* × *nic-11*	IX, IV	36:37:40	0.354	0.342	0.024	17.1
59	*pf-13* × *pab-1*	IX, III	26:30:51	0.477	0.432	0.129	21.6
60	*pf-13* × *nic-11*	IX, IV	21:22:44	0.506	0.500	0.024	25.0
61	*ac-51* × *pf-17*	IX, VII	14: 8:76	0.776	....	....	>33.3
62	*pf-19* × *pf-17*	X, VII	31:33:40	0.385	0.349	0.074	17.4
63	*ac-16* × *thi-4*	X, IV	38:30:57	0.456	0.403	0.136	20.2
64	*ac-16* × *nic-11*	X, IV	18:17:24	0.407	0.424	0.024	21.2
65	*ac-16* × *ac-31*	X, V	37:27:54	0.458	0.303	0.284	15.2
66	*pf-2* × *pab-1*	XI, III	36:32:18	0.209	0.099	0.129	4.9
67	*pf-2* × *thi-4*	XI, IV	41:36:17	0.188	0.065	0.136	3.3
68	*pf-2* × *pf-17*	XI, VII	39:32: 6	0.078	0.004	0.074	< 0.1
69	*ac-21* × *pf-17*	XI, VII	8:12:32	0.615	0.609	0.074	30.5
70	*ac-1* × *pf-15*	VII, III	13: 6:37	0.661‡	....	....	...
71	*ac-1* × *pf-19*	VII, X	35:33:34	0.393‡	....	....	...
72	*ac-157* × *ac-17*	VIII, III	43:44:13	0.130	0.127‡	0.004	6.4

* Second division segregation frequency for locus A was calculated using the formula $T = a + b - \frac{3ab}{2}$, where T is the tetratype frequency, and a and b are the second division segregation frequencies for loci a and b, respectively (PERKINS 1949).

† In crosses 49–69 the second division segregation frequency for locus B was determined from data presented in Table 2.

‡ See text for explanation.

zygotes isolated gave rise to complete tetrads. In addition, data are included from tetrads in which one product was missing. The results from multiple-point crosses are presented in Table 2, those from two-point crosses showing linkage in Table 3, and those from two-point crosses indicating nonlinkage in Tables 4 and 5. The results are summarized in the form of genetic maps in Figure 1.

Linkage: From a diploid zygote nucleus heterozygous for two loci, three tetrad types are possible, *parental ditype* (PD, 0/4 strands recombinant), *nonparental ditype* (NPD, 4/4 strands recombinant), and tetratype (T, 2/4 strands recombinant). PD and NPD tetrads are expected in equal frequency when two loci segregate independently of one another. On the other hand, if the loci are linked the simplest crossover pattern resulting in a NPD segregation is a four-strand double exchange between the two loci. Therefore, linkage of two loci is indicated when the PD:NPD ratio is significantly greater than 1:1 (PERKINS 1953).

In the first demonstration of linkage in *C. reinhardi* five loci and the cen-

TABLE 5

Additional data from two-point crosses demonstrating independence of linkage groups

Cross number	Cross	Linkage groups	Observed number of tetrads PD:NPD:T
73	*arg-1* × *thi-4*	I, IV	9: 9: 7
74	*arg-1* × *pf-14*	I, VI	9:17:34
75	*arg-2* × *pf-17*	I, VII	24:18:45
76	*arg-1* × *thi-1*	I, VIII	9: 7:13
77	*arg-1* × *pf-16*	I, IX	17:22:33
78	*arg-1* × *pf-2*	I, XI	25:20:21
79	*ac-12b* × *thi-4*	II, IV	26:31:23
80	*ac-12b* × *ac-31*	II, V	12:16:18
81	*ac-12* × *pf-14*	II, VI	26:28:57
82	*ac-12* × *pf-17*	II, VII	38:46:32
83	*ac-12b* × *thi-1*	II, VIII	25:18:37
84	*ac-12* × *pf-16*	II, IX	27:37:33
85	*ac-12b* × *ac-16*	II, X	12: 9:20
85a	*ac-12* × *ac-16*	II, X	19:11:16
86	*ac-12a* × *pf-2*	II, XI	31:22:30
87	*pab-1* × *thi-4*	III, IV	20:29: 9
88	*thi-2* × *thi-4*	III, IV	23:17:44
89	*pab-1* × *nic-7a*	III, VI	24:20:37
90	*pab-1* × *pf-17*	III, VII	35:40:12
91	*pab-1* × *thi-1*	III, VIII	16:21:22
92	*pf-15* × *ac-157*	III, VIII	14:28:72
93	*nic-11* × *ac-31*	IV, V	24:21:20
94	*thi-4* × *ac-31*	IV, V	16:12:32
95	*pf-20* × *thi-1*	IV, VIII	11:12:51
96	*thi-8* × *pf-14*	V, VI	33:33:42
97	*ac-31* × *nic-7a*	V, VI	13:14:70
98	*thi-8* × *pf-17*	V, VII	34:35:25
99	*ac-31* × *pf-16*	V, IX	36:29:48
100	*ac-31* × *pf-13*	V, IX	24:24:61
101	*thi-8* × *pf-19*	V, X	35:35:34
102	*thi-8* × *pf-2*	V, XI	44:40:31
103	*pf-14* × *thi-1*	VI, VIII	23:18:27
104	*nic-7* × *thi-1*	VI, VIII	19:13:60
105	*pf-14* × *ac-15*	VI, IX	32:42:42
106	*nic-7* × *pf-13*	VI, IX	16:14:49
107	*nic-7* × *pf-19*	VI, X	20:21:69
108	*pf-14* × *pf-2*	VI, XI	29:38:15
109	*thi-1* × *pf-16*	VIII, IX	24:27:63
110	*thi-1* × *pf-13*	VIII, IX	16:18:64
111	*thi-1* × *pf-19*	VIII, X	33:22:60
112	*thi-1* × *pf-2*	VIII, XI	20:24:31
113	*pf-16* × *ac-16*	IX, X	21:20:34
114	*pf-16* × *pf-2*	IX, XI	36:49:24
115	*pf-19* × *pf-2*	X, XI	45:43:22

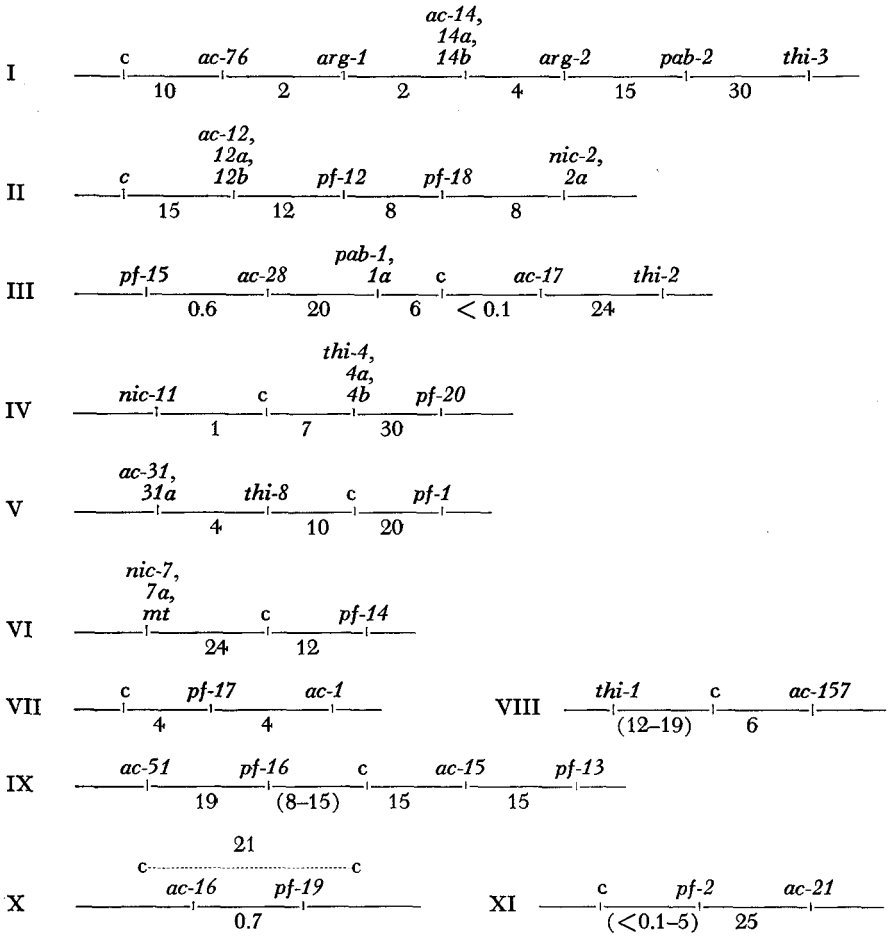

FIGURE 1.—Genetic maps of the eleven linkage groups of *C. reinhardi* based on data from Tables 2, 3, and 4. The centromeres are represented by the letter c.

tromeres were mapped in two linkage groups (EBERSOLD 1954, 1956). Unfortunately, loss of two of these original markers (*pgd* and *slo*) and germination or scoring difficulties with two others (*na* (*ac-5*) and *lg* (*ac-8*)) have prevented their inclusion in the present maps. Subsequently, linkage of *arg-1* to *arg-2* and *thi-1* to *ac-157* was established by EVERSOLE (1956). He also provided evidence for close linkage or allelism of *nic-2* and *nic-2a*.

Gene order in the linkage groups comprising more than two mutant loci was determined directly from three-point and four-point crosses in only two instances (linkage groups I and V). In linkage group I, *ac-76* was localized from two-point data as were the loci in linkage groups II, III, IV, and IX. It is obvious that the validity of combining two-point values for map construction depends in large part on the extent to which recombination frequencies vary from cross to cross. The amount of variation in recombination frequency between several linked loci

can be read directly in Table 2. On the basis of the consistency of these values, we believe that gene sequences determined by combining two-point data are valid.

In addition to the example provided by EVERSOLE, evidence for allelism or close linkage has been found for 15 independently isolated mutant strains at six different loci. Two additional mutant strains, *nic-7* and *nic-7a*, both derived from wild-type mt^+, have shown no recombination with mt^+ from the analysis of over 100 tetrads of each mutant × wild type. In all other cases, close linkage or allelism was indicated by the absence of wild-type recombinants from intercrosses between recurrent mutant strains. Data obtained more recently indicate that *ac-29* is also closely linked to the mating-type locus, since recombinants with mt^+ have not been found and since *ac-29* is 40.5 map units from *pf-14*. H6, H7 (both require nicotinamide), and H5 (*thi-10*) are also approximately 40 map units from *pf-14*, but at the present time it is not known whether they are closely linked to the mating-type locus or to *nic-7*.

Independence of linkage groups: Nonlinkage is indicated by a ratio of 1PD:1NPD or by a deviation significantly greater than 1NPD:4T (PERKINS 1953). The rationale on which the second criterion is based is discussed by BARRATT, NEWMEYER, PERKINS and GARNJOBST (1954, p. 79). Applying these criteria to the data presented in Tables 2, 4, and 5, 11 separate linkage groups have been defined. Whenever possible, crosses involving loci situated close to their centromeres (see below) were selected for presentation. The effect of such a selection is to increase the sensitivity and the reliability of this method of establishing independence (BARRATT *et al.* 1954). The probability that the observed PD:NPD ratios do not deviate significantly from 1:1 and the probability that the observed NPD:T ratios represent a random deviation in excess of 1:4 for each of the 55 possible combinations have been determined. These values are listed in Table 6.

Centromere location: Second division segregation (SDS) frequencies for at least one locus in each linkage group, except IX, X, and XI, were determined from three-point and four-point crosses using the method of WHITEHOUSE (1957). It has been our experience with unordered tetrads that SDS frequency values are more consistent when the loci involved are all represented in the same cross rather than in a series of two-point crosses. Moreover, the values are, in general, reproducible only with data obtained from 100 or more tetrads. It can be seen (Table 2) that the recombination percent (1/2 SDS frequency × 100) between several loci (*arg-1*, *pab-1*, *ac-31*, *thi-4*, and *pf-17*) and their respective centromeres is reasonably consistent from cross to cross. SDS frequencies for these loci and for *ac-12* and *nic-11* calculated from data listed in Table 2 were used as the basis for determining SDS frequencies for loci which have been involved only in two-point crosses (crosses 49–69, Table 4). Using the information thus obtained in combination with the results presented in Tables 2 and 3, the centromeres can be mapped in linkage groups I, II, III, IV, V, VI, IX, and XI. Centromeres of groups VII, VIII, and X have also been mapped as discussed below. In linkage groups I, II, IV, and V the position of the centromeres is probably more reliable than in the other groups since the SDS frequency for at least two loci in each of these groups was determined from three-point and four-

TABLE 6

Independence of linkage groups

Linkage group	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	..	<0.001	<0.001	<0.001	<0.001	<0.02	<0.09	<0.1	<0.001	<0.002	<0.001
II	>0.7 (5)	..	<0.001	<0.001	>0.4	<0.003	<0.001	<0.02	<0.001	<0.02	<0.001
III	>0.3 (5)	>0.3 (5)	..	>0.2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.02	<0.001
IV	1.0 (73)	>0.5 (79)	>0.3 (50)	..	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
V	>0.4 (4)	>0.6 (6)	>0.6 (8)	>0.6 (90)	..	<0.001	<0.001	<0.001	<0.001	<0.003	<0.001
VI	>0.1 (74)	>0.8 (81)	>0.4 (53)	>0.3 (10)	>0.5 (54)	..	<0.001	<0.001	<0.001	>0.4	<0.001
VII	>0.3 (75)	>0.1 (82)	>0.8 (52)	>0.4 (9)	>0.8 (92)	>0.8 (10)	..	<0.001	<0.001	<0.001	<0.001
VIII	>0.6 (76)	>0.2 (83)	>0.8 (72)	>0.2 (12)	>0.1 (93)	>0.4 (97)	>0.1 (12)	..	<0.02	>0.2	<0.001
IX	>0.4 (77)	>0.2 (84)	>0.8 (57)	>0.8 (58)	>0.4 (94)	>0.2 (98)	>0.1 (55)	>0.6 (101)	..	<0.002	<0.001
X	>0.1 (7)	>0.3 (85a)	>0.3 (7)	>0.8 (64)	>0.2 (65)	>0.8 (99)	>0.7 (62)	>0.1 (102)	>0.8 (104)	..	<0.001
XI	>0.4 (78)	>0.2 (86)	>0.6 (66)	>0.6 (67)	>0.6 (96)	>0.2 (100)	>0.4 (68)	>0.5 (103)	>0.1 (105)	>0.7 (106)	..

Upper portion of grid: Probability that NPD:T ratio represents a random deviation in excess of 1:4.
Lower portion of grid: Probability that PD:NPD ratio represents a random deviation from 1:1. Cross numbers from which the values in the table were obtained are in parentheses.

point data rather than from a comparison of SDS frequencies obtained from two-point data.

The position of the centromere with respect to *ac-17* in linkage group III still remains doubtful since the recombination frequencies between the loci in this group are high and since it has not yet been possible to obtain zygotes from a cross involving both *ac-17* and *pab-1*. Placing the centromere to the left of *ac-17* as shown in Figure 1 is, therefore, arbitrary. By comparing the tetratype frequencies from crosses 51, 70 and 62, 71, *ac-1* has been tentatively located distally to *pf-17* with respect to the centromere in linkage group VII. For linkage group VIII, the SDS frequency for *thi-1* was determined from a three-point cross, whereas the SDS frequency for *ac-157* was calculated in a rather indirect manner as follows: The SDS frequency for *pf-17* was used as the basis for calculating the SDS frequency for *ac-17*, which, in turn, was used as the basis for calculating the SDS frequency for *ac-157*. Since both *pf-17* and *ac-17* are very close to their

respective centromeres, the estimate of the SDS frequency for *ac-157* should be reasonably accurate.

DISCUSSION

It can be shown clearly that linkage groups III, IV, V, VI, VIII, and IX are independent of one another. In these groups the centromeres are bounded by loci on both sides, and a representative locus in each group assorts independently of loci located on both sides of the centromere for each of the other groups. It can be shown similarly (although the complete data are not given here) that the above linkage groups are also independent of groups I, II, VII, X, and XI. In these latter groups mutant loci have been localized on only one side of the centromere. The possibility exists that linkage across the centromere has not been detected in certain instances and that there are in reality fewer than 11 linkage groups. Although loci involved in intergroup crosses appear to assort independently, the conditions necessary for making pairwise combinations among these groups have been considered. In no case is fusion possible unless chiasma interference is negative across the centromere. This is true even if it is assumed that $C = 1$ and that positive chromatid interference exists giving rise to only four-strand double exchanges. Assuming an extreme case, that of a double exchange at each meiotic event, fusions can be made only between linkage groups I–II, I–VII, I–X, II–X, and VIII–X. Other fusions are not possible unless positive chromatid interference also exists giving a proportion of NPD segregations greater than 25 percent. Although existing linkage data for loci on opposite sides of the centromere in *C. reinhardi* show no evidence of this type of interference, they do not necessarily rule out its existence in certain chromosomes.

The haploid chromosome number for *Chlamydomonas reinhardi* has been reported variously as 18 ± 2 (SCHAECHTER and DELAMATER 1955), 16 (WETHERELL and KRAUSS 1957), eight (BUFFALOE 1958), and 6–8 (LEVINE and FOLSOME 1959). In a series of photographs of mitosis taken at different focal levels in a vegetative cell (LEVINE and FOLSOME 1959), eight chromosomes are clearly distinguishable, but two darkly stained areas near the cell wall can not be resolved either in the photomicrographs or in the original preparation. Although these two areas have been interpreted as representing two chromosomes, one or both areas may actually be more than a single chromosome. The haploid chromosome number in this material, then, is a minimum of eight. The cytological observations of BUFFALOE and of LEVINE and FOLSOME, therefore, are in a range that is consistent with the number of linkage groups determined from the genetic data presented here.

SUMMARY

Linkage studies in *Chlamydomonas reinhardi* were conducted with the mating-type locus and with 45 mutant loci controlling nutritional requirements and flagellar movement. From tetrad data these loci and the centromeres have been located in 11 linkage groups. Six of these groups (with loci mapped on both sides of the centromere) were shown to be independent of one another. Two or more

of the remaining groups (with loci mapped on only one side of the centromere) may represent loci in the same group. The pattern of chiasma and chromatid interference necessary for making fusions between the latter groups is discussed.

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