The Unusual Sexual Preferences of a Chlamydomonas Mutant May Provide Insight Into Mating-Type Evolution

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ABSTRACT

Chlamydomonas monoica undergoes intraclonal mating-type differentiation (homothallism). Although the species differs in this regard from the more commonly studied heterothallic C. reinhardii, cell-cell interactions and progression of the sexual cycle are similar for many homothallic and heterothallic species of the genus. Regulation of chloroplast gene transmission by the nuclear mating-type alleles (mt+ and mt−) is another common denominator for Chlamydomonas species studied thus far. We have previously reported the use of chloroplast inheritance patterns to identify mutants of C. monoica that have lost the potential to function as the mt+ mating-type. A similar screening procedure led to the isolation of an unusual mutant, mtl-3 whose phenotype is less readily explained. Chloroplast gene transmission patterns in crosses involving mtl-3 suggest that the mtl-3 strain mates preferentially as mt−. However, normal mating efficiencies and high zygospore viability are observed in clonal culture, indicating the unbiased production of functional opposite mating-types. By construction of appropriately marked strains we have been able to show that mtl-3 mt− gametes prefer the mt+ gametes of their own strain. A model is presented which invokes unequal crossing over between highly homologous flagellar agglutinin genes to account for the unusual properties of the mtl-3 strain and for the evolution of mating barriers within the genus.
the opposite cell type. The *swi* mutants of homothallic yeast offer examples of this type of defect (EGEL, BEACH and Klar 1984; STERN, JENSEN and HERKOWITZ 1984). Alternatively, mutations affecting a function unique to one mating-type and essential for sexual reproduction, such as the *mt* mutant strains of yeast (MACkAY and MANNEY 1974; MICHAEL and GUTZ 1987) will effectively block homothallism without directly preventing cells from choosing between the two alternative developmental pathways. In C. *monoica*, for example, *mt* cells may be produced which synthesize a defective *mt* specific flagellar agglutinin, or an abnormal *mt* specific membrane receptor required for cell fusion while the same strain produces normal *mt* cells. Because the *mt* locus of Chlamydomonas acts as a master regulator (reviewed by GOODENOUGH and FERRIS 1987), all loci under *mt* regulation, as well as the *mt* locus itself, if mutated, may result in barriers to homothallism and promote unidirectional heterothallism (i.e., selfing is eliminated and only one mating type participates in crossing).

We have described previously two mating-type limited mutant alleles that affect *mt* specific functions in C. * monoica* (VANWINKLE-SWIFT and HAHN 1986). The *mt*-1 mutant strain continues to produce cells of opposite mating-type which can pair and fuse to form the zygote, the lethal (and therefore selective) effect of the mutation is expressed after mating and involves loss of *mt* derived chloroplast DNA which would have been protected and preserved in the absence of the *mt*-1 mutation (VANWINKLE-SWIFT and SALINGER 1988). The *mt*-2 mutant strain, in contrast, fails to produce mating pairs in clonal culture and may be defective for an early step in *mt* cell type differentiation. Both the *mt*-1 and the *mt*-2 strains can produce viable sexual progeny only when provided with normal *mt* gametes from a different strain.

Here we describe a new mutant strain, *mt*-3 which was selected on the basis of its pattern of chloroplast gene transmission in crosses. Chloroplast gene inheritance in Chlamydomonas is under tight regulation by the nuclear *mt* alleles such that only the chloroplast markers derived from the *mt* parent are effectively transmitted to the zygote progeny (SAGER 1954; VANWINKLE-SWIFT and AUBERT 1983). Chloroplast marker transmission in crosses between *mt*-3 strains and standard homothallic strains suggested that the *mt*-3 strain was consistently acting as the *mt* parent in these crosses. However, the *mt*-3 strain itself continued to show homothallic mating-type differentiation with the subsequent production of viable sexual progeny. We will provide evidence that the *mt*-3 *mt* gametes prefer the *mt* gametes of their own strain, while the *mt*-3 *mt* gametes retain their ability to pair with *mt* cells of other strains as well as their own. We conclude that both mating-types of the *mt*-3 strain must have been altered in some way with regard to cell-cell recognition, resulting in selection for increased inbreeding within the strain as a whole. Thus, although the initial screening procedure was designed to identify barriers to selfing, the *mt*-3 strain instead shows a mating-type-limited barrier only to crossing.

### MATERIALS AND METHODS

**Strains and culture conditions:** All strains used in this study are derived from strain wt135e (VANWINKLE-SWIFT and BURRASCANO 1983) of C. *monoica* (STREHLOW 1929). Table 1A shows the previously constructed genetically marked strains used in this study and their phenotypes; strains constructed during the course of this work are listed in Table 1B, along with their precise derivations. Many of these strains carry recessive lethal zygote-specific mutations (zym) which serve to select against self-matings when performing crosses for genetic analysis (VANWINKLE-SWIFT and BURRASCANO 1983). All strains are routinely maintained

<table>
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<tr>
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<th>References*</th>
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<tr>
<td>zym-6</td>
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<td>mtl-6</td>
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* Phenotypes: a, mating-type independent zygotic lethal; b, *mt* limited zygotic lethal; c, *mt* limited mating defect; d, *mt* limited mating defect; e, Mendelian spectinomycin resistance; f, Mendelian erythromycin resistance; g, uniparentally inherited erythromycin resistance; h, uniparentally inherited streptomycin resistance.

* References: 1, VANWINKLE-SWIFT and BAUER (1982); 2, VANWINKLE-SWIFT and BURRASCANO (1983); 3, VANWINKLE-SWIFT and AUBERT (1983); 4, VANWINKLE-SWIFT and HAHN (1986); 5, VANWINKLE-SWIFT and SALINGER (1988); 6, VANWINKLE-SWIFT and S. Slack, unpublished.
as vegetative cultures on agar solidified Bristol’s medium (BM; Bischoff and Bold 1963) under continuous cool white fluorescent light (5–5000 lux) at 18–22°C. To induce mating and zygote development, vegetative cells are suspended in mating-induction medium (LPN) as described in VanWinkle-Swift and Bauer (1982). Mature zygospores are maintained on BM agar in the dark for 3–10 days prior to induction of germination by return to continuous light. (At this time, the plates are inverted over chloroform for 20 sec to kill unmated cells and select for the zygospores.)

**Mutagenesis and mutant screening:** A strain carrying the zygotic lethal marker zym-6 was mutagenized by growth in the presence of 0.25 mM fluorodeoxyuridine, an effective nuclear mutagen for C. monica (VanWinkle-Swift 1980), following the protocol described by VanWinkle-Swift and Hahn 1986. After 7 days of mutagenesis, the culture was diluted and plated on BM agar for the isolation of individual post-mutagenesis clones. Individual clones were picked by sterile toothpick and transferred to fresh BM agar for additional growth.

Each post mutagenesis clone was then mated to the ery-1 spr-fld-1 zym-13 strain. This strain carries the zym-13 zygotic lethal mutation complementary to zym-6, and a uniparentally inherited chloroplast antibiotic resistance marker, ery-ul conferring resistance to erythromycin. (The spr-fld-1 mendelian spectrinclonin resistance allele was not scored in the preliminary studies.) After allowing 7 days for mating and zygote maturation, an aliquot from each mating was “spot-plated” onto BM agar (25 crosses per plate) and stored in the dark for at least 3 days. The plates were then inverted over chloroform, returned to the light, and after allowing for en masse germination of the zygospores, the progeny lawns were replica-plated to BM supplemented with 300 μg/ml erythromycin. Little or no growth on erythromycin indicated a cross in which the erythromycin resistance allele was not transmitted efficiently.

Post mutagenesis subclones showing preferential transmission of erythromycin resistance were then retested by repeating the cross. An aliquot from the cross was diluted and spread on BM agar for the isolation of individual zygote clones. A sample of at least 100 zygote clones from a given cross was picked by sterile toothpick to fresh BM plates, and replica-plated to erythromycin medium to determine the precise frequency of resistant and sensitive zygote clones. In the initial screening, 3600 post mutagenesis clones were tested; 32 clones were retested; and 1 clone (now referred to as mtl-3 zym-6) showed consistent biased transmission of its chloroplast marker (erythromycin-sensitive allele) in the test cross.

**Characterization of the mtl-3 strain:** As detailed in results, a number of crosses were performed involving the original mtl-3 zym-6 strain to determine the phenotype associated specifically with the mtl-3 mutation (which required removing the zym-6 allele from the strain by outcrossing), to establish linkage relationships with other markers, and to determine any epistatic relationships with other mtl alleles via isolation and characterization of appropriate double mutant strains.

The procedures for zygote clone analysis and for tetrad analysis have been described in detail elsewhere (VanWinkle-Swift and Burrascano 1983; VanWinkle-Swift and Hahn 1988). To score for the mtl-3 allele, tetrad products derived from all crosses were subsequently crossed to the ery-ul zym-1 strain (zym-1 is complementary to all other zym alleles used in the various crosses.) Each cross of second generation tetrad products was “spot-plated” (25 crosses per plate), the zygospores germinated en masse, and the progeny lawns replicated to BM supplemented with erythromycin. Little or no growth of the lawn on erythromycin indicated a tetrad product carrying the mtl-3 allele (and transmitting erythromycin sensitivity preferentially). Tetrad analysis for mapping of mtl-3 relative to zym loci was accomplished by complementation testing of each tetrad product against standard zym tester strains (VanWinkle-Swift and Burrascano 1983). The presence or absence of the mtl-2 allele (resulting in mating only as mt') in tetrad products was determined by testing for zygote formation (light microscopy of 7-day-old LPN cultures) with an mtl-2 tester strain. The presence or absence of the mtl-1 allele was determined by a complementation test for germination of zygospores after crossing each tetrad product to an mtl-1 tester strain.

Ultimately, characterization of the mtl-3 phenotype required estimation of self-mating and crossing efficiencies for various strains. To determine self-mating efficiency, a given strain was incubated in LPN medium for 7 days. An aliquot was then examined by light microscopy. Selfing efficiency is expressed as a percent derived from the ratio: 2(no. of zygotes)/(no. of unmated cells + 2(no. of zygotes)). This approach can be used for all strains whether or not they carry zym alleles because the maturation-defective zygotes (zym) can still be easily recognized as zygotes (VanWinkle-Swift and Burrascano 1983). To determine the crossing efficiency for two strains carrying complementary zym alleles, it was necessary to distinguish between wild-type zygospores and zym zygotes via light microscopy. The crossing efficiency is expressed as a percent derived from the ratio: 2(no. of wild-type zygospores)/(2(no. of zym zygotes) + 2(no. of wild-type zygotes) + no. of unmated cells).

**RESULTS**

**Isolation of the mtl-3 mutant strain:** After growth of the zym-6 zygotic lethal strain in the presence of fluorodeoxyuridine for 7 days, 3600 individual post-mutagenesis clones were isolated. Analysis of the transmission of the chloroplast antibiotic resistance marker, ery-ul in crosses between each postmutagenesis zym-6 clone (erythromycin sensitive) and a complementary zygotic lethal strain carrying a uniparentally inherited erythromycin resistance marker (ery-ul), revealed 32 crosses showing transmission favoring the erythromycin sensitive chloroplast allele. Repeated analysis of these 32 crosses identified a single clone (hereafter referred to as mtl-3 zym-6) which consistently transmitted its chloroplast allele (erythromycin sensitivity) preferentially. By analogy to the mating-type directed transmission of chloroplast markers in Chlamydomonas reinhardtii (Sager 1954; cf. VanWinkle-Swift and Aubert 1983), the mtl-3 zym-6 strain appeared to be acting preferentially as the mt' parent in the test cross.

**Transmission of the ery-ul chloroplast marker in crosses involving the mtl-3 zym-6 strain:** The pattern of chloroplast gene transmission in crosses involving mtl-3 zym-6 was compared to that observed in standard C. monica crosses (Table 2A). As expected for crosses between chloroplast antibiotic resistant and sensitive homothallic strains, control crosses show an approximately equal frequency of zygote clones receiving the
antibiotic resistant, or the sensitive chloroplast allele (VanWinkle-Swift and Aubert 1983). This "bidirectional uniparental inheritance" results from the coproduction of mt" and mt" gametes by both the resistant and sensitive strains, and thus the simultaneous occurrence of reciprocal crosses. [The use of complementary zygote specific lethal alleles (zym) in crosses eliminates self-mating events from the analysis of mixed mating populations.]

In contrast to standard crosses, crosses involving the mtl-3 strain and a homothallic partner produced an excess of zygote clones receiving the chloroplast marker of the mtl-3 parent, i.e., erythromycin-resistant zygote clones were rare (Table 2A). Thus it appears that the mtl-3 mt" gametes and the mt" gametes of the standard homothallic strain carrying the resistance allele are somehow being excluded from the crossing events or are giving rise to nonviable zygotes. This pattern of mtl-3 directed chloroplast marker transmission is not locus specific—a similar bias in transmission is observed for a uniparentally inherited streptomycin resistance allele (Table 2A). The pattern of chloroplast marker inheritance observed in crosses involving mtl-3 is in contrast to that previously described for the mating-type-limited mutants, mtl-1 and mtl-2 (Table 2B and VanWinkle-Swift and Hahn 1986). The mtl-2 mutant strain fails to produce zygotes in clonal culture, appears to mate preferentially as mt", and is thus functionally heterothallic. In crosses to an ery-u1 homothallic strain, erythromycin resistant zygote clones are recovered preferentially as the mtl-2 mt" gametes select mt" partners which carry and transmit the ery-u1 chloroplast allele.

In contrast, mtl-1 strains continue to undergo intraclonal zygote formation (self-mating) as a consequence of continued coproduction of opposite mating-types, but produce homozygous mtl-1 zygospores that are nonviable. In crosses, heterozygous zygospores produced via matings involving an mtl-1 mt" partner fail to germinate, while those produced via mtl-1 mt" partners show normal viability (VanWinkle-Swift and Hahn 1986). The mtl-1 strain has been shown to be defective for the mt" directed protection of chloroplast DNA in zygotes (VanWinkle-Swift and Salinger 1988). The mtl-1 and mtl-2 mutants are similar in that both strains fail to transmit their chloroplast genes effectively in crosses (due to mt" limited gene mutations), and, in this regard, both differ from the mtl-3 strain described here. The pattern of chloroplast marker transmission in crosses suggests that the detrimental effect of mtl-3 is directed toward the mt" gametes of the mutant strain.

Distinguishing between biased, biparental and strictly uniparental chloroplast marker transmission: The occasional transmission of the antibiotic resistance allele from the mtl-3* parent in the test crosses could result from revertants or contaminating nonmutant cells in the original postmutagenesis clone giving rise to a low frequency of normal mt" gametes. Therefore the original mtl-3 zym-6 strain was subcloned and several subclones were tested for chloroplast marker transmission. Transmission of the erythromycin resistance allele was inefficient but detectable for each subclone and ranged from 1–13% among the nineteen mtl-3 zym-6 somatic subclones used as parents (Table 3). Thus chloroplast gene transmission by mtl-3 can be described as strongly biased but not strictly unidirectional (i.e., the mtl-3 strain appears to function as mt" at a low frequency).

Although the mtl-3 zym-6 strain undergoes self-
mating and thus appears to produce mating competent gametes of both mating-types, the transmission of chloroplast markers in crosses suggests that mtl-3 in some way prevents the usual pattern of transmission associated with mt+ gametes. If this were due simply to excess production of mtl-3 mt+ gametes, the self-mating efficiency of the mutant strain would be expected to be reduced to a level well below that of standard strains. Because we have not found this to be the case (data not shown), we have considered the possibility that mtl-3 mt+ gametes, although mating as mt+, may behave as mt+ with regard to chloroplast gene transmission (i.e., they would transmit their chloroplast genomes to the zygote progeny). However, such a model predicts that a mating between an mtl-3 mt+ gamete and a normal mt+ gamete would produce a zygospore receiving chloroplast markers from both parents, i.e., a biparental zygospore. In the absence of subcloning, such a zygospore from our original test crosses (see Table 2) would score simply as erythromycin resistant although the individual biparental zygote clone would also contain erythromycin-sensitive cells. If the mtl-3 mutation were to promote biparental inheritance, the test cross (to a strain carrying the erythromycin resistance allele) should have produced equal frequencies of “resistant” zygospore clones (actually biparental and derived from the mtl-3 mt+ gametes) and erythromycin-sensitive zygote clones (derived from the mtl-3 mt+ gametes). Instead we recovered an excess of erythromycin sensitive zygote clones (Table 1) which can be unambiguously classified as uniparental. Nevertheless, to further rule out the possibility of biparental inheritance, seven erythromycin-resistant zygote clones from the original test cross were removed from nonselective medium and subcloned. Approximately 100 subclones of each were tested for resistance. All subclones scored as erythromycin resistant (data not shown) providing no evidence for biparental gene transmission.

The effect of mtl-3 on other aspects of the sexual reproductive cycle: Although intraclonal mating efficiencies (selfings) can vary greatly from one strain to another (and upon repeated testing of a single strain), the self-mating efficiencies observed for the mtl-3 zym-6 strain were within the range typical of standard homothallic strains (10–60%) and well above those observed for a known homothalism-defective mutant, mtl-2 (0–2%). In this regard the mtl-3 mutant resembles the mtl-1 strain which also retains the ability to self-mate (but produces germination defective homzygotes). Perhaps mtl-3 also exerts its effect at the time of germination rather than on mating per se. However, the chloroplast marker transmission pattern for mtl-3 is opposite that of mtl-1 (see Table 2). Therefore, if the mtl-3 gene function is also essential for germination, this locus must be expressed only in mt+, while mtl-1 is a mt+ specific function.

To test this hypothesis, zygospore germination was evaluated in crosses between the original mtl-3 zym-6 strain and a standard homothallic strain (carrying a complementary zym allele). Only those zygospores derived from matings involving a mtl-3 zym-6 mt+ parent, i.e., 50% of the zygospore sample, are predicted to be nonviable if the mtl-3 defect affects only the mt+ gametes. Zygospore populations derived from an mtl-3 self-mating were also analyzed. (The latter test depends upon the absence of any zygotic lethal (zym) markers and made use of an mt-3 tetrad product derived from the original test cross.) Because the zygospores produced by selfing mtl-3 would all be derived from matings which included the hypothesized defective mtl-3 mt+ parent, they should all fail to germinate. (However, the occasional transmission of chloroplast markers from the mtl-3 mt+ parent in mtl-3 X mtl-3 mt+ crosses suggests a low level of leakiness associated with the mutant phenotype. The leaky nature of the mtl-3 mutation might also allow occasional homozgyous mtl-3 germination). The results of this analysis, shown in Table 4, fail to confirm the presence of a mating-type-limited defect in zygospore germination. Zygospores homozygous or heterozygous for mtl-3 show similar germination frequencies comparable to values obtained for standard strains. Analysis of viability following tetrad dissection of germinated zygospores also failed to provide evidence of a mating-type-limited meiotic defect.

Assessment of linkage and epistasis between mtl-3, a putative defective mt+ specific allele, and mtl-1, an allele associated with an mt+ limited function: If the effect of mtl-3 on chloroplast gene transmission is direct (as opposed to an effect on mating-type interactions or homothallism), an additional assumption must be made: the mtl-3 mt+ gametes must not only be able to transmit their chloroplast genes as if they were mt+ but must also be able to block transmission from a normal mt+ partner (otherwise we would observe biparental inheritance, see previous section). The mtl-1 mutant strain cannot transmit its chloroplast genes effectively, due to an apparent failure to protect its chloroplast DNA in the zygote (VanWinkle-Swift and Salinger 1988). Because this allele does affect chloroplast gene transmission directly and does not alter the ratio of mt+ and mt- gametes in the population, the linkage relationship between mtl-1 and mtl-3, as well as the phenotype of the double mutant were of particular interest.

Zygospores were isolated and dissected from a cross between the mtl-3 zym-6 strain and a strain carrying the mtl-1 mutation. Because mtl-1 is a mt+ limited lethal mutation (VanWinkle-Swift and Hahn 1986), the zygospores which germinate are presumably de-
rived from matings between mtl-3 zym-6 mt^ allele and the mtl-1 strain. Fifty six tetrads from the cross were analyzed. Table 5A shows sample data from the three types of tetrads obtained with regard to the mtl-1 and mtl-3 phenotypes. Each tetrad product was assayed for its ability to promote the test cross served to identify the double mutant and indicate a synergistic interaction between the mtl-1 and mtl-3 mutant alleles.

Although the transmission pattern shown by the mtl-1 mtl-3 double mutant is highly variable—ranging from 0 to 100%—the test crosses for analysis of mtl-1 mtl-3 chloroplast marker transmission always yielded extremely small sample sizes (see Table 5A). Indeed, the difficulty in obtaining viable zygote clones from the test cross served to identify the double mutant tetrads. Applying this approach for defining genotypes and classifying tetrads, standard tetrad analysis could be used to demonstrate the absence of linkage between mtl-1 and mtl-3 (Table 6, A and B).

Crosses involving mtl-1 mtl-3 double mutants were repeated and analyzed for zygospore germination. Table 7 summarizes the effects of mtl-1 and mtl-3 on the germination of zygospores homoyzogous or heterozyzogous for these markers. The small sample sizes obtained for test crossed between mtl-1 mtl-3 double mutant tetrads products and the ery-u1 zym-1 tester strain reflect decreased germination in these crosses and indicate a synergistic interaction between the mtl-1 and mtl-3 mutant alleles.

In a self-mating of mtl-1, very few zygospores are viable (Table 7) because the required mtl-1^ function cannot be provided by either parent (VanWinkle-Swift and Hahn 1986). In a cross to mtl-1^, only about half of the zygospores (heterozygous for mtl-1) germinate (Table 7 and VanWinkle-Swift and Hahn 1986) because only the mtl-1^ mt^ gametes express the essential function and can compensate for the mtl-1 defect of their partners (in fact the defect is irrelevant to their mt^ partners, although essential to the zygote). The very poor germination of heterozygotes produced from a cross between mtl-1mtl-3 double mutants and a mtl-1^ mtl-3^ strain suggests again that mtl-3 causes preferential expression of mt^ limited functions, including that defined by the mtl-1 locus. This preferential expression leads to the selection of mt^ partners which fail to express the essential mtl-1^ allele and, consequently, to the formation of nonviable zygotes. The mtl-3 allele thus appears to be epistatic to mtl-1.

Heterozygosity for mtl-1 and mtl-3 does not, in itself, lead to the detrimental effect on germination. Heterozygotes produced by crossing mtl-1 and mtl-3 (i.e., markers in repulsion) show high viability. In fact, these zygotes show improved germination relative to mtl-1 heterozygotes not carrying an mtl-3 allele (Table 7). The explanation is as follows. In a repulsion cross, mtl-3, acting preferentially as mtl^, will select mtl-1 mt^- partners which fail to express the mtl-1 defect (because this locus is mt^ specific). The resultant zygospores will germinate normally. In a coupling cross, however, mtl-1 mtl-3 gametes acting preferentially as mtl^- (due to the effect of mtl-3) also express the mtl-1 defect and select mtl^- partners unable to compensate for the defect due to the absence of expression of the needed wild-type mtl-1^ function.

mtl-3 mt^- gametes fail to transmit chloroplast genes and obey the usual rules for mating-type-directed uniparental inheritance: Self-mating tests on mtl-3 strains indicated the coproduction of mt^ and mt^- gametes. However, analysis of chloroplast marker transmission in outcrosses suggested that either mtl-3

### Table 4

<table>
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<th>Table 4</th>
<th>Effect of the mtl-3 mutant allele on zygospore germination and postmeiotic viability</th>
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<tr>
<td>Homozygous mtl-3*/mtl-3*</td>
<td>mtl-3 × mtl-3^</td>
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</table>

^ N = the number of zygospores or tetrads analyzed.

^ Complete tetrads are those in which all four meiotic products survive and give rise to a macroscopic colony.

^ Excess cells of the ery-ul spr-fd-1 zym-13 strain were used in the cross to decrease the frequency of zygospores derived from mtl-3 self-mating.

^ The mtl-3 strain is a tetr product from the cross mtl-3 zym-6 × ery-u1 spr-fd-1 zym-13.
TABLE 5
Analysis of epistatic interactions between the mtl-1 and mtl-3
mutant alleles

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<th>Growth on Speciomycin</th>
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<th>Percent Ery-1+ Transmission†</th>
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</tr>
<tr>
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<tr>
<td>C</td>
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</tr>
<tr>
<td>D</td>
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B. Summary of ery-1+ transmission patterns in crosses between tetrad products (erythromycin sensitive) from the cross mtl-3 sym-6 × spr-fd-1 mtl-1 and the ery-1 sym-1 strain (erythromycin resistant)

<table>
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<th>Tetrad product genotype</th>
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</tbody>
</table>

* The presence of the sym-6 or mtl-1 allele was determined by complementation tests in which each tetrad product is allowed to mate with sym-6 and mtl-1 tester strains and scored for the presence (+) or absence (-) of viable zygotes.

† Each tetrad product (erythromycin-sensitive) was crossed to the ery-1 sym-1 (erythromycin-resistant) strain and a sample of zygospore clones (sample size is given in parentheses) from the cross was tested for resistance.

‡ Genotypes for the mtl-1 and mtl-3 loci only are indicated.

mt- gametes are greatly reduced in numbers or these mt- gametes transmit chloroplast markers like mt+ . In an attempt to resolve this question, the mtl-3 strain was crossed to mtl-2, a strain which produces only functional mt- gametes. Because the mtl-3 and mtl-2 effects on chloroplast marker transmission are opposite and easily distinguished from the wild-type recombinant, the double mutant could again be easily identified by process of elimination. Sample tetrad data for defining genotypes and classifying tetrads are given in Table 8A, and the results of linkage analysis (showing that mtl-2 and mtl-3 are unlinked) are presented in Table 6C.

The mtl-2 mtl-3 double mutant fails to transmit its chloroplast allele in the test cross, as expected for a mt+ strain (Table 8B). Thus mtl-2 (conferring the mt+ phenotype) is epistatic to mtl-3. Also as expected, the mtl-2 mtl-3 double mutants do not self-mate (Table 8C) due to the presence of the mtl-2 mutation. However, unexpectedly, the double mutant strains also cross very inefficiently resulting in poor zygote yields in test crosses (Table 8C). This observation, coupled with the evidence that the mt+ gametes of mtl-3 obey normal rules of chloroplast inheritance, made us begin to suspect that the apparent conflict between self-mating tests (implying the presence of functional mt+ gametes in normal frequencies), and tests on chloroplast inheritance (implying an excess of functional mt+ gametes in the mtl-3 strain) might be due to the precise nature of the mating interactions occurring in selfing, vs. crossing populations.

mt-3 mt+ gametes prefer mtl-3 mt+ partners: To determine whether mtl-3 mt- gametes were prone to "self" rather than "cross," a mixed population of three appropriately marked strains was used as the source of zygospores. Included in this ménage à trois were: (a) a strongly homothallic mtl-3 strain carrying a Mendelian mutation (spr-fd-1) conferring spectinomycin resistance; (b) a functionally heterothallic (non-self-mating) mt- strain (mtl-2) carrying a Mendelian erythromycin resistance allele (ery-10); and (c) a functionally heterothallic (non-self-mating) mt+ strain (mtl-6). VANWINKLE-SWIFT and S. SLACK, unpublished results). As indicated in Table 9, the segregation ratios for the Mendelian markers observed in tetrads iden-
Table 7: Effect of mtl-1 and mtl-3 mutant alleles on zygospore germination

<table>
<thead>
<tr>
<th>Zygospore genotype*</th>
<th>Derivation</th>
<th>Percent germination (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtl-1* mtl-3*</td>
<td>(a) Self-mating of wild type</td>
<td>95 (120)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td>(b) zym-6 × ery-1 × yzm-1</td>
<td>82 (51)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td>(a) mtl-1 zym-6 tetrad products</td>
<td>66 (59)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td>(b) spr-fd-1 zym-6 × yzm-1</td>
<td>45 (69)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td>(c) mtl-1 zym-6 × yzm-13</td>
<td>33 (52)</td>
</tr>
<tr>
<td>mtl-1 mtl-3*</td>
<td>(a) self-mating of mtl-1 tetrad products (from the cross mtl-3 zym-6 × spr-fd-1 mtl-1)</td>
<td>0 (114)</td>
</tr>
<tr>
<td>mtl-1 mtl-3*</td>
<td>(b) self-mating of the spr-fd-1 mtl-1 strain</td>
<td>0 (34)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td>(a) mtl-3 zym-6 × ery-1 × yzm-1</td>
<td>77 (100)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td>(b) mtl-3 zym-6 tetrad products (from the cross mtl-3 zym-6 × spr-fd-1 mtl-1)</td>
<td>98 (82)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td>(c) mtl-3 zym-6 × yzm-13</td>
<td>76 (75)</td>
</tr>
<tr>
<td>mtl-1 mtl-3*</td>
<td>Self-mating of mtl-3 tetrad products (from the cross mtl-3 zym-6 × spr-fd-1 mtl-1)</td>
<td>0 (95)</td>
</tr>
<tr>
<td>mtl-1 mtl-3*</td>
<td>(a) mtl-1 zym-6 × ery-1 × yzm-1</td>
<td>70 (104)</td>
</tr>
<tr>
<td>mtl-1 mtl-3*</td>
<td>(b) mtl-1 zym-6 × yzm-13</td>
<td>82 (98)</td>
</tr>
<tr>
<td>mtl-1 mtl-3*</td>
<td>(a) mtl-1 zym-6 × ery-1 × yzm-1</td>
<td>0 (48)</td>
</tr>
<tr>
<td>mtl-1 mtl-3*</td>
<td>(b) mtl-1 zym-6 × yzm-13</td>
<td>0 (54)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td>mtl-1 mtl-3 zym-6 tetrad products</td>
<td>2.8 (36)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td>crossed to ery-1 × yzm-1</td>
<td>5.9 (34)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td></td>
<td>14 (49)</td>
</tr>
<tr>
<td>mtl-1 mtl-3*</td>
<td></td>
<td>2.2 (46)</td>
</tr>
<tr>
<td>mtl-1* mtl-3*</td>
<td></td>
<td>6.1 (33)</td>
</tr>
</tbody>
</table>

* Genotypes are designated only with regard to the mtl-1 and mtl-3 loci.

N = number of zygospores analyzed for each cross; in some cases more than one tetrad product of the given genotype was used to obtain the diploid zygospore genotype—hence more than one calculation of percent germination is given.

Heterozygosity at both mtl-1 and mtl-3 loci produced by a cross with the markers in coupling, i.e., mtl-1 mtl-3 × mtl-1 mtl-3*.

Heterozygosity at both mtl-1 and mtl-3 loci produced by a cross with the markers in repulsion, i.e., mtl-1 mtl-3* × mtl-1 mtl-3.

...tifies the precise mating event which produced each zygospore analyzed. In two separate experiments, the mtl-3 mt" gametes showed an eightfold preference for mtl-3 mt" gametes as compared to mtl-6 mt" gametes. The mtl-2 mt" gametes also showed a preference for mtl-3 mt" gametes, but it was much weaker. We do not know whether this reflects an inherent strength of mtl-3 mt" gametes (in addition to the apparent weakness of mtl-3 mt") or simply variation in the efficiency of gametogenesis for different strains. In mating induction medium, the cells undergo several divisions before pairing is initiated. Furthermore, once pairing does occur, it is asynchronous. As a consequence we cannot precisely control the numbers of competent gametes of the various strains available at a given point in time. Nevertheless, the considerable excess of matings involving mtl-3 mt" gametes, and the remarkable similarity in the data from the two experiments support the hypothesis that the participation of mtl-3 mt" gametes in selfing vs. crossing events is not equivalent. It is difficult to explain such biased behavior without assuming that both the mt" and the mt" gametes of the mtl-3 strain are in some way altered relative to the mating phenotypes of other strains.

DISCUSSION

The mtl-3 mutation was first recognized by its effect on chloroplast gene transmission in crosses. The chloroplast marker (erythromycin sensitivity) carried by the mtl-3 mutant strain was transmitted preferentially, suggesting that this strain functioned preferentially as the mt" partner in crosses with wild-type strains. We initially assumed that a barrier to homothallism, i.e., the absence of functional mt" gametes in a clonal population of mtl-3 cells would also be evident. Thus, the continued self-mating exhibited by the strain was unexpected.

The apparent conflict between conclusions reached on the basis of self-mating tests (both mating-types appear to be functional) and those based on chloroplast marker transmission in crosses (mt" gametes are apparently defective or reduced in frequency) can be resolved if the mtl-3 mt" gametes interact differently with the mt" gametes of their own strain than with those of standard strains. We have provided evidence here that although the mtl-3 mt" gametes can mate effectively with mt" gametes of their own strain, they mate inefficiently with strains not carrying the mtl-3 mutation. As a consequence, when a cross is performed between the mtl-3 strain and a strain carrying a distinctive chloroplast marker, the matings which occur will be primarily between mtl-3 mt" gametes and the mt" gametes of the strain carrying the chloroplast resistance marker. Since it is the chloroplast marker of the mt" parent which is transmitted in Chlamydomonas (SAGER 1954; McBRIEDE and McBRIEDE 1975), the cross shows inefficient transmission of the resistance allele.

Taking into account the mt" specific limitation on crossing of mtl-3 strains, the phenotypes of the mtl-1 mtl-3 double mutant can also be readily explained. In a cross involving an mtl-1 mtl-3 parent, the double mutant will cross effectively only as mt"; however, this is also the mating-type in which the zygotic lethal effect of mtl-1 is expressed (VAN WINKLE-SWIFT and HAHN 1986). Consequently, very few of the zygospores from this cross will germinate (see Table 7).
A. Sample raw data for the classification of tetrads from the cross mtl-3 zym-6 × ery-ul spr-fd-1 mtl-2

<table>
<thead>
<tr>
<th>I.D. No.</th>
<th>× sym-6</th>
<th>× mtl-2</th>
<th>Percent ery-ul transmission</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>10A</td>
<td>-</td>
<td>-</td>
<td>91 (450)</td>
<td>mtl-2 mtl-3*</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>+</td>
<td>2 (350)</td>
<td>mtl-2* mtl-3</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>+</td>
<td>7 (457)</td>
<td>mtl-2* mtl-3</td>
</tr>
<tr>
<td>D</td>
<td>-</td>
<td>-</td>
<td>100 (390)</td>
<td>mtl-2 mtl-3*</td>
</tr>
</tbody>
</table>

B. Summary of mtl-2 and mtl-3 effects on chloroplast marker transmission: Pooled data from tetrad products derived from the cross: mtl-3 zym-6 × ery-ul spr-fd-1 mtl-2

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of products analyzed</th>
<th>Self-mating efficiency (%)</th>
<th>Percent ery-ul transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtl-2* mtl-3*</td>
<td>17</td>
<td>Range: 10-73</td>
<td>Mean: 42.3 ± 14.4</td>
</tr>
<tr>
<td>mtl-2 mtl-3*</td>
<td>15</td>
<td>Range: 0-20</td>
<td>Mean: 0.3 ± 0.6</td>
</tr>
<tr>
<td>mtl-2* mtl-3</td>
<td>15</td>
<td>Range: 0-20</td>
<td>Mean: 6.1 ± 7.3</td>
</tr>
<tr>
<td>mtl-2 mtl-3</td>
<td>24</td>
<td>Range: 0-2</td>
<td>Mean: 0.2 ± 0.5</td>
</tr>
</tbody>
</table>

C. Effect of mtl-2 and mtl-3 on self-mating and crossing efficiency: analysis of tetrad products from the cross mtl-3 sym-6 × ery-ul spr-fd1 mtl-2

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tetrad product</th>
<th>Percent selfing</th>
<th>Wild type</th>
<th>sym</th>
<th>Percent crossing*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtl-2* mtl-3*</td>
<td>2E</td>
<td>6.8</td>
<td>12</td>
<td>20</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>10B</td>
<td>9.4</td>
<td>10</td>
<td>28</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td>7C</td>
<td>23.9</td>
<td>29</td>
<td>39</td>
<td>42.6</td>
</tr>
<tr>
<td></td>
<td>10A</td>
<td>51.1</td>
<td>16</td>
<td>16</td>
<td>50.0</td>
</tr>
<tr>
<td>mtl-2 mtl-3*</td>
<td>1C</td>
<td>0</td>
<td>4</td>
<td>25</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>2C</td>
<td>1.3</td>
<td>10</td>
<td>20</td>
<td>38.3</td>
</tr>
<tr>
<td></td>
<td>7A</td>
<td>2.9</td>
<td>6</td>
<td>33</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>10C</td>
<td>2.1</td>
<td>18</td>
<td>17</td>
<td>51.4</td>
</tr>
<tr>
<td>mtl-2 mtl-3</td>
<td>1D</td>
<td>0</td>
<td>2</td>
<td>46</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>6A</td>
<td>2.9</td>
<td>1</td>
<td>42</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>3B</td>
<td>1.3</td>
<td>1</td>
<td>74</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>7D</td>
<td>4.1</td>
<td>0</td>
<td>65</td>
<td>0</td>
</tr>
</tbody>
</table>

* See footnote, Table 5.

The % crossing is the % of the zygote sample derived from outcrossing (see MATERIALS AND METHODS).

The mtl-2* mtl-3 tetrad products selected for analysis also carried the sym-6 zygotic lethal allele (complementary to sym-1) to select against mtl-3 selfings.
The behavior of the \textit{mtl-2} \textit{mtl-3} double mutant is explained as follows: When the \textit{mtl-3} allele is combined with \textit{mtl-2}, a mutation which prevents formation of \textit{mt} \textsuperscript{+} gametes (VanWinkle-Swift and Hahn 1986), a double mutant is generated that can only produce \textit{mt} \textsuperscript{-} gametes. This strain will of course be unable to self-mate. However, the strain will also fail to cross efficiently with strains not carrying the \textit{mtl-3} allele since the \textit{mtl-3} mutation causes the \textit{mt} \textsuperscript{-} gametes to prefer \textit{mt} \textsuperscript{+} gametes of their own strain.

To explain the preference shown by \textit{mtl-3} \textit{mt} \textsuperscript{+} gametes for the \textit{mt} \textsuperscript{+} gametes of their own strain, one must assume that the \textit{mtl-3} \textit{mt} \textsuperscript{+} gametes are somehow recognizably different from standard \textit{mt} \textsuperscript{+} gametes. The inefficient crossing of \textit{mtl-3} \textit{mt} \textsuperscript{+} gametes also clearly indicates that the \textit{mtl-3} \textit{mt} \textsuperscript{-} gametes are different from standard \textit{mt} \textsuperscript{-} gametes. In other words, the \textit{mtl-3} mutation appears to have altered both mating types, but in a way that still allows them to recognize each other effectively. The alteration also allows \textit{mtl-3} \textit{mt} \textsuperscript{+} gametes to continue to recognize standard \textit{mt} \textsuperscript{-} gametes, while the \textit{mtl-3} \textit{mt} \textsuperscript{-} gametes appear to be more severely altered.

Before proposing a model to explain how such altered recognition patterns might have been produced, an additional property of the \textit{mtl-3} strains needs to be considered. In crosses involving the original \textit{mtl-3} strain, tetrads products carrying the \textit{mtl-3} allele (as determined by preferential transmission of erythromycin sensitivity in a test cross) were occasionally recovered which failed to self-mate at all, but crossed efficiently (as \textit{mt} \textsuperscript{+}). We do not know whether this results from a further alteration of the \textit{mtl-3} \textit{mt} \textsuperscript{+} cell type such that it cannot be recognized by its own \textit{mt} \textsuperscript{-} gametes, or a further alteration of the \textit{mtl-3} \textit{mt} \textsuperscript{-} gametes rendering them unable to mate with any strain. Whatever the explanation, these F1, \textit{mtl-3} strains cross effectively and exclusively as \textit{mt} \textsuperscript{+} and have a phenotype more easily scored than the original \textit{mtl-3} phenotype (which requires analysis of chloroplast gene transmission). We have not yet attempted to quantify precisely the occurrence of these self-sterile \textit{mtl-3} tetrads products, but they are relatively uncommon (<10% of the \textit{mtl-3} tetrads products).

Furthermore when a self-sterile \textit{mtl-3} tetrads product is used in a subsequent cross, an occasional \textit{mtl-3} tetrads product is produced in the next generation which has regained the potential for self-mating while retaining the characteristic \textit{mtl-3} \textit{mt} \textsuperscript{+} directed bias in chloroplast marker transmission. Again, most \textit{mtl-3} tetrads products have the self-mating phenotype of their immediate \textit{mtl-3} parent (whether it is a self-mater, or a self-sterile).

Although the selfing potential of the \textit{mtl-3} strain thus shows a certain degree of meiotic instability, the phenotype of each class of \textit{mtl-3} strain has remained mitotically stable over the five years we have spent attempting to understand this genetic locus. Meiotic instability of the \textit{mtl-3} phenotype is also evidenced by the frequent difficulty we have in demonstrating 2:2 segregation for the \textit{mtl-3} phenotype (biased chloroplast gene transmission) in tetrads derived from crosses involving self-mating \textit{mtl-3} strains. Often only 1 tetrads product can be clearly classified as \textit{mtl-3} (only tetrads which showed clear 2:2 segregation have been used in the analyses reported here). It is important to note that this loss of the \textit{mtl-3} phenotype can be observed even in tetrads derived from self-mating of \textit{mtl-3}.

We feel that all of these abnormalities can be most easily explained by assuming that frequent chromosomal rearrangement may be occurring within a genetic region that determines recognition between

\begin{table}
\centering
\caption{Evidence for preferential selfing of \textit{mtl-3} cells in a mixed population: (\textit{spr-fd-1} \textit{mtl-3}) \textit{+} (\textit{ery-10} \textit{zym-6} \textit{mtl-2}) \textit{+} (\textit{mtl-6})}
\begin{tabular}{lccc}
\hline
Possible matings & Predicted R:S & No. of & Percent of \\
 & ratios\textsuperscript{*} & matings\textsuperscript{b} & matings \\
\hline
\textit{spr-fd-1} \textit{mtl-3} (\textit{mt} \textsuperscript{+}) & 4:0 & 0:4 & (a) 40 44 \\
\textit{x} \textit{spr-fd-1} \textit{mtl-3} (\textit{mt} \textsuperscript{+}) & (b) 49 47 \\
\textit{spr-fd-1} \textit{mtl-3} (\textit{mt} \textsuperscript{+}) & 2:2 & 2:2 & (a) 30 33 \\
\textit{x} \textit{ery-10} \textit{zym-6} \textit{mtl-2} (\textit{mt} \textsuperscript{-}) & (b) 34 32 \\
\textit{spr-fd-1} \textit{mtl-3} (\textit{mt} \textsuperscript{-}) & 2:2 & 0:4 & (a) 5 6 \\
\textit{x} \textit{mtl-6} (\textit{mt} \textsuperscript{+}) & (b) 6 6 \\
\textit{ery-10} \textit{zym-6} \textit{mtl-2} (\textit{mt} \textsuperscript{-}) & 0:4 & 2:2 & (a) 15 17 \\
\textit{x} \textit{mtl-6} (\textit{mt} \textsuperscript{+}) & (b) 16 15 \\
\hline
\end{tabular}
\textsuperscript{*} R = antibiotic resistant (\textit{spr}, spectinomycin, \textit{ery}, erythromycin); S = antibiotic sensitive.
\textsuperscript{b} The No. of matings = the number of dissected tetrads yielding the predicted segregation ratios for the mendelian markers (a) and (b) are results from two experiments run simultaneously but using independently isolated \textit{ery-10} \textit{zym-6} \textit{mtl-2} strains.
\end{table}
opposite mating-types. Recognition between Chlamydomonas cells of opposite mating-type occurs by way of mating-type-specific surface flagellar proteins. These flagellar agglutinins—long, linear glycoproteins—have been isolated and purified from *C. reinhardtii* (Adair, HWANG and GOODENOUGH 1983; GOODENOUGH et al. 1985) and from two incompatible syngens of *C. eugametos* (MUSGRAVE et al. 1981; KLIS et al. 1985; SAMSON et al. 1987a,b). Although the overall length of the molecules isolated from opposite mating-types or from different species may be similar or different (GOODENOUGH et al. 1985; VAN DEN ENDE, KLIS and MUSGRAVE 1988), the sexual flagellar agglutinins of Chlamydomonas share a common molecular morphology: a globular head region likely to be involved in the actual cell-cell recognition events (GOODENOUGH et al. 1985); a flexible shaft region (the length and flexibility of which may vary between species, or mating-types; VAN DEN ENDE, KLIS and MUSGRAVE 1988); and a terminal hook that is involved in anchoring the agglutinin to the flagellar membrane (GOODENOUGH et al. 1985).

The remarkable similarity in molecular morphologies, and the fact that antibodies raised against the flagellar agglutinin of one mating-type often also recognize the agglutinin of the opposite mating-type (GOODENOUGH et al. 1985; HOMEAN et al. 1988) suggest that these proteins share sequence homologies. It follows that the genes which encode the two agglutinins will also be similar in sequence. The evolution of mating-type specific agglutinins may well have been initiated by the duplication of a single progenitor gene (whose function may have been in cell wall structure/assembly based on the morphological and antigenic similarities between present day flagellar agglutinins and cell wall glycoproteins; GOODENOUGH and HEUSER 1985; MUSGRAVE et al. 1983). Once the duplication had occurred, mutations arising independently in the two copies could give rise to the distinctive flagellar surface proteins that form the basis for compatability/incompatibility within the genus.

Mutants that are defective for sexual flagellar agglutination have been obtained from *C. reinhardtii* (GOODENOUGH, HWANG and WARREN 1978; HWANG, MONK and GOODENOUGH 1981). Some of the responsible mutations show tight linkage to the *mt* locus itself, while others simply show mating-type limited expression but no linkage to *mt*. Although agglutination mutants fail to produce the functional agglutinin (GOODENOUGH et al. 1985), it is not yet known which, if any, carry defects in the structural genes for the agglutinins, and which carry mutations that affect agglutinin gene expression or post-translational processing of a precursor agglutinin. Until this question is resolved, the relative map positions of the structural agglutinin genes in *mt* and *mt* strains of *C. reinhardtii* will remain unknown. Even less is known in the case of *C. monica*.

To account for homothallism in *C. monica*, we assume that the genes for both the *mt* and the *mt* flagellar agglutinins must coexist in individual cells of our wild-type strain. If these homologous genes are closely linked, a single mutational event (deletion or intrachromosomal rearrangement) could lead to the simultaneous alteration of the agglutinins of both mating-types. These changes might allow continued cell-cell recognition between the altered cell types, while abolishing recognition of wild type by one or both mutant mating-types (as we propose for *mt*-3 strains). Depending upon the precise nature of the deletion, cell-cell recognition might be more drastically altered resulting in complete sterility (a common phenotype found in all our mutant searches). Alternatively, a single mutational event could alter both mating-types if a single agglutinin gene actually encodes both agglutinins. In this case, differential post-transcriptional or post-translational processing would underline agglutinin mating-type specificity. A mutation within a shared domain could alter both mating-types, while one which interferes with processing might affect the sexual preferences of one mating-type more than the other.

Although a deletion, as proposed above, would be expected to lead to a stable, nonrevertible phenotype, intrachromosomal rearrangement promoted by the existence of homologous gene sequences might generate a less stable phenotype. Unequal sister chromatid exchange, or intrasstrand recombination could lead to novel mating phenotypes—through dosage effects (extra copies of one or both agglutinin genes, or removal of one copy) as well as potential sequence alterations. In this regard, the observations of KUNZ et al. (1984) demonstrating the induction of mitotic unequal sister chromatid exchange in yeast under conditions of thymidylate biosynthesis inhibition are particularly noteworthy. The *mt*-3 mutant described here was recovered following exposure to fluorodeoxyuridine, a known inhibitor of thymidylate synthetase (SANTI and McHENRY 1972; SANTI, McHENRY and SOMMER 1974).

The variety of mating phenotypes that could be generated by intrasstrand exchange, or unequal crossing over (mitotic or meiotic), would increase proportionately if each mating-type specific agglutinin were itself present in duplicate copies (a reasonable means of increasing the number of agglutinin molecules synthesized during a brief gametogenesis period). Additional variations can be envisioned if the agglutinin proteins contain internal repeat regions, as TARTOF (1988) has recently discussed for other proteins with extracellular function. The difference in the length of the shaft portion of the *mt* and *mt* agglutinins of
C. eugametos (SAMSON et al. 1987b) might, for example, have arisen through such intramolecular duplication.

According to the model we are developing here, the occasional additional variation in mtl-3 mating phenotype recovered in tetrad products suggests that unequal crossing over between duplicated genes may be continuing in subsequent meioses rather than simply being an isolated event that gave rise to the original mtl-3 mutant strain. However, one might expect recombination to be actively suppressed in regions containing gene duplications to prevent rampant intrachromosomal rearrangements (including deletions and inversions). It is perhaps noteworthy that the mt locus of C. reinhardtii is known to lie within a region of crossover suppression (GILLHAM 1969).

If the phenotypes arising in mtl-3 crosses are a consequence of reduced suppression of recombination, one may ask whether the mtl-3 mutation itself led directly to this release of suppression (e.g., by deletion of a chromosomal region critical to establishing a chromatin structure which blocks recombination), or whether an earlier mutation occurring in the strain "set the stage" for the mtl-3 mutational event (i.e., subsequent unequal crossing over between homologous agglutinin genes).

Our method of fluorodeoxyuridine mutagenesis involves prolonged exposure to the mutagen and only partial inhibition of growth during this period. Certainly the opportunity existed for multiple mutational events prior to the isolation of individual clones. In fact, continuing work with several mtl-3 strains constructed during the course of this study has recently revealed the presence of another "mutation." This mutation, presently referred to as dupZ1 (K. P. VANWINKLE-SWIFT, unpublished results), has the unusual effect of promoting apparent high frequency conversion at the zym-1 locus. Preliminary data indicate that this apparent conversion (deviation from 2:2 segregation for zym-1 alleles in 10–20% of the tetrads analyzed), is not true conversion, but rather results from duplication of the zym-1 locus. The responsible "mutation," dupZ1 is not linked to either mtl-3 or to zym-1.

We are presently searching for other loci which may respond similarly to dupZ1—none have yet been found (although independent mutant alleles of zym-1 respond similarly). Interestingly, the zym-1 locus (involved in early events of zygote maturation) is also noteworthy in being a hotspot for mutagenesis, whether via ethyl methanesulfonate, ultraviolet irradiation, or fluorodeoxyuridine (VANWINKLE-SWIFT and BAUER 1982; VANWINKLE-SWIFT and BURRASCANO 1983; K. P. VANWINKLE-SWIFT, unpublished results).

Continuing work with the mtl-3 and dupZ1 strains may help to clarify the role of chromosomal rearrangement in promoting Chlamydomonas speciation through mating-type divergence and the possible significance of crossover suppression for holding such divergence in check. We also look forward to continuing work by others on the nature of the precise molecular interactions between mating-type specific flagellar agglutinins in heterothallic species (where such studies are more feasible), and to the cloning and sequencing of the agglutinin genes themselves. Only with this kind of information will we be able to move from hypotheses to precise predictions as to the relationships between molecular/chromosomal rearrangement and cell-cell recognition in Chlamydomonas.

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LITERATURE CITED


