GENETIC ANALYSIS OF MATING LOCUS LINKED MUTATIONS IN *CHLAMYDOMONAS REINHARDII*

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ABSTRACT

The mating-type (*mt*) locus of *Chlamydomonas reinhardii* has been analyzed using four mutant strains (*imp-1*, *imp-10*, *imp-11* and *imp-12*). All have been shown, or are shown here, to carry mutations linked to either the plus (mt^+) or the minus (mt^-) locus, and their behavior in complementation tests has allowed us to define several distinct functions for each locus. Specifically, we propose that the mt^+ locus contains the following genes or regulatory elements: a locus designated *sfu*, which is necessary for sexual fusion between gametes; a locus designated *upp* (uniparental plus), which controls aspects of chloroplast gene inheritance and perhaps also zygote maturation; and a locus designated *sad*, which functions in sexual adhesion. The mt^- locus also contains a *sad* locus as well as a gene or regulatory element designated *mid*, which is necessary for the minus dominance in mt^+/mt^- diploids.

THE mating-type locus of *Chlamydomonas reinhardii*, which exists as two apparent alleles (mt^+ and mt^-) on linkage group (LG) VI, has been shown to control gametic differentiation (SMITH and REGNERY 1950; SAGER 1955) and organelle gene inheritance (SAGER 1954; GILLHAM 1969; CHUR-DER and CHIANG 1974; BOYNTON *et al.* 1984). A single locus could control such diverse phenotypes in two basic ways: it could specify one or more regulatory proteins that activate other (structural) genes, or it could represent a cluster of (structural) genes that behave as a single allele because recombination between them is disallowed. Available genetic data indicate that both models are applicable. Thus several "sex-limited" genes that specify plus gametic traits are unlinked to mt^+ and yet are expressed only in gametes that carry mt^+ (GOODENOUGH, HWANG and WARREN 1978), and a second set of unlinked genes involved in generating the minus gametic phenotype requires mt^- for their expression (FOREST and TOGASAKI 1975). These observations suggest that each *mt* "allele" encodes regulatory proteins that act to "turn on" or "turn off" the sex-limited genes. On the other hand, a number of loci on LG VI that are presumably not involved in generating the mating phenotype (*e.g.*, acetate-, nicotinamide-, and thiamine-requirement) fail to recombine with *mt*, indicating a general suppression of recombination in the *mt* region (EBERSOLD *et al.* 1962; GILLHAM 1969). Moreover, at least two gene loci involved with gametic traits have shown tight linkage to *mt* (GOODENOUGH, HWANG and MARTIN 1976; HWANG, MONK

and GOODENOUGH 1981), suggesting (but not proving) that the *mt* locus might include a group of gamete-specific genes held together by recombinational suppression. Taken together, therefore, it would appear that the *mt* locus could contain both structural genes and regulatory genes that affect the expression of unlinked loci.

The present study was designed to expand our understanding of the *mt* locus by further analysis of mating-related mutations. The first of these mutations, *imp-11*, was originally isolated from a uv-mutagenized mt^- strain, and is capable of uv-induced reversion to the minus phenotype (GOODENOUGH, DETMERS and HWANG 1982). Previous studies have shown this mutant to display most of the mating phenotypes of a plus gamete: it undergoes sexual agglutination only with minus gametes, and its mating structure elongates via actin filaments, again a plus trait. However, the mutant is unable to undergo sexual fusion, apparently because its mating-structure membrane lacks a "fringe" of coat material present on the wild-type mating structure (GOODENOUGH, DETMERS and HWANG 1982). Genetic analysis of this mutation is crucial to understanding mating-type determination. Here we document that *imp-11* is closely linked to *mt*. We also compare and contrast its patterns of chloroplast gene transmission with *imp-1*, a mutation linked to mt^+ that sexually agglutinates as plus, also fails to produce fringe material and is also fusion-defective (GOODENOUGH, DETMERS and HWANG 1982). Interestingly, these mutants fail to complement one another.

In addition, we analyze the complementation properties of *imp-10* and *imp-12*, two apparent alleles that map to a locus (*sad-1*) closely linked to mt^- (HWANG, MONK and GOODENOUGH 1981). These mutants are unable to agglutinate sexually, indicating that a gene essential for minus agglutinin production lies within or near mt^-. Here we show that both *sad-1* isolates can be complemented by mt^+, by the mutated mt^+ (*imp-1*), and by the mutated mt^- (*imp-11*). Therefore, the information necessary for minus agglutinin production that is encoded by the *sad-1* locus is unaltered by the *imp-11* mutation and is contained within the mt^+ locus. The analyses of these mutations allow us to propose that both mating-type "loci" contain several functional genes.

MATERIALS AND METHODS

Diploid strain construction: Diploid strains were constructed using polyethylene glycol-induced cell fusions (MATAGNE, DELTOUR and LEDOUX 1979; GALLOWAY and HOLDEN 1984) of the haploid strains listed in Table 1. Table 2 lists the diploid genotypes, the parents used in the diploid construction and the average diploid cell volumes (determined by ocular micrometer measurements of cell diameter of 20 random cells). Note that *imp-1* and *imp-11* are written as alleles of mt^+ and mt^-, respectively; a more accurate nomenclature, $mt^+_{imp\text{-}1}$ and $mt^-_{imp\text{-}11}$ was deemed needlessly cumbersome for this and subsequent tables.

Since the original strain carrying the *imp-11* mutation lacked selectable markers and also could not be mated, it was necessary to obtain a spontaneous secondary mutation to allow positive selection for diploids following fusions with other strains. The phenotype selected was resistance to fluoroacetamide (fam^r), while utilizing urea as a nitrogen source. This mutation also confers an inability to grow on media with acetamide as the sole nitrogen source (acm^-) (HODSON and GRESSHOFF 1979; R. C. HODSON, personal communication). It is therefore possible to select for either

TABLE 1

Haploid strains

Strain designation	Nuclear and chloroplast genotypes	Average cell volume (μm^3)
1 CC-1148	*imp-11*	146
2 CC-1862[a]	*imp-11, acm⁻ (fam^r)*	139
3 CC-1868[a]	*arg-7, imp-1 (mt⁺)*	153
4[a]	*arg-2, cw-15, mt⁺, sr-u-sm2, er-u-11*	164
5[a]	*arg-2, cw-15, mt⁻, sr-u-sm2, er-u-11*	120
6 WU-101[a]	*arg-7, nic-7, ac-29a, pf-14, mt⁻, er-u-37*	152
7 CC-1866	*arg-2, thi-10, mt⁺, sr-u-sm2*	151
8 CC-1849	*arg-7, mt⁺, hrb-u-ar207*	180
9 CC-1412[a]	*arg-7, mt⁺, hrb-u-dr2, sr-u-sm2*	132
10	*arg-7, nic-13, mt⁺, hrb-u-br202*	157
11	*arg-7, nic-13, mt⁻, hrb-u-br202*	147
12	*arg-2, nic-13, cw-15, mt⁻, er-u-11*	166
13	*arg-2, nic-7, mt⁺*	124
14	*arg-2, thi-10, mt⁺, sr-u-sm2, er-u-11*	166
15 CC-1310[a]	*arg-7, imp-12 (mt⁻)*	153
16 CC-1865[a]	*arg-2, imp-1 (mt⁺)*	180
17 CC-1867[a]	*arg-2, imp-10 (mt⁻)*	161
18 CC-620[b]	R3, *mt⁺*, high mating efficiency wild-type	125
19 CC-621[b]	NO, *mt⁻*, high mating efficiency wild-type	115

"CC" strains are available (by the given number) from the Chlamydomonas Genetics Center, Department of Botany, Duke University, Durham, North Carolina 27706. WU-101 is from D. E. HOURCADE (1983). Genotypes are wild-type unless designated. The *arg-2* and *arg-7* markers are complementing alleles of the same gene (arginine requiring) located 18 map units from the centromere of chromosome *I*. The mating-type cluster, located on chromosome VI, includes *thi-10, nic-7, ac-29a, mt⁺, mt⁻* and mating defective (impotent) mutations (*imp-10* and *imp-12*, which are *mt⁻* linked, and *imp-1*, which is *mt⁺*-linked). The *pf-14* (paralyzed flagella) marker is located on the opposite arm of chromosome *VI*. A mutation in the *nic-13* locus (4 map units from the centromere of chromosome X) produces nicotinamide auxotrophy. *sr-u-sm2, er-u-11, er-u-37, hrb-u-dr2, hrb-u-ar207* and *hrb-u-br202* are chloroplast mutations conferring resistance to either streptomycin, erythromycin, diuron, atrazine or bromacil.

[a] Used to construct diploids for complementation tests.

[b] Used to determine mating efficiency.

the mutant (*fam^r*) or the wild-type (*Acm⁺*) traits. Moreover, the *acm⁻* mutation that we obtained was recessive, allowing selection of diploids carrying the *imp-11* and *acm⁻* mutations.

Culture conditions and media: Cultures were routinely grown at room temperature with continuous "daylight" fluorescent illumination on a Tris phosphate medium (1.5% agar) (SURZYCKI 1971). Media were supplemented with 10 mM sodium acetate and 100 μg/ml arginine, 1 μg/ml nicotinamide, or 5 μg/ml thiamine as needed. For fluoroacetamide resistance, the NH_4Cl in the medium was replaced by 150 mM fluoroacetamide and 500 μM urea. To select for the *Acm⁺* phenotype,

TABLE 2

Diploid strains

Haploid parent strains	Nuclear genotype	Phenotype selected	Average cell volume (μm^3)
A. 2 + 7	*imp-11/mt*$^+$, *arg-2/+*, *acm*$^-$*(fam*^{r}*)/+*, *thi-10/+*	acm$^+$, thi$^+$, arg$^+$	355
B. 2 + 6	*imp-11/mt*$^-$, *arg-7/+*, *nic-7/+*, *pf-14/+*, *ac-29a/+*, *acm*$^-$*(fam*^{r}*)/+*	acm$^+$, nic$^+$, arg$^+$, ac$^+$	360
C. 3 + 7	*imp-1/mt*$^+$, *arg-7/arg-2*, *thi-10/+*	arg$^+$, thi$^+$	266
D. 3 + 5	*imp-1/mt*$^-$, *arg-2/arg-7*, *cw-15/+*	arg$^+$	292
E. 10 + 12	*mt*$^+$*/mt*$^-$, *arg-2/arg-7*, *cw-15/+*, *nic-13/nic-13*	nic$^-$, arg$^+$	320
F. 8 + 4	*mt*$^+$*/mt*$^+$, *arg-2/arg-7*, *cw-15/+*	arg$^+$	304
G. 13 + 14	*mt*$^+$*/mt*$^+$, *arg-2/arg-2*, *nic-7/+*, *thi-10/+*	thi$^+$, nic$^+$, arg$^-$	358
H. 11 + 12	*mt*$^-$*/mt*$^-$, *arg-2/arg-7*, *cw-15/+*, *nic-13/nic-13*	nic$^-$, arg$^+$	345

Diploids were produced by fusions of the numbered haploid parent strains from Table 1.

NH_4Cl was replaced by 10 mM acetamide. Chloroplast resistance markers were scored by growth on medium containing either 100 μg/ml erythromycin, 200 μg/ml streptomycin, 3 μM diuron, or 1 μM bromacil.

Crosses and scoring of progeny phenotype: Diploid gametes were produced by nitrogen starvation of diploid strains. Mating and tetraploid zygote maturation followed practices standard for diploid zygotes (EBERSOLD and LEVINE 1959). Tetraploid zygotes were germinated on media containing NH_4^+ as the nitrogen source plus all amino acid and vitamin supplements to allow growth of any progeny segregating recessive auxotrophic phenotypes. Germination frequencies were normal. Dissected tetrads were grown into colonies and were replica-plated to determine auxotrophic and resistance phenotypes of nuclear and chloroplast alleles. Compared with crosses between haploids and diploids, tetrad lethality was relatively low (100 complete tetrads out of 146 dissected), and instances of meiotic abnormalities were relatively rare. Specifically, in certain crosses, one diploid parent was heterozygous for the *nic-7*, *thi-10* or *ac-29a* mutant alleles. These mutations would not be expected to be expressed by any of the diploid progeny as long as meiosis had occurred normally. In fact, two out of 88 tetrads contained progeny displaying one of these recessive traits, and in both cases the tetrad had other abnormalities.

Analysis of tetraploid meioses: To obtain linkage data for the *imp-11* mutation, two diploid strains were crossed and marker segregation was analyzed in meioses of tetraploid zygotes. A detailed discussion of tetraploid meiotic segregation patterns can be found in LEOPOLD (1956a). Briefly, in tetraploid meioses without recombination between a given marker (*A* or *a*) and its centromere on any of the four homologues (*A/A/a/a*), only two types of tetrads are expected (4*A/a*:0 and 2*A/A*:2*a/a*), and these are predicted to occur in a 2:1 ratio. For centromere-distal markers, on the other hand, recombination frequencies in tetraploid yeast are found to be so high that second-division segregation approaches 100% (LEOPOLD 1956b). On the assumption that this also holds true for Chlamydomonas tetraploids, then in addition to the 4*A/a*:0 and the 2*A/A*:2*a/a* classes, centromere-unlinked markers are expected to yield an additional class, namely, 2*A/a*:1*A/A*;1*a/a*, and the three types of tetrads are predicted to occur in a 4:1:4 ratio (LEOPOLD 1956b). Figure 1 diagrams such an outcome for a cross of two diploid parents, each carrying a recessive *imp* mutation.

Determination of mating phenotypes: Mating phenotypes of progeny (imp, plus, minus) were distinguished by their ability to agglutinate and by their mating efficiency—determined by the percentage of quadriflagellate cells out of 200 cells observed after a 30-min mating with the high-mating-efficiency strains CC-620 and CC-621. (Quadriflagellate cells formed by mating are morphologically distinct from the occasional cell with aberrant flagellar number found in unmated

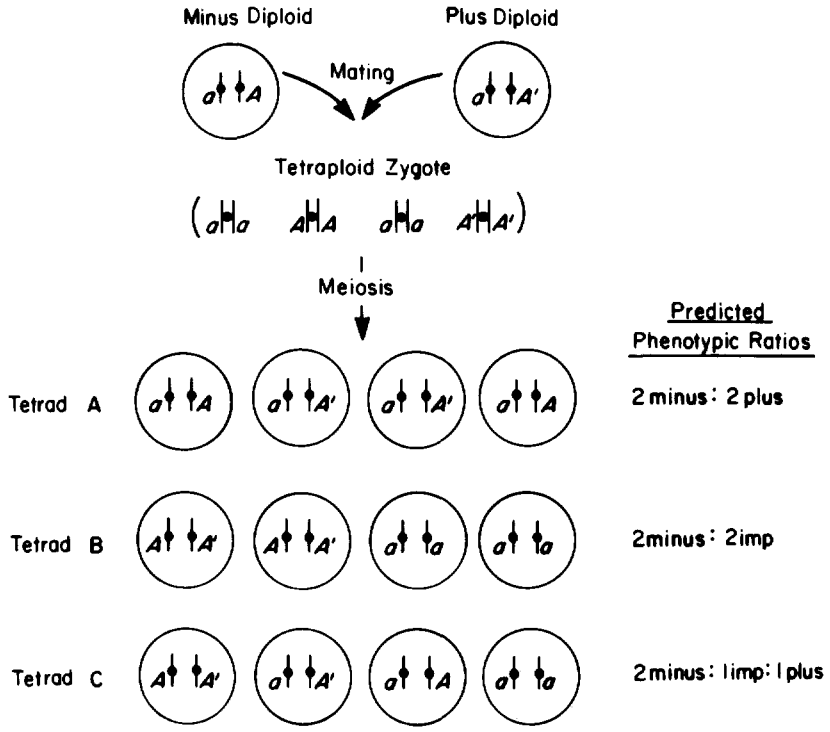

FIGURE 1.—Predicted segregation of a mating-type linked mutation. The mt^+ (A'), mt^- (A) and recessive, defective mating-type (a) loci segregate in meiosis to produce tetrads with three different types of phenotypic ratios. If the *mt* locus, 36 map units from its centromere (SMYTH, MARTINEK and EBERSOLD 1975) undergoes 100% second-division segregation, tetrads A, B, and C should occur in a 4:1:4 ratio. All three tetrads arise by several types of recombinational and segregational events.

cultures.) The haploid *imp-11* strain consistently produces <2% quadriflagellates in such a test. Diploid strains of all genetic compositions have reduced mating efficiencies, compared with haploid wild-type, and were therefore retested to obtain unambiguous results if mating efficiencies were between 2 and 5%.

RESULTS

Genetic analysis of imp-11: The first experiments performed in this study were designed to determine the genetic linkage and segregation patterns of the *imp-11* mutation. Since *imp-11* affects many aspects of the gametic phenotype—minus cells come to acquire plus gametic traits, and fringe material is absent from an otherwise plus-like mating structure—it was important to learn whether or not *imp-11* was recessive, whether it mapped to the *mt* locus and whether it behaved as a single or as a multiple mutation. Such information, moreover, was essential to the interpretation of the complementation tests described in subsequent sections. Since *imp-11* only rarely (<2%) fuses with minus gametes in sexual mating mixtures, and the resultant quadriflagellate cells never mature into zygotes (suggesting that the *imp-11* mutation may in-

terfere with zygote maturation), these analyses have all been carried out using cells fused together via polyethylene glycol (PEG) (MATAGNE, DELTOUR and LEDOUX 1979).

When haploid *imp-11* strains are fused with haploid mt^+ cells via PEG, the resultant diploids are invariably sexually-competent plus strains. Conversely, the fusion of *imp-11* and mt^- haploid yields sexually competent minus strains. As neither fusion yields imp cells, we conclude that *imp-11* is recessive in both mating-type backgrounds.

The plus phenotype of the diploids formed between *imp-11* and mt^+ cells allows one to rule out one model for the *imp-11* mutation. Because the original mutation occurred in an mt^- cell, this model proposed that the mt^- locus remained intact, but the *imp-11* mutation somehow prevented its normal expression. Because *imp-11* is recessive, the model predicted that, if it were not a mating locus alteration, then in a diploid with mt^+ (the genotype written as $+/imp\text{-}11$, mt^+/mt^-), the mt^- locus should exert its normal dominance over mt^+ (EBERSOLD 1967), and the strain should be sexually minus. Because these diploids are sexually plus, we conclude that the *imp-11* strain no longer carries a normal mt^- locus (diploid genotype written as $imp\text{-}11/mt^+$).

To learn whether the *imp-11* phenotype can be directly attributed to this altered mt^- locus or whether multiple mutations are involved, the linkage and segregation patterns of *imp-11* were analyzed. As detailed in MATERIALS AND METHODS, such analyses must be performed using tetraploid zygotes that undergo meiosis to yield four diploid zygospores. To demonstrate that the segregation patterns worked out for tetraploid yeast (LEOPOLD 1956b) hold true as well for Chlamydomonas, a number of tetraploid strains were constructed carrying known genetic markers, and their meiotic products were analyzed. As summarized in Table 3, the hypothesis of 100% second-division segregation, yielding 4:1:4 tetrad ratios (see also Figure 1), is fully applicable for all the Chlamydomonas markers except *nic-13*, which has been shown to be closely linked to its centromere (HASTINGS *et al.* 1965). Since the *mt* locus is centromere-unlinked (SMYTH, MARTINEK and EBERSOLD 1975), it is expected to show this same 4:1:4 segregation pattern (Figure 1).

Table 4, cross (a) shows the data obtained from tetraploids constructed from sexual matings between $imp\text{-}11/mt^+$ diploids and $imp\text{-}11/mt^-$ diploids. Three types of tetrads are produced when these tetraploid cells undergo meiosis: 2 plus:2 minus; 2 imp:2 minus; and 2 minus:1 imp:1 plus (Figure 1). In analyzing these diploid phenotypes, it is essential to bear in mind that the wild-type mt^- locus is dominant to mt^+ (EBERSOLD 1967), the mt^- locus contributed by *imp-11* is recessive to mt^+ (phenotype of PEG-induced diploids) and any additional mutation responsible for the *imp* phenotype is also recessive (phenotype of PEG-induced diploids). When these constraints are imposed on the phenotypes, then the possible genotypes that could yield the ratios in Table 4, cross a, become quite limited, and the data conform well to the hypothesis that *imp-11* is a recessive allele cosegregating with *mt*. More specifically, we propose that *imp-11* represents a mutation in the mt^- locus that converts it from a

TABLE 3

Segregation of markers in tetrads from tetraploid zygotes

Parental genotype		Tetrad frequencies (A)	(B)	(C)	χ^2
		4F:0f	2F:2f	3F:1f	
1. *fam*^{r}/+ × *fam*^{r}/+	Observed	19	4	15	
	H_1 Expected	21.5	8.9	7.6	8.63 ($P < 0.05$)
	H_2 Expected	16.9	4.2	16.9	0.29 ($P > 0.7$)
		4A:0a	0A:4a	2A:2a	
2. *arg-7/arg-2*	Observed	14	4	12	
×	H_1 Expected	17.0	7.0	6.0	6.30 ($P < 0.05$)
arg-7/arg-2	H_2 Expected	13.3	3.3	13.3	0.06 ($P > 0.95$)
		4A:0a	2A:2a	3A:1a	
3. *arg-7/arg-2*	Observed	8	0	7	
×	H_1 Expected	8.5	3.5	3.0	6.65 ($P < 0.05$)
arg-2/+	H_2 Expected	6.7	1.7	6.7	0.85 ($P > 0.7$)
4. *arg-7/arg-2*	Observed	5	0	7	
×	H_1 Expected	6.8	2.8	2.4	9.14 ($P < 0.05$)
arg-7/+	H_2 Expected	5.3	1.3	5.3	0.77 ($P > 0.5$)
		4N:0n	2N:2n	3N:1n	
5. *nic-13/nic-13*	Observed	7	5	2	
×	H_1 Expected	8.6	4.0	1.4	0.21 ($P > 0.9$)
Nic$^+$/*Nic*$^+$	H_2 Expected	6.2	1.5	6.2	8.22 ($P < 0.05$)

The phenotypes of the tetrads (A), (B) and (C) are abbreviated. Capital letters refer to wild-type phenotypes (F, fluoroacetamide sensitivity; A, arginine prototrophy; N, nicotinamide prototrophy), and the lower case stands for recessive phenotypes (f, fluoroacetamide resistance, etc.). Thus, in the first cross, all members of a tetrad may be fluoroacetamide sensitive (4F:0 in tetrad (A)), one member may be resistant (3F:1f in tetrad (C)) or two may be resistant (2F:2f in tetrad (B)). The first null hypothesis (H_1) is based on detectable linkage of the marker to the centromere. For the first four crosses, an arbitrary recombination frequency (chosen based on recombination frequencies in diploid zygotes) was used to predict a 0.20 frequency for tetrad (C). Consequently, the predicted frequency of tetrad (A) was 0.56 (0.66 minus half the frequency of (C)), and the frequency of (B) was 0.33–0.10. H_1 for the fifth cross used a lower predicted frequency (0.10) for tetrad class (C) because the *nic-13* marker is centromere-proximal (4 map units in diploid zygotes). The second null hypothesis (H_2) used the same predicted ratios (4(A):1(B):4(C)) for all five crosses.

dominant to a recessive form. Chi-square analysis (Table 5, no. 1) supports this hypothesis.

The linkage data in Table 4 also serve to rule out other, more complex, hypotheses that could be envisioned. For example, one might propose that *imp-11* is a double mutation (unlikely in any case considering its revertability (GOODENOUGH, DETMERS and HWANG 1982) wherein the original *mt*$^-$ locus changed to a normal *mt*$^+$ locus and a second mutation, unlinked to *mt*, modifies *mt*$^+$ expression to produce the *imp* phenotype. In this case, the modified phenotype would occur in the diploid zygospores only when the zygospore is homozygous for *mt*$^+$ *and* homozygous for the second mutation. This greatly reduces the frequency at which imp progeny would be expected, and the hypothesis fits the data poorly (Table 5, nos. 2 and 3). The probability that a

TABLE 4

Segregation of mating phenotypes in tetraploid meiosis

Cross	Frequency of tetrad types		
	(A) 2 minus : 2 plus	(B) 2 imp : 2 minus	(C) 2 minus : 1 imp : 1 plus
a. *imp-11/mt⁻* × *imp-11/mt⁺*	20	5	13
b. *imp-11/mt⁻* × *imp-1/mt⁺*	5	2	6
c. *imp-1/mt⁻* × *imp-11/mt⁺*	7	3	4

The first two diploid strains listed in Table 2 (A and B) were used for cross a. Cross b used diploid strains B and C. In cross c, the parental strains were D and A.

TABLE 5

Chi-square analysis of imp-11 *segregation*

H_o	Expected tetrad ratios (A) : (B) : (C)	χ^2 (2 d.f.)
1	4 : 1 : 4	1.10, $P > 0.50$
2	13 : 1 : 4	6.41, $P < 0.05$
3	37 : 1 : 16	21.9, $P < 0.01$

Tetrads A, B and C have the phenotypes shown in Figure 1. χ^2 tests are used to test three null hypotheses: (1) *mt⁻* was altered to a defective form; (2) *mt⁻* was converted to *mt⁺*, and a second locus that is centromere-linked alters expression; (3) same as (2), except that the second locus is not linked to its centromere. In the double mutation models the second locus is recessive and must be homozygous along with a homozygous *mt⁺* genotype to produce the imp phenotype. This requirement reduces the expected frequency of tetrad B from one in nine to one in 18 (or 1/6 × 1/3) if the second marker is linked to its centromere or one in 54 (or 1/6 × 1/9) if it is unlinked.

mating-locus alteration and a second unlinked mutation are both required in the homozygous condition to produce the *imp* phenotype is even more remote and was not calculated.

Absence of complementation between imp-11 *and* imp-1: Diploids were next constructed to compare the properties of *imp-11* with *imp-1*, a mutation selected in an *mt⁺* background and linked to *mt⁺* (GOODENOUGH, HWANG and MARTIN 1976), which expresses the same nonfusing fringeless phenotype as *imp-11* (GOODENOUGH, DETMERS and HWANG 1982). PEG-induced *imp-1/mt⁺* and *imp-1/mt⁻* diploids behaved as sexually competent plus and minus gametes,

TABLE 6

Frequency of biparental (BP) transmission of chloroplast genes in tetraploid zygotes

Cross	No. of zygotes	% BP	Chloroplast markers	Days after mating
a. mt^+/mt^+ (F) × mt^+/mt^- (E)	30	100	*sr-u-sm2, hrb-u-br202*	5–7
b. mt^+/mt^+ (F) × mt^-/mt^- (H)	40	22.5	*sr-u-sm2, hrb-u-br202*	5
c. $mt^+/imp\text{-}1$ (C) × $imp\text{-}1/mt^-$ (D)	12	91.7	*sr-u-sm2, er-u-11*	7–15
d. $mt^+/imp\text{-}11$ (A) × $imp\text{-}1/mt^-$ (D)	18	88.9	*sr-u-sm2, er-u-11*	7
e. mt^+/mt^+ (G) × $imp\text{-}11/mt^-$ (B)	48	22.9	*sr-u-sm2, er-u-37*	6–8
f. $mt^+/imp\text{-}1$ (C) × $imp\text{-}11/mt^-$ (B)	24	12.5	*sr-u-sm2, er-u-37*	7
g. $mt^+/imp\text{-}11$ (A) × $imp\text{-}11/mt^-$ (B)	46	23.9	*sr-u-sm2, er-u-37*	7–10

Full parental genotypes (A through H) are shown in Table 2. Chloroplast resistance markers are shown for the plus and minus (mating phenotype) parents, respectively. These include resistances to streptomycin *(sr-u-sm2)*, erythromycin *(er-u-37, er-u-11)* and bromacil *(hrb-u-br202)*. BP inheritance was deduced when tetrad progeny displayed phenotypes (including sensitivities) from both parents. Similarly, uniparental inheritance was presumed when the phenotypes of only one parent were detected. No progeny were observed that had uniparental inheritance from the minus parent. "Days after mating" were the number of days between mating and zygote germination.

respectively, indicating that *imp-1* is also recessive to wild-type alleles. Table 4, crosses b and c, show that when tetraploids were created by matings between *imp-1*-containing diploids and *imp-11*-containing diploids and the resultant meiotic products were scored, many of the progeny had the impotent phenotype. Since either mutant allele was present only once in the tetraploid, this indicates that a cell which receives the two different mutant alleles has a mutant phenotype; that is, *imp-1* and *imp-11* fail to complement one another. In fact, the ratios of mutant and wild-type phenotypes are the same as if there were two mutant alleles of the same type in the original tetraploid.

Effect of imp-1 *and* imp-11 *on chloroplast gene inheritance*: Finally, crosses were performed to ask whether either the *imp-1* or *imp-11* mutation has any effect on the transmission of chloroplast genes. The slight leakiness of the haploid *imp-1* strain permits crosses with haploid mt^- strains. The rare zygotes that form have >90% uniparental transmission of chloroplast genes from the *imp-1* strain (data not shown), indicating that the *imp-1* defect has no effect on chloroplast inheritance. Since *imp-11* is nonleaky, this question must be studied in crosses of heterozygous diploids. Previous investigators have shown that when heterozygous diploids (phenotypically minus) are crossed with homozygous mt^+ diploids, the frequency of biparental chloroplast inheritance is >60% (MATAGNE and MATHIEU 1983; EVES and CHIANG 1984), whereas when homozygous mt^- diploids are crossed with homozygous mt^+ diploids, the frequency of biparental transmission is much lower (MATAGNE and MATHIEU 1983). Similar frequencies are obtained using our wild-type diploid strains (Table 6, crosses a and b). Furthermore, when the phenotypically minus diploids also carry the *imp-1* mutation ($mt^-/imp\text{-}1$), a high level of biparental transmission continues to be observed (Table 6, cross c), again demonstrating that the *imp-1* mutation has no effect on this feature of the *mt* locus. If the *imp-11* mutation is introduced into the tetraploids as a heterozygous diploid with a normal mt^+

TABLE 7

Complementation of imp *mutations*

	mt$^-$	*mt*$^+$	*imp-1*	*imp-10*	*imp-11*	*imp-12*
mt$^-$	minus					
mt$^+$	minus	plus				
imp-1	minus	plus	imp			
imp-10	minus	minus	minus	ND		
imp-11	minus	plus	imp	ND	imp	
imp-12	minus	minus	minus	imp	minus	ND

ND, not determined. imp (impotent), plus and minus were the mating phenotypes of diploids. The phenotype of *imp-11/imp-1* diploids was the ability to agglutinate as plus, but with defective sexual cell fusion. The dominance relationship was not determined. *imp-10/imp-12* diploids had the nonagglutinating minus phenotype.

locus, it also has no effect on chloroplast gene transmission (Table 6, cross d). If, however, *imp-11* is introduced as a heterozygous diploid with a normal *mt*$^-$ locus (Table 6, crosses e, f and g), the proportion of biparental zygotes is dramatically reduced to 12–24%, similar to the frequencies reported when homozygous *mt*$^-$ diploids are crossed with homozygous *mt*$^+$ diploids. We conclude, therefore, that the defective mating locus generated by the *imp-11* mutation is recessive to the *mt*$^+$ locus with respect to chloroplast gene transmission and behaves like a normal *mt*$^-$ allele in this regard. In other words, the *mt*$^-$ mutation has allowed expression of several gamete-specific plus traits (agglutination, mating-structure) but has not converted the *mt*$^-$ locus into a locus that behaves like *mt*$^+$ with respect to control over chloroplast gene inheritance (nor with respect to expression of the plus-specific mating-structure fringe).

These observations suggest that it may be possible to select for mutations that affect the ability of *mt*$^+$ to control chloroplast gene inheritance by screening for tetraploids that yield low numbers of biparental progeny.

Complementation analyses of mt*-linked mutations*: Table 7 shows the results of pairwise complementation tests between the various mating-type linked mutations in our collection, performed by creating diploids using PEG-induced cell fusion. The *mt*$^-$ allele is seen to be dominant to all other mating-type "alleles." The *imp-1* and *imp-11* alleles are noncomplementing, and the *imp-10* and *imp-12* (*sad-1*) alleles, which prevent the expression of normal minus agglutinability (COLLIN-OSDOBY and ADAIR 1985), are shown to be recessive in diploids and to be noncomplementing as well. Most interesting, all available *mt* "alleles" are able to complement both *imp-10* and *imp-12*, generating gametes that mate as normally agglutinating minus cells.

DISCUSSION

Several aspects of the data yield new insights on the genetic control of heterothallic mating type in *C. reinhardii*. First, *imp-11* superficially appeared to be a mating-type switch; that is, from minus to a defective plus phenotype. However, complementation tests and the crosses of heterozygous diploids show that there are defects in more than one plus-specific trait. The *imp-11* strain,

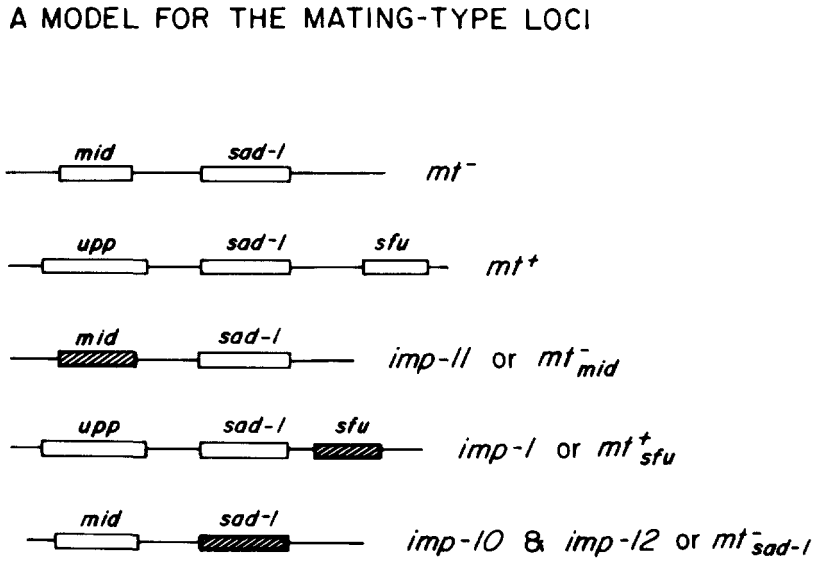

FIGURE 2.—A model for the mating-type loci. The data indicate the existence of at least three mating-related gene functions in the mt^+ locus and two in the mt^-. The shaded regions represent the functions altered or defective in each of the mating locus-linked impotent isolates.

in fact, lacks the necessary information to become wild-type plus—as illustrated by the fact that mutagenesis can produce a reversion to the minus phenotype, but it cannot produce the normal plus phenotype (GOODENOUGH, DETMERS and HWANG 1982). In this respect the *imp-11* mutation resembles the phenotype of the *matα2* mutation in yeast that produces a defective **a** phenotype (MACKAY and MANNEY 1974). For these reasons we propose that *imp-11* is a mutation in function of the mt^- "locus," and our model for the mating-type loci (Figure 2) is constructed on this basis.

In the traditional nomenclature used for Chlamydomonas, mating type is represented by a simple pair of alleles, mt^+ and mt^-; however, the data presented here indicate the existence of several closely linked mating-related functions in the mating locus region. Therefore, in Figure 2 we propose a new nomenclature to designate each of these mutated functions, using a subscript of the mutated locus below the mating type affected. The defects in the various mutants and the functions delineated are detailed as follows.

First, we demonstrate that strains carrying *imp-11* or *imp-1* mutations can complement the sexual adhesion (*sad-1*) mutations, *imp-10* and *imp-12*, extending the earlier observation (HWANG, MONK and GOODENOUGH 1981) that the wild-type mt^+ locus can also complement these mutations. Two explanations can be offered for these results. One is that *sad-1* genes are linked to both mt^+ and mt^- and control an aspect of agglutinin biosynthesis that is common to both plus and minus gametes. This explanation predicts that it should be possible to isolate mt^+-linked mutations affecting plus flagellar sexual agglutinability, although no such mutations have yet been recovered in repeated screens. A second possibility is that *sad-1* genes are associated with both the

mt^+ and mt^- loci, but are only expressed in cells that contain an mt^- locus, the mt^- locus specifying a "*sad-1* activator." Since both explanations predict that *sad-1* is present in both *mt* loci, this feature is incorporated into the mating-locus model presented in Figure 2. Mutations in *sad-1* are written as $mt^-_{sad\text{-}1}$.

The model in Figure 2 also assumes the existence of a gene in the mt^+ locus designated *sfu* for sexual fusion, a gene marked by the *imp-1* mutation (mt^+_{sfu}). Since we show that the *imp-1* mutation is recessive, yet cannot be complemented by *imp-11*, it can be assumed that the *imp-11* strain lacks a functional *sfu* gene. This presumably explains its lack of mating-structure fringe material (GOODENOUGH, DETMERS and HWANG 1982).

The defective mating locus in the *imp-11* strain is shown to be recessive to both mt^+ and mt^-. This observation leads to the proposal that the normal mt^- locus contains a region designated *mid* for minus dominance, and that this region has been rendered dysfunctional by the mutation in the *imp-11* isolate (mt^-_{mid}). Although it is not known why mt^- is normally dominant to mt^+, and therefore no specific functions for *mid* can be cited, an obvious possibility is that *mid* acts to repress the expression of mt^+ sex-limited genes and perhaps also to elicit the expression of mt^- sex-limited genes. An mt^- cell with a dysfunctional *mid* would proceed to express the sex-limited plus genes (*e.g.*, flagellar agglutinins), but would be incapable of expressing any genes encoded exclusively in the mt^+ locus (*e.g.*, the *sfu* gene). Such a speculation for the genesis of the *imp-11* phenotype was previously offered (GOODENOUGH, DETMERS and HWANG 1982). The present report provides genetic evidence to support this speculation, since we show that *imp-11* lacks two mt^+-specific traits, but we stress that the actual function(s) of *mid* remain to be elucidated.

The final genetic activity known to be associated with the mating-type loci confers mt^+ cells with the ability to transmit their chloroplast genomes uniparentally to meiotic products >90% of the time. In heterozygous (mt^+/mt^-) diploids, this trait is manifested as an increased frequency of biparental transmission of chloroplast genomes (MATAGNE and MATHIEU 1983; EVES and CHIANG 1984). There are two previously reported mutations (*mat-1* in a minus and *mat-2* in a plus strain) (SAGER and RAMANIS 1974) that produced altered chloroplast inheritance patterns interpreted as resulting from mating locus-linked mutations. However, the *mat-1* strain is probably diploid and is definitely disomic for chromosome *VI*, having both mt^+ and mt^- loci (N. W. GILLHAM, personal communication), which explains its altered chloroplast inheritance patterns. The *mat-2* strain is no longer available for analysis. In the original report (SAGER and RAMANIS 1974), neither *mat-1* nor *mat-2* produced complete tetrads when crossed with normal haploid strains, suggesting that the altered chloroplast inheritance patterns were due to the ploidy of the presumed mutants and not to defects in genes affecting organellar inheritance. We show here that the *imp-1* mutation does not affect this uniparental plus (*upp*) function, indicating that the lesion affecting the *sfu* gene does not extend into *upp*. The defective mating locus carried by *imp-11* has no *upp* activity, as expected if it derives from an mt^- locus. Thus, we have no mt^+_{upp} mutations in this

proposed locus, but have documented that a *upp* function exists in mt^+ separable genetically from *sfu*.

The real function of *upp* is unknown, but it is possibly a regulatory locus related both to chloroplast gene inheritance and the zygote maturation process. Three observations have led us to suggest this possibility: (1) When a newly formed diploid bypasses zygote maturation in favor of vegetative growth, the uniparental chloroplast transmission system is disrupted (GILLHAM 1978; VANWINKLE-SWIFT 1976). (2) The *imp-11* mutation arose in a minus strain, and although this strain is able to form quadriflagellate cells at low frequency when mated with mt^- cells, unlike the *imp-1* strain it never produces zygotes. Apparently *imp-11* lacks some capability vital to zygote maturation in addition to its other defects. (3) VANWINKLE-SWIFT (1984) reports a zygote maturation-defective mutation in the homothallic species *Chlamydomonas monoica* with properties that could be explained by a defect in a similar gene function.

There are some similarities between the mating-type system in *C. reinhardii* and the **a**/α system in *Saccharomyces cerevisiae*. Like yeast, the Chlamydomonas mating locus may contain closely linked regulatory genes. Unlike yeast, however, we have no evidence of possible homothallism. There are other Chlamydomonas species that are homothallic (BURRASCANO and VANWINKLE-SWIFT 1984; VANWINKLE-SWIFT and AUBERT 1983), but *C. reinhardii* may either have lost this potentiality or never have acquired it. Although we have made repeated screens to obtain mating-type switches in several strains of *C. reinhardii*, we have never found a *bona fide* switch ($<5 \times 10^{-7}$, data not shown).

The mating-locus model presented in Fig. 2 is minimal in that additional functions controlled by *mt* may be revealed by new *mt*-linked gene mutations. Meanwhile, the hypothesis that *imp-11* represents a mutated mt^- locus leads to an important deduction; namely, that all the plus phenotypes displayed by *imp-11* gametes are specified by genes that lie outside the mt^+ locus or are present in both mating-type loci. It is already clear that two loci unlinked to mating type control plus flagellar agglutinability (GOODENOUGH, HWANG and WARREN 1978); it can now be predicted that any additional genes specific for this trait will also map outside of mt^+ or be a part of both mating-type loci. Similarly, information for the construction of a doublet zone and an actin-filled fertilization tubule, both traits restricted to plus gametes (GOODENOUGH and WEISS 1975), is predicted not to reside exclusively in mating locus genes. Therefore, while the model in Figure 2 is minimal, the mt^+ locus is not expected exclusively to encode many more structural genes affecting gametic traits. Whether the mt^- locus is similarly constructed awaits the isolation of a mutation in a mt^+ cell that produces a quasi-minus phenotype, equivalent to the defective plus phenotype of *imp-11*.

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