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Linkage Group XIX of *Chlamydomonas reinhardtii* Has a Linear Map

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ABSTRACT

Linkage group XIX (or the UNI linkage group) of *Chlamydomonas reinhardtii* has been reported to show a circular meiotic recombination map. A circular map predicts the existence of strong chiasma and chromatid interference, which would lead to an excess number of two-strand double crossovers during meiosis. We have tested this prediction in multipoint crosses. Our results are consistent with a linear linkage group that shows positive chiasma interference and no chromatid interference. Chiasma interference occurs both within arms and across the centromere. Of the original loci that contributed to the circular map, we find that two map to other linkage groups and a third cannot be retested because the mutant strain that defined it has been lost. A second reported unusual property for linkage group XIX was the increase in meiotic recombination with increases in temperature during a period that precedes the onset of meiosis. Although we observed changes in recombination frequencies in some intervals on linkage group XIX in crosses to CC-1952, and in strains heterozygous for the mutation *ger1* at 16°, we also show that our strains do not exhibit the previously observed patterns of temperature-sensitive recombination for two different pairs of loci on linkage group XIX. We conclude that linkage group XIX has a linear genetic map that is not significantly different from other Chlamydomonas linkage groups.

A number of unusual genetic properties have been described for linkage group XIX, which has also been referred to as the UNI linkage group, in the biflagellate alga *Chlamydomonas reinhardtii* (Ramanis and Luck 1986; Dutcher 1986). First, nearly all of the identified genes have a mutant phenotype that affects either flagellar assembly or function. Second, meiotic recombination was reported to increase with temperature during a period that precedes the initiation of meiosis. Finally, the meiotic linkage map was reported to be circular (Ramanis and Luck 1986; Dutcher 1986). Among the 10 linkage groups tested, linkage group XIX was the only linkage group to exhibit these properties.

Because of the predominance of flagellar mutations on this linkage group, it was suggested that this linkage group may correspond to a basal body chromosome (Ramanis and Luck 1986; Hall, Ramanis and Luck 1989). While this hypothesis does not require that linkage group XIX be located within the basal body, the question of whether DNA exists within basal bodies has been the subject of debate for some time [reviewed in Fulton (1970) and Johnson and Rosenbaum (1991)]. A basal body location for linkage group XIX is supported by the observation that DNA sequences that map to linkage group XIX hybridize to Chlamydomonas basal bodies *in situ* (Hall, Ramanis and Luck 1989). However, other workers have been unable to detect DNA in Chlamydomonas basal bodies either by 4′,6-diamidino-2-phenylindole (DAPI) fluorescence (Kuroiwa *et al.* 1990) or immunocytochemical methods (Johnson and Rosenbaum 1990). In addition, we have found that the copy number of this linkage group is inconsistent with a basal body location (Johnson and Dutcher 1991).

The reported circularity of linkage group XIX is perhaps its most remarkable property (Ramanis and Luck 1986; Dutcher 1986). Linkage group XIX is the only linkage group in any organism that has been claimed to exhibit both a circular meiotic linkage map and Mendelian segregation. Circular chromosomes have been detected in maize, Drosophila and Saccharomyces (McClintock 1939; Morgan 1933; Klar *et al.* 1983), but they are highly unstable through both meiosis and mitosis and result in elevated levels of inviability among the progeny. This instability results from the formation of dicentric circular molecules during meiotic recombination that break or missegregate during the first division of meiosis (Novitski 1955) or in subsequent mitotic divisions (Haber, Thorburn and Rogers 1984). However, a circular linkage map does not necessarily imply a physically circular chromosome and molecular evidence suggests that linkage group XIX corresponds to a linear molecule (Hall, Ramanis and Luck 1989).

A circular linkage map could be generated for a linear chromosome by restrictions on the number and placement of crossovers on a linear molecule (Stahl

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1967). The restriction on recombination that will generate a circular map from a linear molecule is the same one that is required to keep recombination between two circular molecules from generating a dicentric circular chromosome. Any crossover between two chromatids must be balanced by a second crossover between the same two chromatids elsewhere along the length of the chromosome. Consequently, every pair of linkage group XIX chromatids should be involved in an even (or zero) number of crossover events if its linkage map is circular. Such a coordination of meiotic crossover events has not been described for any other linkage group.

If linkage group XIX has a circular linkage map, then it should exhibit both an excess of double crossovers relative to single crossovers (negative chiasma interference) as well as an excess of two-strand double exchanges over three- and four-strand double exchanges (negative chromatid interference). We have tested the prediction of both negative chiasma and chromatid interference for linkage group XIX. Tetrads from crosses in which three or more linkage group XIX markers can be scored simultaneously were used to estimate the patterns of recombination on this linkage group. We find that the map of linkage group XIX is linear, shows no chromatid interference, and exhibits positive chiasma interference both within arms and across the centromere.

MATERIALS AND METHODS

Chlamydomonas strains: The wild-type strain used was 137c, mt$^+$, and is designated CC-125 by the Chlamydomonas Genetics Center (HARRIS 1989). CC-124 is a *mt*$^-$ strain that was isolated from the same zygote as CC-125. The interfertile strain utilized in the restriction fragment length polymorphism mapping was CC-1952 and is *mt* (GROSS, RANUM, and LEFEBVRE 1988). Cells were grown in medium I as described by SAGER and GRANICK (1953) with the modifications of HOLMES and DUTCHER (1989). Mutant strains were isolated in a 137c background and unless indicated otherwise were maintained in our laboratory stock collection. Drug resistance phenotypes were scored by growth on plates in the following manner: *apm1*, 15 μM oryzalin (JAMES *et al.* 1988); *can1*, 2.8 mM canavanine; *TUN1-3*, 4 μM tunicamycin; *act1*, 64 μM cycloheximide; *ery1*, 136 μM erythromycin; *ani1*, 60 μM anisomycin; *sr1*, 86 μM streptomycin; and *maa4*, 1.16 mM 5-methylanthranilic acid. The auxotrophic markers *arg2* and *arg7* were grown with 2.8 mM arginine HCl and 1 mM ammonium nitrate, which is 1/10 the normal amount. The markers *ac17*, *nic1* and *y1* were scored as described previously (DUTCHER *et al.* 1991).

Flagellar motility defects were scored after a minimum of one day and a maximum of 5 days of growth in constant light in liquid medium supplemented with acetate. When two or more mutations that each have a motility defect were present in a cross the phenotypes of at least three of four meiotic progeny from a tetrad were scored on a light microscope with a 40× phase objective. Cells carrying the *uni1* mutation were identified by the predominance of uniflagellate cells (HUANG *et al.* 1982). The allele *uni1-1* was used in this work because it results in the highest percentage of uniflagellate cells. The *uni1-3* mutation was used in one cross and behaved similarly. Cells carrying the *fla10-14* mutation were indistinguishable from wild-type cells at 21°, but greater than 99% of the cells lost their flagella after 24 to 36 hr at 32° (LUX and DUTCHER 1991). The *pf10* strain shows an abnormal flagellar beat stroke that can be recognized microscopically (DUTCHER, GIBBONS and INWOOD 1988). The *pf27* mutant strain has paralyzed flagella, which only twitch sporadically (HUANG *et al.* 1981). The *pf29* mutant strain has a slow swimming phenotype and cells accumulate near the bottom of the tube (RAMANIS and LUCK 1986). The *pf29* strain was obtained from the Chlamydomonas Genetics Center collection, where it is designated CC-1924, and from D. J. L. LUCK (Rockefeller University). The *sun1* mutation increases the percentage of biflagellate cells in a strain carrying the *uni1* mutation (HUANG *et al.* 1982). The extent of suppression varies considerably with the temperature and the density of the cultures. Consequently, the *sun1* phenotype was always scored twice, at times about 24 hr apart. Except in crosses to determine the linkage between the *sun1* and *uni1* mutations, the *sun1* phenotype was scored in progeny from homozygous *uni1* zygotes. Three strains with the *sun1* and *uni1-1* mutations were used; they were from our stock collection, the Chlamydomonas Genetics Center (CC-1925), and D. J. L. LUCK. Crosses among these three strains gave no recombinants in 213 tetrads. References for the isolation of mutations used in this work can be found in HARRIS (1989), with the exception of the mutant strains *fla20* (DUTCHER 1986), *TUN1-3* (DUTCHER and GIBBONS 1988) and *maa4* (DUTCHER *et al.* 1991, 1992).

Genetic analysis: Standard methods for mating and tetrad dissection were employed (LEVINE and EBERSOLD 1960; HARRIS 1989). Crosses were kept at 21° from time of zygote formation until at least 24 hr after tetrad dissection. The only exceptions were the crosses to test the effects of temperature on recombination, in which the zygotes were placed at 16° or 32° 18 hr after mating. Meiosis was initiated and completed at 21°. Distances between linked markers were calculated by the method of PERKINS (1949). Centromere distances were calculated by the method of WHITEHOUSE (1950) using either *pf27* (on consolidated linkage group XII/XIII) or *y1* (on consolidated linkage group XVI/XVII) or both as centromere markers (DUTCHER *et al.* 1991). In a cross in which *pf10*, *pf27* and *y1* were all present, *pf27* was calculated to be 2 centimorgans (cM) from its centromere as reported previously by BOLDUC, LEE and HUANG (1988). No recombinants between *y1* and its centromere were detected in 290 tetrads from this cross.

Molecular techniques: DNA was isolated as described (JOHNSON and DUTCHER 1991). The 600-bp *Eco*RI *Bgl*I fragment from the plasmid pGullH (JOHNSON and DUTCHER 1991) was used as the hybridization probe for identifying copies of the transposon Gulliver (FERRIS 1989) in genomic blots. The plasmid pAM43 was isolated from a library of size-selected 0.71.3-kb fragments of CC-125 genomic DNA digested with the enzymes *Apa*I and *Sac*I and ligated to the plasmid vector pGEM7Z (Promega), which had been digested with *Apa*I and *Sac*I (JOHNSON 1991). The 700-bp insert in pAM43 hybridizes to a 2.8-kb band on blots of *Pvu*II digested CC-125 DNA and to two bands of 2.3 and 1.3 kb in CC-1952 DNA. Initial assignment of this DNA sequence to linkage group XIX was achieved by observing the retention of the CC-125 pattern in congenic strains that contained most of their genome from the *C. reinhardtii* strain CC-1952. The construction of these congenic strains and their use in identifying DNA sequences on linkage group XIX are described in JOHNSON and DUTCHER (1991). DNA was digested with *Pvu*II, electrophoresed on two separate 1% agarose gels, transferred to nylon membranes and

TABLE 1

Linkage group XIX mapping data

Loci	No. of tetrads[a] PD	NPD	T	Distance (cM)[b]
FLA10 PF10	1040	6	928	24
UNI1 PF10	75	7	562	47
APM1 PF10	86	26	1082	52
UNI1 PF10	504	2	503	26
APM1 FLA10	719	6	952	29
APM1 UNI1	994	0	84	4
FLA11 APM1	121	0	0	<0.5
FLA11 FLA10	61	0	70	27
PF10 CEN[c]	380	383	145	6[c]
FLA10 CEN	318	290	386	19[c]
UNI1 CEN	24	33	162	37[c]
APM1 CEN	49	51	501	42[c]

[a] PD = parental ditype tetrad; NPD = nonparental ditype tetrad; T = tetratype tetrad.

[b] Distances in centimorgans (cM) are calculated using [(T + 6NPD)/(PD + NPD + T)] × 50 (PERKINS 1949).

[c] The centromere of linkage group XIX was monitored using *PF27*, which is a locus on linkage group XII/XIII 2 cM from its centromere. Substituting this value into the equation of Whitehouse (1950), centromere distances are calculated from the formula: [(T/PD + NPD + T) − 0.04)/0.94] × 50.

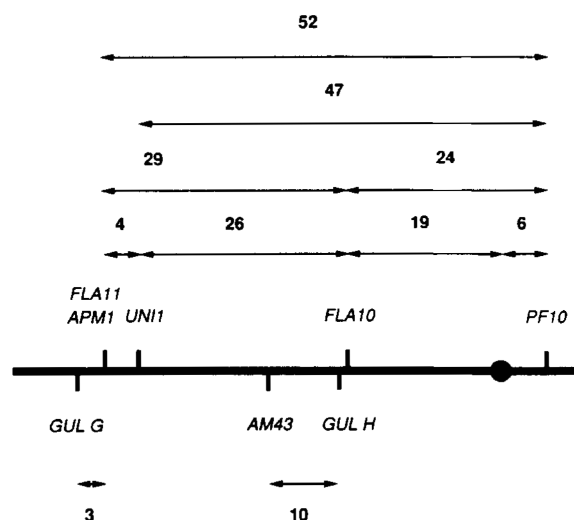

FIGURE 1.—Map of linkage group XIX. The phenotypic markers are presented above the line. The distances between loci are in centimorgans and are from crosses of strains derived from the CC-125 parent. The data are from Table 1. The molecular markers are presented below the line and are from crosses of CC-125 derived strains and CC-1952. The data are from Table 2.

hybridized as previously described (JOHNSON and DUTCHER 1991).

RESULTS

Multipoint crosses: To construct multiply marked strains the *fla10*, *fla11*, *pf10*, *uni1* and *apm1* mutations were used because they have distinguishable phenotypes. These loci span approximately one-half of the 100-cM circular map described for linkage group XIX (RAMANIS and LUCK 1986; DUTCHER 1986; JAMES *et al.* 1988). The tetrad data generated for these markers during the course of this work are summarized in Table 1 and the resulting genetic map for the *APM1* to *PF10* interval is diagrammed in Figure 1. For these markers the map distances are additive and the map is linear. No recombinants were detected between the *FLA11* and *APM1* loci in 121 tetrads examined (Table 1). Therefore, the *FLA11* locus was not used to study recombination patterns.

By including a marker tightly linked to the centromere of another linkage group it is possible to map the linkage group XIX centromere as an additional marker in multipoint crosses. We used both the *pf27* marker on linkage group XII/XIII and the *y1* marker on linkage group XVI/XVII. The calculated centromere distances place the centromere between the *FLA10* and *PF10* loci (Table 1). This order differs from the published maps of linkage group XIX (RAMANIS and LUCK 1986; DUTCHER 1986), which put the *PF10* locus between the centromere and the *FLA10* locus. This placement of the centromere between the *FLA10* and *PF10* loci is supported by three point cross data from crosses in which *fla10*, *pf10* and the centromere marker *y1* were heterozygous (data not shown).

A total of 1710 tetrads from 18 independent crosses were analyzed to estimate the frequency and strand composition of double exchanges in the interval between the *APM1* and *PF10* loci. These crosses involve different combinations of at least three of the five linkage group XIX markers. No tested combination of these markers led to reduced viability or produced a synthetic phenotype. Of the tetrads generated in these multipoint crosses, 2% were unscorable because the tetrads contained fewer than four viable meiotic progeny. This frequency of unscorable tetrads is comparable to what we have observed for crosses involving markers on other linkage groups.

A molecular map in a first generation congenic cross: To increase the number of intervals on linkage group XIX that could be monitored for interference patterns, we included three loci defined by cloned DNA sequences. These molecular markers include

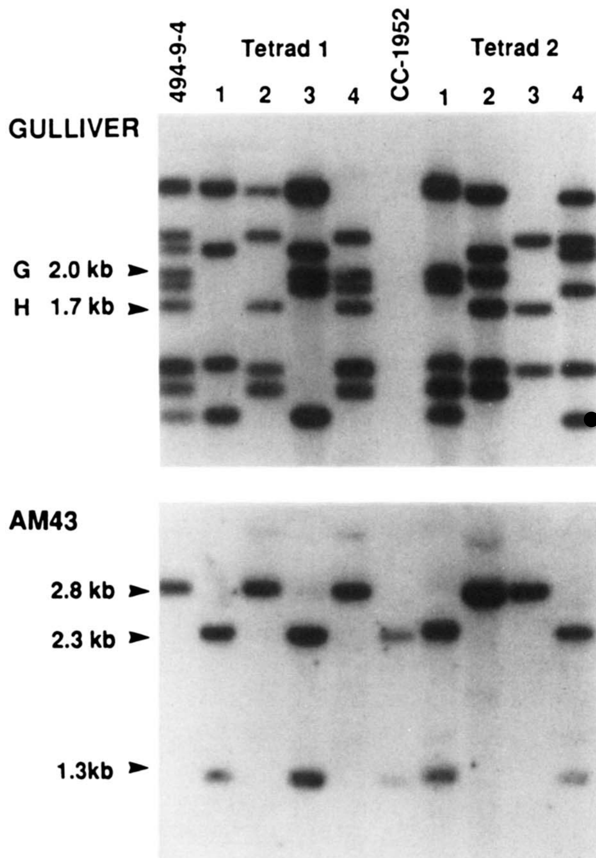

FIGURE 2.—Hybridization patterns of the Gulliver and AM43 probes. The hybridization patterns of Gulliver and AM43 sequences to *Pvu*II-digested DNA from 494-9-4 strain (*pf10 fla10 uni1 apm1 y1* in a CC-125 background), DNA from strain CC-1952, and in DNA from two tetrads resulting from a cross between these two strains. Lane assignments: lane 1, 494-9-4 DNA; lanes 2–5, DNA from the four members of tetrad 1; lane 6, CC-1952 DNA; lanes 7–10, DNA from the four members of tetrad 2. Duplicate filters containing these DNA samples were hybridized to either the 600-bp Gulliver probe (upper panel) or the 700-bp AM43 sequence (lower panel). The Gulliver sequence hybridizes to about 12 bands in the CC-125 parent but is absent in the CC-1952 strain (FERRIS 1989). The two bands labeled G and H map to linkage group XIX (JOHNSON and DUTCHER 1991). The AM43 sequence hybridizes to a 2.8-kb band in the CC-125 parent and to bands of 2.3 and 1.3 kb in CC-1952. In both tetrads the Gulliver H and AM43 sequences cosegregate (parental ditype tetrads), while Gulliver G segregates in a tetratype pattern from them. In both cases the five phenotypic markers segregated in a fashion consistent with a single crossover between *UNI1* and *FLA10*.

two copies of the transposon Gulliver (FERRIS 1989; JOHNSON and DUTCHER 1991) and one randomly selected fragment of single-copy genomic DNA, which has been designated AM43. These molecular probes detect easily scorable restriction fragment length polymorphisms in crosses between CC-125 derived strains and strain CC-1952 (GROSS, RANUM and LEFEBVRE 1988). The hybridization patterns of these sequences to our multiply marked parental strain, the CC-1952 strain, and two tetrads from this cross are shown in Figure 2.

The segregation of the molecular markers was followed in the progeny of a first generation congenic cross of a *apm1 uni1 fla10 pf10 y1* strain to the CC-1952 strain. The five phenotypic markers were scored in 177 tetrads from this cross. Most regions in CC-125 × CC-1952 hybrid crosses appeared to follow the same patterns as observed in the progeny of the CC-125 derived parental crosses. A total of 54 tetrads from the cross of the multiply marked strain to CC-1952 was scored for the segregation of the three molecular markers. Tetrad ratios for pairs of these three markers, as well as the closest phenotypic marker, are presented in Table 2. Gulliver H is 1 cM centromere distal of the *FLA10* locus, Gulliver G is 3 cM centromere distal of the *APM1* locus, and AM43 maps between the *APM1* and *FLA10* loci (Figure 1).

One difference in the genetic map was observed in these hybrid crosses. The genetic distance between the *APM1* and *UNI1* loci was about 2.5 times greater than observed in crosses between CC-125 derived strains. A similar increase was observed in 243 tetrads from three additional independent crosses of *uni1 apm1* strains to CC-1952.

We also found that the increased level of recombination in the *APM1FLA10* interval first detected in CC-125 × CC-1952 crosses continued to be detected in crosses between strains that were increasingly homozygous for regions of the CC-1952 genome (Table 3). Progeny with the *apm1*, *pf10*, mt^+ and *GER2* markers from the first generation cross to the CC-1952 parent were crossed again to CC-1952 (JOHNSON and DUTCHER *et al.* 1991). This process was repeated two additional times to generate a four generation congenic pedigree. The *APM1-FLA10* distance in each congenic cross remains significantly higher than the distance detected in CC-125 × CC-124 derived crosses (Table 3). The *APM1-PF10* distance, however, remained the same in the congenic and CC-125 × CC-124 crosses.

A molecular map in a fourth generation congenic strain: In order to further characterize the unusual recombination patterns on linkage group XIX in congenic crosses, we examined recombination patterns in a CC-125 × CC-1952 congenic strain that had experienced multiple recombination events in its derivation. Strain 18204 is a congenic strain that carries the genetic markers *apm1* and *pf10* from the CC-125 parent, but has the CC-1952 alleles for the three molecular markers described above. This strain must have incurred a two strand double exchange between the *APM1* and *PF10* loci, as well as an additional single exchange between Gulliver and *APM1* during its construction. We have examined the Gulliver G to *PF10* interval using three genetic markers and the three molecular markers in a cross of 18204 to a CC-125 derived strain with the *uni1-1* mutation (Table 2). There is increased recombination in this cross. The interval from Gulliver G to *PF10* showed PD:NPD:T ratios of 4:2:24. The two nonparental ditype (NPD)

TABLE 2

Mapping data for the molecular markers

Loci	No. of tetrads[a] PD	NPD	T	Distance (cM)	No. of tetrads[b] PD	NPD	T	Distance (cM)
GULG APM1	51	0	3	3	26	0	4	7
GULH FLA10	53	0	1	1				
AM43 FLA10	43	0	11	10				
GULH PF10					11	0	19	32
AM43 PF10					6	2	23	57/unlinked
GULG AM43	23	0	31	29	6	0	24	40
AM43 GULH	44	0	10	9	23	2	5	28
GULG GULH	13	0	41	38	4	1	27	52/unlinked

[a] Data are from the cross of ***pf10 fla10 uni1 apm1 y1*** GULG GULH AM43 (494-9-4) to CC-1952.
[b] Data are from a cross of ***pf10 apm1***, a third generation congenic strain (18204) to ***uni1*** GULG GULH AM43.

TABLE 3

Effect of congenic crosses on *APM1-FLA10* recombination frequency

Loci		CC-125[a]	Congenic generations[a] 1°	2°	3°	4°
APM1	*FLA10*					
No. tetrads		719:6:952	95:8:217	10:1:63	10:0:52	44:1:101
Map distance		29	41	47	42	37
P value[b]			(3.3×10^{-8})	(2.0×10^{-6})	(1.2×10^{-4})	(1.0×10^{-2})
APM1	*PF10*					
No. tetrads		86:26:1082	8:5:195	6:2:66	9:2:93	9:4:84
Map distance		52	54	53	50	56
P value[b]			(0.20)	(0.91)	(0.85)	(0.34)
FLA10	*PF10*					
No. tetrads		1040:6:978	232:0:172	36:0:25	59:0:53	46:0:40
Map distance		24	21	21	24	23
P value[b]			(0.05)	(0.47)	(0.82)	(0.83)

[a] The CC-125 column contains data from crosses of CC-125 derived parents. The 1° generation congenic column contains data from crosses between CC-125 derived strains and CC-1952. The 2° generation column contains data from crosses of 1° generation strains by CC-1952. The 3° generation column contains data from crosses of 2° generation strains by CC-1952. The 4° generation column contains data from crosses of 3° generation strains by CC-1952.
[b] The *P* value was calculated for each interval by χ^2 analysis. Each congenic generation was compared to the sum of the generation being tested and the CC-125 crosses. The null hypothesis tested is whether the two sets of data come from the same distribution. For the *FLA10-APM1* interval, the null hypothesis is rejected for each congenic generation. For the *PF10-APM1* and the *PF10-FLA10* intervals, the null hypothesis is accepted.

tetrads involved at least a four-strand double exchange between AM43 and Gulliver H; no NPD tetrads were observed in the first generation congenic crosses (Table 2). χ^2 analysis suggests the Gulliver G-AM43 interval is statistically different between the two crosses.

While the data above show that recombination frequencies along some intervals of linkage group XIX differ in crosses to CC-1952 strains, the linkage group XIX map remains most easily explained by a linear construct. This heterozygosity effect may not act uniquely on linkage group XIX. There is evidence that distances between loci on linkage group VI are different in crosses in which CC-125-derived strains are crossed to the polymorphic *C. reinhardtii* strain CC-1373 (Ranum *et al.* 1988).

Markers on the other side of linkage group XIX: The three molecular markers increased the number of intervals on linkage group XIX that could be monitored simultaneously, but together the eight loci mapped in Figure 1 covered only 55 cM of the approximately 100-cM original circular map. Additional markers were required in order to determine interference patterns on the other side of the circular map. Only three loci were mapped to the other one-half of the linkage group XIX map; they are *SUN1* (Huang *et al.* 1982), *PF29* (Ramanis and Luck 1986) and *FLA20* (Dutcher 1986).

The *sun1* mutation partially suppresses the uniflagellate phenotype of a *uni1* mutant strain (Huang *et al.* 1982), but has no phenotype in a wild-type background. In attempting to construct strains carrying *sun1* in combination with other linkage group XIX markers we discovered that the *sun1* mutation was unlinked to them (Table 4). The *sun1* mutation was tested for linkage to markers on nine other linkage groups and it was determined that the *SUN1* locus maps 22 cM from the *ACT1* locus on linkage group II

TABLE 4

Linkage data for the *PF29* and *SUN1* loci

Loci	No. of Tetrads PD	NPD	T	Linkage
SUN1 UNI1	17	14	153	Unlinked
SUN1 FLA10[a]	86	68	96	Unlinked
SUN1 APM1[a]	38	17	402	—[c]
SUN1 ACT1[a,b]	81	0	65	22 cM
PF29[d] FLA10	12	8	20	Unlinked
PF29 PF10	39	39	10	Unlinked
PF29 ERY1[d]	85	0	49	18 cM
PF29 PF2[d]	85	0	1	1 cM
PF29 PF28[d]	85	0	3	2 cM

[a] This cross was homozygous for the *uni1* mutation.

[b] This locus maps to linkage group II.

[c] Nonlinkage is usually indicated by an equal number of parental and nonparental ditype tetrads. By χ^2 analysis the number of parental ditype and nonparental ditype are not equal ($P = 0.005$). However, the mapping crosses with the *ACT1* locus strongly suggest that the *SUN1* locus maps to linkage group II and not to linkage group XIX. No linkage was observed in crosses of *apm1* by act1 (15:12:64; PD:NPD:T). Viability in these mapping crosses was greater than 90%, which suggests that the *SUN1* locus had not been involved in a translocation event between linkage groups XIX and II.

[d] This locus maps to linkage group XI; additional data are presented in Dutcher *et al.* (1992).

(Table 4). This was true for both a *sun1 uni1* strain from our stock collection (HR5) as well as strains from the Chlamydomonas Genetics Center (CC-1925) and from the laboratory of D. J. L. LUCK. The *sun1* mutation is tightly linked to its centromere (157:139:10 in crosses to *pf27*), but we did not determine the map order of *SUN1*, *ACT1* and the centromere. There are no other mapped loci in this region that may be allelic to the *sun1* mutation (HARRIS 1989).

Cells with the *pf29* mutation (CC-1924) exhibit an abnormal flagellar beat stroke and swim poorly (RAMANIS and LUCK 1986). We found that the *pf29* phenotype in liquid media is more pronounced at 32° than at 21°; all the cells are at the bottom of the tube. We observed that the *PF29* locus is tightly linked to its centromere, as reported by RAMANIS and LUCK (1986), but found that it is unlinked to the *FLA10* and *PF10* loci on linkage group XIX. Crosses of *pf29* to mapping strains showed it was linked to the *ERY1* locus on linkage group XI (Table 4) and was tightly linked to the *PF2* and *PF28* loci on this linkage group. However, the recovery of recombinants between *pf29* and the latter two mutations suggests that it is not allelic at either locus. We have determined that these loci map in the order *PF28-PF2-PF29* (DUTCHER *et al.* 1992).

The *FLA20* locus was identified by a single mutant allele, whose phenotype was the loss of flagella after 36–48 hr at 32° (DUTCHER 1986). This phenotype has been referred to as delayed temperature-sensitive flagellar assembly. In mapping experiments, we discovered that the strain designated *fla20* in our stock collection instead mapped to the *FLA10* locus and exhibited the phenotype of the *fla10-14* allele. All other strains in our collection thought to contain the *fla20* mutation also contain this same mutation. Our attempts to reisolate the *fla20* mutation have been unsuCC-essful. We do not know whether the *fla20* mutation was a *fla10* allele that was mismapped or whether the original *fla20* strain was lost and contaminated with a *fla10-14* strain during the course of maintaining our stock collection. The latter alternative seems most likely. Examination of our stock records suggests that a subset of *fla10* strains were mislabeled in 1988. In summary, the loci previously mapped to this region have been reassigned to other linkage groups, to the linear portion of the linkage group XIX map, or lost. Therefore the linear map of linkage group XIX in Figure 1 represents the approximate current limits of this linkage group defined by available loci.

Chiasma interference: To confirm the linearity of linkage group XIX, we investigated its patterns of recombinational interference. Chiasma interference is the ability of one crossover event to influence the probability of a second crossover on the same chromosome (MULLER 1916). In a multipoint cross, the coefficient of coincidence is the ratio of the frequency of simultaneous crossover events in two nonoverlapping intervals to the expected number of crossover events, which is the product of the frequencies of crossover events in each of these intervals independent of the other (WEINSTEIN 1918). In the absence of chiasma interference the coefficient of coincidence will approximate 1.

The expected number of double crossovers for each pair of intervals is determined from the frequency of recombination within each pair of intervals. The expectations can be calculated from all the tetrad data (Table 1) or from just the tetrads in which at least three markers were heterozygous. We have calculated the expectations in four different ways, but find that the specific method makes very little difference to the

TABLE 5

Chiasma interference on linkage group XIX

Intervals compared	No. of tetrads scored	No. of observed double crossovers	No. of expected double crossovers[a]	Coefficient of coincidence[b]
PF10-FLA10 FLA10-UNI1	577	30	131	0.23
PF10-FLA10 FLA10-APM1	913	54	230	0.23
FLA10-UNI1 UNI1-APM1	591	2	20	0.10
PF10-CEN[c] *CEN*[c]*-FLA10*	376	2	20	0.10
PF10-FLA10 UNI1-APM1	428	6	9	0.67

[a] The expected number of double crossovers is the product of the probabilities of a crossover in each of the two intervals times the total number of tetrads examined. The probabilities of a crossover in each individual interval were calculated from the same crosses in which the number of double crossovers were observed.

[b] The coefficient of coincidence is the number of observed crossover events to the expected number of crossover events in the absence of interference.

[c] The linkage group XIX centromere (*CEN*) was mapped with respect to two independent centromere markers (*pf27* and *y1*). Both were present in the crosses.

TABLE 6

Chromatid interference on linkage group XIX

Crosses[a]	No. of tetrads scored	No. of double crossovers	Strand composition 2	Strand composition 3	Strand composition 4	P^b
CC-125	1710	63	15	36	12	0.46
1° generation congenic	596	43	11	23	9	0.82

[a] Both parents in a CC-125 cross are derived from the 137c strain. In a first generation congenic cross, the parents are CC-125 derivatives and CC-1952.

[b] The *P* value is calculated by χ^2 analysis. The null hypothesis that values fall into a ratio of 1:2:1 was accepted in both cases.

final value of the coefficient of coincidence.

The calculated coefficients of coincidence for various pairs of intervals on linkage group XIX are given in Table 5. The values are all less than one, which indicates positive chiasma interference. The presence of one crossover event suppresses the probability of additional crossovers. The coefficient of coincidence calculated for the intervals on opposite sides of the centromere is 0.1. This suggests that the centromere is not a block to the spread of the chiasma interference.

Chromatid interference: If the selection of chromatids in each of the two exchanges of a double crossover were an independent event, then double crossovers would be expected to fall into a 1:2:1 ratio of two-, three- and four-strand double exchanges. A circular linkage map should exhibit an excess of two-strand double exchanges. Of 1710 tetrads examined, 63 had a crossover in more than one interval (Table 6). Those 63 give a ratio of 15:36:12 for the two-, three- and four-strand double crossovers, respectively. A χ^2 analysis indicates that this is not significantly different from the expected ratio of 1:2:1 ($P = 0.46$), and therefore there is no evidence of chromatid interference within the *PF10-APM1* interval of linkage group XIX (Table 6). This was also true of the same interval in a first generation CC-125 × CC-1952 congenic cross (Table 6).

Temperature-sensitive recombination: The previously reported effects of temperature on recombination frequency for linkage group XIX were most dramatic in crosses involving the *sun1* mutation. The *SUN1-UNI1* cross showed the greatest differences in the work by RAMANIS and LUCK (1986) and the *SUN1-CEN* interval showed the greatest change in the work by DUTCHER (1986). However, the *SUN1* locus maps to linkage group II (Table 2). Therefore, we have reinvestigated the effects of temperature on recombination in the intervals *fla10-pf10* and *apm1-uni1*. We have examined the effects of temperature in two genetic backgrounds. In the course of other work, we have identified a recessive, cold-sensitive meiotic mutation in our laboratory strain, 137*mt*⁻, which was derived from CC-124. We have named the locus *GER1* for *ger*mination defective (LUX 1990). Although this mutation has no obvious effect on the completion of meiosis in heterozygous strains, it might affect recombination frequencies when heterozygous. We measured recombination frequencies in strains heterozygous for the *ger1* mutation and homozygous for the wild-type allele. Except in one cross, no significant effect of temperature was observed on the recombination frequencies in either genetic background (Table 7). In the $ger1^-/GER1^+$ cross at 16° there is a statistically elevated level of recombination in the *APM1-UNI1* interval. This is the only cross that shows a difference from the 21° homozygous cross and the alteration is in the opposite direction from the previously reported temperature effects.

TABLE 7

Temperature effects on pairs of linkage group XIX loci

Loci	Germination genotype[a]	Map distance in cM at temperature		
		16°	21°	32°
PF10 FLA10	*GER1⁺/GER1⁺*	89:0:59 19.9	135:0:112[b] 24	60:0:52 23.2
PF10 FLA10	*ger1⁻/GER1⁺*	82:0:63 21.7	112:0:77 20.4	95:0:87 22.4
APM1 UNI1	*GER1⁺/GER1⁺*	123:0:17 6	170:0:19[c] 4	130:0:11 3.9
APM1 UNI1	*ger1⁻/GER1⁺*	150:0:23 6.6	136:0:19 6.1	158:0:13 3.8

[a] The *ger1⁻* mutation was present in the CC-124 (137 *mt⁻*) strain in our laboratory. It is a recessive cold-sensitive meiotic mutant. Homozygous strains produce less than one viable tetrad in 10^4 zygotes (LUX 1990).

[b] The *P* value was calculated for each cross by χ^2 analysis. Each cross was compared to the sum of the cross being tested and the 21° data presented in this table or the data from Table 1 for the *PF10-FLA10* interval. The null hypothesis tested is whether the two sets of data come from the same distribution. The null hypothesis was accepted using either the data for 21° *GER1⁺/GER1⁺* homozygous crosses for this interval or from Table I.

[c] The *P* value was calculated for each cross by χ^2 analysis. Each cross was compared to the sum of the cross being tested and the 21° data presented in this table or the data from Table 1 for the *FLA10-APM1* interval. The null hypothesis tested is whether the two sets of data come from the same distribution. The null hypothesis was rejected for the 16° *ger1⁻/GER1⁺* cross ($P = 0.016$). The null hypothesis was accepted for the remainder of the crosses using either the data for 21° *GER⁺/GER1⁺* homozygous crosses for this interval or from Table 1.

DISCUSSION

Genetic properties of linkage group XIX: We conclude that the earlier reports demonstrating a circular map and temperature-sensitive recombination frequencies for linkage group XIX (RAMANIS and LUCK 1986; DUTCHER 1986) are not reproducible. Two loci that originally were mapped to linkage group XIX, *SUN1* and *PF29*, now map to linkage groups II and XI, respectively. These two loci and *FLA20*, a locus with no extant mutant alleles, were essential in establishing the circularity of linkage group XIX. Based on mapping data presented in this paper, we now argue that the genetic map of linkage group XIX is linear. In addition, we have also been unable to repeat the published observations that recombination frequencies on this linkage group are sensitive to changes in temperature prior to the onset of meiosis (RAMANIS and LUCK 1986; DUTCHER 1986). Our recent findings agree with those of JAMES (1989) and co-workers, who also examined the frequency of recombination in the *apm1-uni1* interval when zygotic cells were shifted to 17° or to 33° during the sensitive period preceding meiosis. They found a map distance of 7.1 cM at 33° ($n = 126$ tetrads) and a map distance of 5.6 cM at 17° ($n = 116$ tetrads). These differences are not significantly different from one another ($P = 0.68$).

We are unsure how the earlier circular maps arose. It is possible that the circular map was generated due to the mislabeling of strains or systematic scoring errors. Both possibilities are difficult to exclude, but all known isolates of the *pf29* and *sun1* mutations define the same loci. Although fewer tetrads were analyzed in the previous studies the linkage reported between the *sun1* and the *pf29* mutations to other mutations on linkage group XIX was nevertheless statistically significant (HUANG *et al.* 1982; RAMANIS and LUCK 1986; DUTCHER 1986). It remains a possibility that culture conditions at Rockefeller University influence the behavior of these loci.

Another possible explanation is that *SUN1* and *PF29* now map to other linkage groups because of chromosomal translocation events. However, no unusually high levels of lethality are detected in crosses involving these strains (Table 4). If the translocations involved essential genes, then some tetrads would be expected to contain inviable meiotic progeny because they are nullisomic for essential genes (MCBRIDE and GOWANS 1969; CHALEFF and FINK 1980). Furthermore, two separate translocation events would have been required, because *SUN1* and *PF29* no longer map to the same linkage group (18:19:0; Table 4).

A final possibility is that the *sun1* or *pf29* mutant alleles, or some other locus, acts to influence patterns of recombination on linkage group XIX. We have shown that crosses between *C. reinhardtii* strains CC-125 and CC-1952 can result in altered recombination frequencies for one interval on linkage group XIX (Table 2). However, this alteration does not create a circular map. In addition, crosses heterozygous for alleles of the *GER1* locus show elevated recombination frequencies at 16° for linkage group XIX (Table 7). In neither of these backgrounds does recombination increase with temperature as was previously reported.

The reported circularity of linkage group XIX and the temperature-sensitive pattern of recombination clearly set it apart from the other Chlamydomonas linkage groups (RAMANIS and LUCK 1986; DUTCHER 1986). In the absence of these properties, it is worth considering whether linkage group XIX is genetically unique. The apparent clustering of genes that have flagellar phenotypes is not absolute. We have identi-

fied three loci (*MAA5, MAA10, MAA11*) on linkage group XIX that can be mutated to confer resistance to 5-methyl anthranilic acid (DUTCHER *et al.* 1992). In many organisms, this compound is converted into the toxic amino acid analog 5-methyltryptophan by the tryptophan biosynthetic enzymes. None of these *maa* mutations have phenotypes that affect flagellar assembly or function. Because there are now genes on linkage group XIX not involved in flagellar assembly or function, the significance of the apparent clustering of these genes on linkage group XIX is not clear. This finding does not rule out the possibility that linkage group XIX is a genetic remnant of the basal body's endosymbiotic ancestor, but it does suggest that other reasons for the clustering phenomenon need to be considered. One possible explanation is that the apparent clustering is simply an artifact of mapping primarily mutations that exhibit flagellar phenotypes.

Interference patterns on linkage group XIX: The recombination events we detected on linkage group XIX show a five to ten fold reduction in the observed number of double crossovers relative to the expected number of double crossovers. This is in accord with the results of EBERSOLD and LEVINE (1959), who examined recombination events on the right arm of linkage group I. As in their study, we find that the chiasma interference decreases with increasing interval size or with distance between monitored intervals. Therefore, chiasma interference in Chlamydomonas appears to follow the pattern observed in most other organisms [reviewed in JONES (1987) and KING and MORTIMER (1990)].

The patterns of recombination on linkage group XIX of *C. reinhardtii* do not fit the expectations for a circular linkage group. One prediction of a circular map is strong chromatid interference; the number of two-strand double exchanges should be much greater than the number of three- and four-strand double exchanges. We were unable to find any evidence of chromatid interference on linkage group XIX. The double crossovers were distributed in the 1:2:1 ratio expected if each pair of homologous chromatids is equally likely to be involved in a crossover event, and if the involvement of a chromatid pair in one crossover does not affect its probability of being involved in a second crossover. EBERSOLD and LEVINE (1959) observed the same 1:2:1 ratio on the right arm of linkage group I. In general, there is little evidence to support the existence of chromatid interference in any fungal organism (summarized in FINCHAM, DAY and RADFORD 1979) or by half-tetrad analysis in Drosophila (ANDERSON 1925; NOVITSKI 1954).

Our data show that PERKINS' (1949) mapping function is appropriate for mapping genes in *C. reinhardtii.* This equation assumes both that exchange events never involve more than two simultaneous crossovers and that chromatid interference does not exist. Because these assumptions appear to be closely met, we believe this mapping function is more appropriate for calculating distances between linked loci in this organism than the commonly used equation $[(NPD + 0.5T)/((PD + NPD + T)] \times 100$ (HARRIS 1989).

One unexpected finding in the present study is that chiasma interference extends across the centromere in linkage group XIX. The effect of the centromere on chiasma interference is varied. In Drosophila, maize, and Neurospora adjacent intervals that contain a centromere do not show chiasma interference; centromeres act as blocks to the spread of interference [reviewed in SYBENGA (1975)]. In contrast, the centromere does not block chiasma interference in Podospora (KUENEN 1962). In *Saccharomyces cerevisiae,* both outcomes have been observed. When the two intervals are large (>30 cM), the centromere acts as a block to chiasma interference (HAWTHORNE and MORTIMER 1960); this may reflect the decrease in interference with distance. When smaller intervals are examined, there is positive chiasma interference (SNOW 1979) or negative chiasma interference (SYMINGTON and PETES 1988). The centromere of linkage group XIX falls into the class that does not act as a block to interference. This is one of the few examples of positive interference extending across the centromere and clearly demonstrates that centromeric regions are not obligate blocks to the establishment of interference.

Because EBERSOLD and LEVINE (1959) did not examine interference across the linkage group I centromere, we do not know whether our observation that chiasma interference extends across the linkage group XIX centromere is true for other Chlamydomonas centromeres as well. Indirect evidence suggests that it is. Analysis of the available mapping data (HARRIS 1989) suggests that map distances are as additive across centromeres as they are within linkage group arms. This would not be true if the centromere were a block to strong chiasma interference. Additionally, there is not a dramatic difference in the frequency of NPD tetrads observed for intervals that span the centromere compared to those that do not.

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