Interaction Between Mendelian and Non-Mendelian Genes. Regulation of the Transmission of Non-Mendelian Genes by a Mendelian Gene In *Chlamydomonas reinhardtii*

(recombination/mutagenesis/organelle genes)

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ABSTRACT The non-Mendelian genetic element of a given mating type of *Chlamydomonas reinhardtii* was first doubly marked by successive mutageneses and then transferred into a number of offspring cells of the opposite mating type. In subsequent crosses of these offspring clones, the transmission pattern of the original non-Mendelian markers was analyzed. The results indicate that the mating-type gene of a given cell regulates the transmission and perhaps the recombination of the non-Mendelian genes regardless of whether the non-Mendelian markers were isolated originally in the same cell or obtained from another cell of the opposite mating type through genetic transmission. The regulation of the transmission of non-Mendelian genes in *C. reinhardtii* is thus apparently different from the autonomous control of the transmission of non-Mendelian mitochondrial genes in yeast by a genetic factor located on the mitochondrial genome.

Besides having a Mendelian genetic system that consists of 16 linkage groups and nearly 100 known mutations (1, 2), *Chlamydomonas reinhardtii* possesses a non-Mendelian (*nM*) inheritance system, which was first recognized by Sager (3, 4). Genes of this *nM* system are usually transmitted primarily from one of the parents to all the meiotic progeny. The mutational, segregational, and recombinational events of the *nM* genes also differ in specific details from the Mendelian genes (3). It is the predominantly uniparental transmission pattern, however, that distinguishes the *nM* from the Mendelian genes. A linkage relationship has been reported for most of the *nM* genes in *C. reinhardtii* (2). In spite of suggestions that the cytological origin of the *nM* genes of *C. reinhardtii* may be either the chloroplast or mitochondrion, this question has not been satisfactorily resolved (3, 7, 8).

Recently it has been found that a genetic factor ω , responsible for "mitochondrial sexuality," appears to determine the polarity or preferential transmission of the *nM* genes of yeast from ω^+ parents to the diploid progeny, and this ω factor segregates independently of and appears to be distinct from the Mendelian gene for mating types a and α (9, 10). In view of this development in yeast mitochondrial genetics, we now ask whether the transmission pattern of the *nM* genes is in any way dependent on the particular mating type of *C. reinhardtii*, from which an *nM* marker was first isolated. Systematic and well-controlled experiments designed specifically for study of the transmissional aspect of *nM* genes have been

scarce (4). In fact, no deliberate effort has been made to ascertain whether the transmission of *nM* markers originally isolated in the mt^- wild-type strain may be different from the *nM* markers isolated in the mt^+ strain. The experiments reported in this communication were designed to distinguish between the following alternatives: (1) the transmission of *nM* genes in *C. reinhardtii* is regulated autonomously by a factor that may be a part of the *nM* genetic element (irrespective of its cytological location) or (2) such transmission is controlled by a Mendelian gene, the "mating type."

We first marked an *nM* genetic element in a given mating type with at least two mutations by successive mutageneses. This marked *nM* genetic element was then crossed into a number of offspring of opposite mating type. A certain proportion of the offspring carrying the double *nM* markers (the "reconstituted" mutants) should still have possessed either an intact *nM* genetic element or the same controlling factor (if it exists) originally present in a particular mating type before the crossing. This paper reports the results of the transmission pattern of the *nM* genes in these "reconstituted" mutants in subsequent crosses. Our results suggest that control of the transmission of *nM* genes in *C. reinhardtii* differs from the autonomous regulation of the transmission of yeast mitochondrial genes; the transmission of these *nM* genes is apparently controlled by a Mendelian gene. Furthermore, the constant interaction between the mating-type gene and the *nM* genes is not necessarily limited to the transmission of the *nM* genes. The recombinational *nM* genes also appear to be influenced by the mating-type gene.

MATERIALS AND METHODS

Strains, Media, and Culture Conditions. The basic strains of *C. reinhardtii* (obtained through the courtesy of Drs. N. W. Gillham and R. P. Levine and from the Indiana Univ. Culture Collection) used in this study are described in Table 1. The Mendelian mutants, *nic-7* and *ac-29a* (see Table 1), which are completely linked to and almost inseparable from the mt^+ and mt^- genes, respectively (5), were used in specific mating-type tests to determine the mating type of all strains and clones employed in this study. The details of the growth conditions for vegetative culture, induction of gametogenesis and zygote maturation have been reported previously (11-13). The media used in these experiments and their abbreviations are as follows: C = high-salt minimal medium (12); N = C plus 0.75 $\mu\text{g/ml}$ of nicotinamide; NO_3 = C minus NH_4Cl

Abbreviations: *nM*, non-Mendelian (abbreviations for *C. reinhardtii* strains and genotypes are listed in Table 1).

TABLE 1. Basic *Chlamydomonas reinhardtii* strains used

Strains	Genotypes		Phenotypes
	Mendelian	Non-Mendelian	
No. 89	mt ⁻ , NO ₃ ⁺		Wild type
No. 90	mt ⁺ , NO ₃ ⁺		Wild type
nic-7	mt ⁺ , NO ₃ ⁺ nic-7 ⁻ (nic)		Nitcotinamide-requiring and 3-acetylpyridine-sensitive
ac-29a	mt ⁻ , NO ₃ ⁻ ac29a ⁻ (ac)		Yellow on C plate, no growth on NO ₃ plate
Spr-273	mt ⁺ , NO ₃ ⁻	Sp ^r	No growth on NO ₃ plate, resistant to 100 µg/ml of spectinomycin (originally isolated from an mt ⁺ wild type)
Ery-2-y	mt ⁻ , NO ₃ ⁻	E ^r	No growth on NO ₃ plate, resistant to 100 µg/ml of erythromycin (originally isolated from an mt ⁻ wild type)

plus 292 µg/ml of KNO₃; ap = C plus 75 µg/ml of 3-acetylpyridine; S = C plus 500 µg/ml of streptomycin; E = C plus 100 µg/ml of erythromycin; and Sp = C plus 100 µg/ml of spectinomycin (Upjohn Co.). Antibiotics were sterilized by filtration through Millipore filters and added to solid media that had been autoclaved and cooled.

Genetic Analyses. Methods for the dissection and analysis of tetrads and pedigree studies, as well as single-strand analysis, have been described by Ebersold and Levine (14), Gillham (15), and Sager and Ramanis (16).

RESULTS

1. Isolation of the "Reconstituted" Mutants. The double-antibiotic-resistant *nM* mutants ES-1 and SSp-1 were isolated from the single *nM* mutants Ery-2-y and Spr-273 (Table 1), respectively, by low-level streptomycin mutagenesis with a frequency of less than 10⁻⁸.

Under slightly different conditions of zygotic maturation, other investigators have reported that the zygotes of the

same strains we used (11, 12) germinated into four zoospores. We found, however, that the majority of zygospores would not germinate to release four zoospores. Instead, a mixture of four and eight zoospores of varying proportions was consistently observed at zygotic germination. For the sake of consistency, we therefore chose to employ the present experimental conditions, under which the zygotic germination terminated, with the simultaneous extrusion of eight zoospores from at least 95% of the zygospore population (11, 12).

With regard to the segregation of Mendelian genes, the eight zoospores were shown to be derived always from four zoospore pairs (the members of each pair being identical) in all 258 tetrads analyzed. Without exception, all Mendelian genes exhibited strictly 4:4 normal segregation pattern (Table 2). The independent assortment of a pair of unlinked Mendelian genes, NO₃ and nic-7 (1), was also evident from the fact that in nine of our ten crosses the parental ditypes and nonparental ditypes were roughly equal (Table 3). One striking exception did occur; in cross 7 (Table 3) among the nine tetrads, only parental ditype—but neither nonparental ditype nor tetratype—was obtained. The possibility that the *nM* genes carried by this particular "reconstituted" mutant, nic ES-24, were the cause for this deviation in the independent assortment of unlinked Mendelian genes is currently under investigation.

The transmission pattern of the doubly marked *nM* mutants was analyzed first by multiple-point crosses. Regardless of single or double combinations, all three *nM* markers used showed typical non-Mendelian transmission. Each of the 12 tetrads analyzed in cross 13 (mt⁺ Sp^r × mt⁻ E^r S^r) exhibited 0:8 segregation for E^r and S^r and 8:0 segregation for Sp^r (Table 2), and all of the 28 other tetrads involving E^r and S^r exhibited the same 0:8 segregation for E^r and S^r (crosses 1 and 2, Table 2). In cross 8 (mt⁺ S^r Sp^r × mt⁻ ac-29a), all 18 tetrads gave 8:0 segregation for S^r and Sp^r. From single zygote colony analysis, the frequency of exceptional zygotes transmitting the mt⁻ parental E^r S^r markers was 0.44–1.19%. This low-frequency transmission of mt⁻ parental *nM* genes into the progeny is characteristic of these *nM* genes in *C. reinhardtii* and is in agreement with previous reports (2, 5).

TABLE 2. Tetrad analysis from multiple-point crosses

Cross no.	Cross		Number of tetrads analyzed	Mendelian genes				Non-Mendelian genes		
	mt ⁺	mt ⁻		mt ⁺ : mt ⁻	ac ⁺ : ac ⁻	nic ⁺ : nic ⁻	NO ₃ ⁺ : NO ₃ ⁻	E ^r :E ^s	S ^r :S ^s	Sp ^r :Sp ^s
1	90	×	ES-1	7	4:4	—	4:4	0:8	0:8	—
2	nic-7	×	ES-1	21	4:4	—	4:4	0:8	0:8	—
3	nicES-3	×	89	39	4:4	—	4:4	8:0	8:0	—
4	nicES-3	×	Spr-273	18	4:4	—	4:4	8:0	8:0	0:8
5	nicES-16	×	89	9	4:4	—	4:4	8:0	8:0	—
6	nicES-22	×	89	11	4:4	—	4:4	8:0	8:0	—
7	nicES-24	×	89	9	4:4	—	4:4	8:0	8:0	—
8	SSp-1	×	ac-29	18	4:4	4:4	—	—	8:0	8:0
9	90	×	acSSp-15	23	4:4	4:4	—	—	0:8	0:8
10	90	×	acSSp-12	22	4:4	4:4	—	—	0:8	0:8
11	90	×	acSSp-14	20	4:4	4:4	—	—	0:8	0:8
12	90	×	acSSp-19	25	4:4	4:4	—	—	0:8	0:8
13	Spr-273	×	ES-1	12	4:4	—	—	0:8	0:8	8:0
14	nic-7	×	SSp-2	24	4:4	—	4:4	—	0:8	0:8

TABLE 3. *Tetrad analysis of multiple-point crosses involving two Mendelian genes*

Cross no.*	Mendelian gene pair	Observed parental ditype	Number of tetrads	
			Non-parental ditype	Tetratype
2	nic-NO ₃	7	7	7
3	nic-NO ₃	6	6	27
5	nic-NO ₃	3	3	3
6	nic-NO ₃	3	5	3
7	nic-NO ₃	9	0	0
9	ac-NO ₃	8	6	9
10	ac-NO ₃	5	6	11
11	ac-NO ₃	4	6	10
12	ac-NO ₃	8	8	9
14	nic-NO ₃	6	8	10

* See Table 2 for parental genotypes and other segregation results.

The original *nM* genetic element of the *mt*⁻ strain now marked by the two antibiotic resistant genes *E*^r and *S*^r was transferred into a number of *mt*⁺ cells by crossing with *nic*-7. After extensive subcloning of the zoospore progeny, 20 independent clones of the genotype *mt*⁺ *nic*-7, *E*^r *S*^r were isolated (*nicES*-3, *nicES*-14, etc). Among these 20 "reconstituted" mutants three were NO₃⁺ and 17 were NO₃⁻. Conversely, the *nM* genetic element that was originally present in the *mt*⁺ strain and now marked by *S*^r and *Sp*^r was transferred into *mt*⁻ cells by the cross, *SSp*-1 × *ac*-29a. In accordance with the expected transmission of *nM* genes, *S*^r and *Sp*^r of *SSp*-1 were transmitted simultaneously into every meiotic product (Table 2, cross 8). After extensive subcloning of the zoospore progeny, 10 "reconstituted" mutant clones (i.e., *acSSp*-12, *acSSp*-14, etc.) of the desired genotype *mt*⁻, *ac*-29, *S*^r *Sp*^r were isolated.

2. *Transmission of the mt*⁺ *Non-Mendelian Genes That Originated from mt*⁻ *Strain*. When the newly "reconstituted" *mt*⁺ mutants carrying double-antibiotic-resistant *nM* genes originating in the *mt*⁻ strain were crossed with wild-type *mt*⁻ strain (IU no. 89), the simultaneous transmission of these two *nM* genes into all the offspring was evident for all such "reconstituted" mutants tested. The results of tetrad analysis indicated that in each of these five crosses all offspring were *E*^r *S*^r, with no exception among a total of 312 meiotic products in 39 tetrads (crosses 3-7, Table 2). Single-strand analysis of these types of crosses revealed that, with the size of the zoospore progeny population ranging from 1260 to 5797, every zoospore progeny clone was *E*^r *S*^r (i.e., nos. 3, 5, 16, and 17 in Table 4). It is thus clear that regardless of the origin of the *nM* genes, as long as they were in an *mt*⁺ cell, simultaneous transmission into all meiotic progeny invariably occurred.

3. *Genetic Transmission of mt*⁻ *Non-Mendelian Genes That Originated from mt*⁺ *Strain*. When the "reconstituted" *mt*⁻ mutants carrying multiple *nM* genes originating in the *mt*⁺ strain were crossed with a wild-type *mt*⁺ strain, the transmission of the *nM* genes into offspring was exceedingly rare. In four such crosses (Table 2, crosses 9-12), the transmission of either *S*^r or *Sp*^r gene was less than one in 20-25 tetrads or 160-200 meiotic products. In the same four crosses, the frequency of exceptional zygotes, which transmitted the *mt*⁻ *nM* markers *Sp*^r and *S*^r simultaneously into the zygote colonies was only 1.92-3.74%, and the frequency of zoospore progenies carrying *S*^r *Sp*^r was 1.34% in one of the crosses (Table 4). In a few representative cases, the genetics of the meiotic progeny resulting from a few exceptional zygotes was analyzed individually. Table 5 summarizes the results obtained from six such individual zygotes in two independent crosses. From any given exceptional zygote, the frequency of zoospore progenies carrying any *nM* gene from the "reconstituted" *mt*⁻ parents was never 100% but varied from 3.96 to 93.34%; for the *S*^r *Sp*^r genotype, the frequency varied from 3.5 to 87.88%. The frequency of simultaneous transmission of both *S*^r and

TABLE 4. *Single-strand analysis of multiple-point crosses*

Cross no.	Cross			Total clone analyzed	Frequency (%)							Frequency of zygote colonies with <i>mt</i> ⁻ genes (%)	Total zygote colonies analyzed
	<i>mt</i> ⁺	×	<i>mt</i> ⁻		<i>E</i> ^r	<i>S</i> ^r	<i>Sp</i> ^r	<i>E</i> ^r <i>S</i> ^r	<i>E</i> ^r <i>Sp</i> ^r	<i>S</i> ^r <i>Sp</i> ^r	<i>E</i> ^r <i>S</i> ^r <i>Sp</i> ^r		
2	<i>nic</i> -7	×	<i>ES</i> -1	10,946	10.94	0.237	—	0.191	—	—	—	1.19	4201
3	<i>nicES</i> -3	×	89	5,797	0	0	—	100	—	—	—	ND*	ND
4	<i>nicES</i> -3	×	<i>Sp</i> -273	2.19 × 10 ⁴	ND	ND	ND	ND	ND	ND	0.00355	0.285	2800
5	<i>nicES</i> -16	×	89	3,372	0	0	—	100	—	—	—	ND	ND
9	90	×	<i>acSSp</i> -15	3,420	—	ND	ND	—	—	1.34	—	3.74	115
10	90	×	<i>acSSp</i> -14	ND	—	ND	ND	—	—	ND	—	1.92	52
11	90	×	<i>acSSp</i> -12	ND	—	ND	ND	—	—	ND	—	2.19	91
12	90	×	<i>acSSp</i> -19	4,265	—	0	0	—	—	0	—	2.72	147
13	<i>Sp</i> -273	×	<i>ES</i> -1	6.688 × 10 ⁷	ND	ND	97.5	0.01175	ND	ND	8.97 × 10 ⁻⁶	0.444	3600
15	<i>SSp</i> -1	×	<i>Ery</i> -2-y	13,460	3.47	3.2	0.11	2.73	5.82	85.36	1.25	20.67	387
16	<i>nicES</i> -14	×	89	1,260	0	0	—	100	—	—	—	ND	ND
17	<i>nicES</i> -23	×	89	2,460	0	0	—	100	—	—	—	ND	ND

* ND, not determined.

TABLE 5. *Single-strand analysis of exceptional zygote colonies from multiple-point crosses*

Cross number*	Total zygotes screened	Exceptional zygote colonies	Frequency (%)				Total cells analyzed
			S ^r	Sp ^r	S ^r Sp ^r	NO ₃	
9	115	I	0.733	0.0523	64.1	50	3,819
		II	0.611	0.114	63.4	50	2,617
		III	0.367	0.0919	3.5	50	32,612
		IV	1.94	0.81	8.9	50	1,234
12	147	I	7.42	2.4	72.78	49.65	3,676
		II	4.59	0.87	87.88	50	5,488

* See Tables 2 and 4 for parental genotypes and other segregation results.

Sp^r from the mt⁻ parent into the zoospore progeny was always much higher than the single transmission of either S^r or Sp^r, which resulted from a recombinational event between the mt⁻ and mt⁺ parental *nM* genes. The transmissional frequency of S^r alone from the mt⁻ parent was consistently higher (2.39- to 14-fold) than that of Sp^r alone (Table 5), which suggests that a high rate of nonreciprocal recombination favoring the preservation of S^r marker may have been operative.

4. *Other Results on the Transmission of nM Genes.* To study the transmission of *nM* genes, in addition to the main experimental design outlined in part 1 of the *Results* section, we utilized a supplementary scheme in which the *nM* genetic element marked by a single *nM* marker Sp^r in an mt⁺ cell was transferred first into an mt⁻ cell. This mutant mt⁻ Sp^r-273 was crossed with mt⁺ clones before (cross 4, Table 2) and after a second marker S^r was added to the *nM* genetic element (cross 14, Table 2). In cross 4 (mt⁺ E^r S^r × mt⁻ Sp^r), all 18 tetrads exhibited 8:0 segregation for E^r and S^r and 0:8 segregation for Sp^r (Table 2) while the frequency of exceptional zygotes was 0.285% (Table 4). In cross 14 (mt⁺ × mt⁻ S^r Sp^r), the entire 24 tetrads gave 0:8 segregation for S^r and Sp^r. These results once again indicate that no matter how antibiotic-resistant *nM* markers were obtained in a given clone, the mating-type gene in that particular clone controls the transmission of such *nM* markers.

DISCUSSION

In order to probe the interaction between the mating-type gene and the *nM* genes, we conducted a much broader analysis of the transmission pattern of the *nM* genes than previously undertaken. This analysis included the use of multiple *nM* markers and the independent isolation of these markers in both mt⁺ and mt⁻ strains in such a way that well-controlled reciprocal crosses with multiple *nM* markers could be analyzed. To minimize the possible exclusion of any genetic mechanism that could lead to low frequency exceptional transmission of the mt⁻ parental *nM* genes to the progeny, the "reconstituted" mutants carrying the mt⁻ parental *nM* markers were selected at random from a large population of exceptional zygotes (4201 zygotes, Table 4, cross 2). Moreover, repeated subclonings of a given zoospore progeny colony on selective plates were carried out before isolating any "reconstituted" mutants in our present investigation, since the zoospores were not pure with respect to the *nM* genes (2, 5, 18).

The original rationale for our present investigation was

based on the observation that the transmission of yeast mitochondrial genes into diploid progeny was regulated by a genetic factor located on the organelle rather than on the nuclear genetic linkage group (9, 10). Therefore, critical crosses were designed to ascertain whether a similar regulatory factor(s) exists in the *nM* genetic linkage group in *C. reinhardtii*. A given *nM* genetic element was marked with two different antibiotic-resistant mutations to facilitate the recovery of such an *nM* genetic element in the offspring of the opposite mating type. Nonetheless, in those crosses from which the "reconstituted" mutants were isolated, rare recombinational events could occur and lead to the separation of double *nM* markers from the same regulatory factor (if it exists) originally present in a particular mating type prior to the crossing. The fact that all 12 "reconstituted" mutants analyzed exhibited mating-type-controlled uniparental transmission of these *nM* markers argues strongly that the *nM* genetic element in *C. reinhardtii* does not contain its own regulatory factor that determines the preferential transmission of the *nM* genes on the same linkage group. To conclude otherwise would require the unlikely assumption that none of these 12 "reconstituted" mutants still possessed the original intact *nM* genetic element on which the double-antibiotic-resistant mutations were first isolated.

Thus, unlike the yeast mitochondrial genes, the transmission of the *nM* genes in *C. reinhardtii*, regardless of their cytoplasmic location, is not regulated autonomously but is controlled by the Mendelian gene for the mating type that is located on one of the 16 Mendelian genetic linkage groups. Mendelian genes in the mating type region of chromosome VI tend to be extremely tightly linked (5). Several other observations suggest that the mt⁻ and mt⁺ may not be alleles but are genes with distinct functions (5). It is possible, therefore, that another Mendelian gene(s) tightly linked to the mt⁺ gene actually regulates the preferential transmission of *nM* genes. For the sake of convenience, but not precluding the possibility mentioned above, we shall continue to use the simple term "mating-type gene" in the context of its interaction with the *nM* genes.

Although it has been suggested that these *nM* genetic determinants in *C. reinhardtii* may be located in the chloroplast (2, 18, 19), the critical causal relationship between the *nM* genes and the chloroplast DNA remains to be established by experimental means; it is still not known whether all of the 26-52 repetitive copies of the chloroplast genome (22-24) are equivalent in their genetic competence at all times throughout the life cycle of *C. reinhardtii*. It is possible that during

meiosis, in which the recombination and segregation of parental chloroplast genomes occur, only a very small number of the 52 repetitive chloroplast genomes (e.g., less than 5–10%) would be genetically competent. As a result, their fate is not necessarily the same as the genetically incompetent majority whose behavior in meiosis is what is detectable biochemically with the analytical methods thus far employed (19–21). Even in such studies the most crucial control experiment—to demonstrate that the mt^- parental chloroplast DNA behaves differently under the ultraviolet-induced biparental transmission of nM genes (18) as compared with the normal maternal transmission—has not yet been performed.

Among the three mM mutations used, the S^r and Sp^r markers exhibited the typical characteristics of the predominantly uniparental transmitting nM genes and were transmitted into the progeny from the mt^- parents at consistently low frequency and at exceedingly high frequency from the mt^+ parent. A characteristic linkage relationship between S^r and Sp^r markers has also been demonstrated. The progenies of exceptional zygotes always contained a great excess of the mt^- parental genotype $S^r Sp^r$ over the recombinants (Table 5), which was also true in cross 15 (Table 4) where both S^r and Sp^r genes were present in the mt^+ parent. On the other hand, the properties of the E^r marker differed somewhat from the S^r and Sp^r markers. An extremely high frequency of recombination between E^r and S^r markers in the zoospore progeny was obtained when these two markers were carried by the mt^- parents (cross 2, Table 4), although the frequency of exceptional zygotes resulting from this coupling cross with $mt^+ E^s S^s$ (cross 2, Table 4) was normal (1.19%). The fact that the recombinants $E^r S^s$ and $E^s S^r$ were more frequent than the parental genotypes $E^r S^r$ and $E^s S^s$ in this cross suggests that either the mutation E^r was not linked with the S^r and Sp^r mutations or the E^r marker may have been under a specific and somewhat different control by the mating type gene, perhaps because of the particularly sensitive location of the E^r marker on the postulated nM linkage group.

The mating-type gene appeared to have a certain effect on the recombination of nM genes as well. The frequency of triple antibiotic resistant $E^r S^r Sp^r$ genotype among the offspring resulting from the cross, $mt^+ E^r S^r \times mt^- Sp^r$, was roughly 400-fold higher than the reciprocal cross, $mt^+ Sp^r \times mt^- E^r S^r$ (crosses 4 and 13, Table 4). The frequency of exceptional zygotes was comparable (0.285% vs. 0.444%) and the selection (if it exists), either intercellular or on agar plate, for this triple resistant ought to have been the same for these two reciprocal crosses. These results suggest that the recombination among the three nM genes may have also been influenced by the mating-type gene in a manner either analogous or supplementary to its effect on the transmission of the nM genes. Moreover, among the zoospore progenies of exceptional zygotes resulting from cross $mt^- S^r Sp^r \times mt^+$ (Table 5), the genotype $S^r Sp^s$ was invariably higher than for $S^s Sp^r$ (from 14- to 2.39-fold higher). If this difference is not

entirely due to the possible selection for different genotypes within the zygote colony, it may be related to the arrangement of these nM markers on the nM genetic linkage group and thus their recombination frequencies with the $mt^+ nM$ element, since it is possible that only those S^r and Sp^r markers that recombined with the $mt^+ nM$ genetic element would survive in the zoospore progeny.

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