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Growth responses of the marine photosynthetic ciliate *Myrionecta rubra* to different cryptomonad strains

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ABSTRACT: The photosynthetic *Myrionecta rubra* (=*Mesodinium rubrum*) is a cosmopolitan ciliate that frequently feeds on free-living cryptomonad species in diverse marine environments. Hence, *M. rubra* might feed on a wide range of cryptomonad species. To explore this possibility, we investigated the growth responses of *M. rubra* strain MR-MAL01, which was starved for 20 d, to diverse cryptomonad strains CR-MAL01, CR-MAL02, CR-MAL03, CR-MAL04, CR-MAL05, and CR-MAL06. Phylogenetic analysis of the aligned 18S rRNA sequences from cryptomonads suggested that the strains CR-MAL01, CR-MAL02, CR-MAL04, and CR-MAL05 were included in cryptomonad Clade 2, while the strains CR-MAL06 and CR-MAL03 were included in cryptomonad Clade 3, with high bootstrap value (98%) and posterior probability of 1. Intriguingly, *M. rubra* strain MR-MAL01 grew better in the presence of cryptomonad strains CR-MAL01, CR-MAL02, CR-MAL03, CR-MAL04, and CR-MAL05 (0.16 to 0.40 d^{-1}) than in the absence of a cryptomonad strain (0.011 d^{-1}), indicating that *M. rubra* could grow in the presence of diverse cryptomonad prey. However, *M. rubra* (MR-MAL01) did not grow better in the presence of cryptomonad strain CR-MAL06. Further comparisons of the growth rates of MR-MAL01 showed that cryptomonads in Clade 3 might be less favored than those in Clade 2 by the starved *M. rubra*. Here, we demonstrated that MR-MAL01 has preferences with regard to potential cryptomonad prey strains, suggesting that the growth of MR-MAL01 may be partially affected by the availability of preferred cryptomonad prey types.

KEY WORDS: *Myrionecta rubra* · Cryptomonads · Growth rates · 18S rRNA sequences

INTRODUCTION

The marine phototrophic ciliate *Myrionecta rubra* Jankowski 1976 (=*Mesodinium rubrum* Lohmann 1908) is a common and cosmopolitan species (Crawford 1989). The 'functionally autotrophic' ciliate (Ryther 1967, Sieburth et al. 1978) frequently forms massive blooms in eutrophicated marine environments (Taylor et al. 1971, Lindholm 1985). Using novel laboratory strains of this 'unculturable' ciliate that feeds on cryptophyte prey (Gustafson et al. 2000, Yih et al. 2004a) and bacterial cells (Myung et al. 2006), its applicability as a live feed (Yih et al. 2004b), its growth and photophysiology (Johnson & Stoecker 2005, Hansen & Fenchel 2006), the function and maintenance of cryptophyte plastids (Hansen & Fenchel 2006, Johnson et al. 2006), and the phylogenetic position of *M. rubra* (Johnson et al. 2004) have recently been explored. Kleptoplastidy (i.e. plastids derived from ingested prey) in *M. rubra* has been discussed over the last few years as a means to acquire and maintain photosynthetic capacity (Gustafson et al. 2000, Yih et al. 2004a, Johnson et al. 2006). Hansen & Fenchel (2006), however, have argued that plastids of *M. rubra* are

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really associated with a permanent symbiosis. Therefore, it is possible that the 3 reported strains of *M. rubra* might not have developed through the same paths of plastid evolution (Hansen & Fenchel 2006).

Diverse species of cryptomonads are found in marine habitats and *Myrionecta rubra* might possibly feed on multiple species of cryptomonads. Thus, it appears that *M. rubra* may retain plastids from various cryptomonads and tend to prefer a particular type of cryptomonad. However, the preferential growth of *M. rubra* in the presence of various cryptomonads remains to be determined. To evaluate this possibility, we investigated the growth responses of *M. rubra* strain MR-MAL01, which was starved for 20 d and then supplied with prey cells of 6 cryptomonad strains (CR-MAL01, CR-MAL02, CR-MAL03, CR-MAL04, CR-MAL05, and CR-MAL06). We observed that *M. rubra* strain MR-MAL01 exhibited a preference for particular strains among the 6 cryptomonad strains, and that the growth of *M. rubra* may occasionally be influenced by the availability of its preferred prey types in the phototrophic plankton community.

MATERIALS AND METHODS

Clonal cultures of cryptomonads and *Myrionecta rubra*. Clonal cultures of *M. rubra* MR-MAL01 and a cryptomonad CR-MAL01 were established and maintained as described in a recent study (Yih et al. 2004a). Briefly, single cells were isolated using Pasteur capillary pipettes from water samples collected at Gomso Bay, Korea (35° 40′ N, 126° 40′ E). Each isolated *M. rubra* MR-MAL01 cell was washed 3 times with enriched f/2 seawater media, and subsequently incubated under continuous illumination. Every 5 to 6 d, cryptophyte prey (CR-MAL01) were added to readjust the proportion of cryptophytes to *M. rubra* MR-MAL01 cells to a 5:1 ratio. The other 5 cryptomonad strains were also cultured by isolating single cells from samples of Korean coastal waters (see Table 1). All the strains were kept at 15°C and 30 psu in enriched f/2 seawater media (Guillard & Ryther 1962). The same seawater was always used for the repeated subculturing of cryptomonads and *M. rubra* MR-MAL01 in order to ensure that the cultures were maintained under similar water conditions. The cryptomonads were provided a continuous illumination of 25 µE m^{-2} s^{-1}, whereas *M. rubra* MR-MAL01 was provided with an illumination of 60 µE m^{-2} s^{-1} (Yih et al. 2004a). No cultures were axenic. However, the contribution of bacterivory to the growth of *M. rubra* MR-MAL01 in terms of cell carbon (ingestion of 53 bacteria h^{-1} by 1 ciliate; Myung et al. 2006) was estimated to be not significant. Further, our vast preliminary data indicated that our cryptomonad strains did not take up fluorescently-labeled (i.e. 5-[4,6-dichlorotriazin-2-yl] aminofluorescein [DTAF]) bacteria.

Growth rates of *Myrionecta rubra* and cryptomonad prey species. The experimental *M. rubra* culture was starved of cryptomonad prey for 20 d and used in a batch culture experiment to estimate growth rate. After 20 d, each experimental cryptomonad culture (1.0×10^4 cells ml^{-1}) was offered as prey to the starved *M. rubra* culture (1.0×10^3 cells ml^{-1}) under continuous illumination (60 µE m^{-2} s^{-1}). The abundances of *M. rubra* and its cryptomonad prey in subsamples obtained daily from duplicate 500 ml polycarbonate (PC) bottles were determined by counting the cells in 1 ml Sedgewick-Rafter slides.

The specific growth rates of *Myrionecta rubra* and the cryptomonad prey obtained from each experimental bottle were calculated by averaging the daily growth rates (DGR) using:

$$DGR = \ln(S_{t2}/S_{t1})/(t_2 - t_1) \quad (1)$$

where $t_2 - t_1 = 1$ d, and S_{t1} and S_{t2} are cell concentrations in consecutive subsamples.

Molecular sequencing. Nucleic acids were extracted and purified using hexadecyltrimethyl ammonium bromide (CTAB) and organic extractions, as described by Ausubel et al. (1999). Cells were harvested from 100 to 250 ml samples of the cultures by centrifugation (10 min at 12 000 × *g*). Amplification of 18S rRNA genes was performed using standard PCR protocols with eukaryote-specific primers EukA and EukB (Medlin et al. 1988). The reaction mixture contained 50 to 100 ng of DNA, 0.2 mM deoxynucleoside triphosphate, each primer at a concentration of 0.3 µM, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 40 mM KCl, and 2.5 U *Taq* DNA polymerase (Bioneer). PCR-amplification was performed according to the following protocol: an initial denaturation step (5 min, 94°C) was followed by 30 cycles consisting of denaturation (45 s, 94°C), annealing (1 min, 55°C), and extension (3 min, 72°C), with a final 10 min extension step at 72°C. The size of the PCR products was approximately 1.5 kb for *Myrionecta rubra* strain MR-MAL01 and approximately 1.8 kb for the 6 cryptomonad strains CR-MAL01, CR-MAL02, CR-MAL03, CR-MAL04, CR-MAL05, and CR-MAL06, and this was confirmed by agarose gel electrophoresis. The amplified products were purified using a PCR purification kit (Bioneer) according to the manufacturer's instructions, and then ligated into the prepared pGEM-T Easy vector supplied with the pGEM-T Easy Vector System (Promega) according to the manufacturer's protocols. Plasmid DNA from putative positive colonies was harvested using a Bioneer plasmid purification kit (Bioneer). Typically, 5 to 8 positive clones from each strain were partially sequenced using the T7

promoter sequencing primer (i.e. 5′-AATACGACTCACTATAG-3′) derived from the cloning vector, and all partial sequences (approximately 700 bp) were subsequently identified by a BLASTN search. They were putatively identified as members of cryptomonads. Among the positive clones, including the identified partial sequences, 1 to 2 positive clones were selected and completely sequenced using both the SP6 promoter sequencing primer (i.e. 5′-ATTTAGGTGACACTATAG-3′) derived from the cloning vector and a eukaryote-specific primer (i.e. 1209R; 5′-GGGCATCACAGACCTG-3′). Sequencing was performed with an Applied Biosystems automated sequencer (ABI 3730xl) at Macrogen in ROK. The 18S rRNA gene sequences from the cultured samples were deposited in GenBank under the following accession numbers: MR-MAL01 (EF195734), CR-MAL01 (EF195735), CR-MAL02 (EF195736), CR-MAL03 (EF195737), CR-MAL04 (EF195738), CR-MAL05 (EF195739), and CR-MAL06 (EF195733).

Phylogenetic analysis. The 18S rRNA gene sequences obtained from the cultures were compared with the sequences of related taxa obtained from the GenBank database using a BLASTN search. The sequences were manually aligned using the 18S rRNA secondary structure (Van de Peer et al. 2000). Phylogenetic analyses were performed using the following 2 data sets: (1) 33 representative sequences in alveolates, and (2) 31 representative sequences in cryptomonads. Only homologous positions in the 18S rRNA gene sequences were used for all the phylogenetic analyses. *Prorocentrum micans* and Glucocystophyta (*Cyanophora paradoxa* and *Glaucocystis nostochinearum*) were used as outgroups for the alveolates and cryptomonads, respectively. The 1344 position of unambiguously aligned sites was retained for phylogenetic analysis of alveolates and outgroups. For the phylogenetic analysis of cryptomonads, the conserved 1524 position was considered as an 'unambiguously aligned site'. These alignments are available on request.

Phylogenetic trees were inferred by the maximum likelihood (Felsenstein 1981) method using PAUP* 4b10 (Swofford 1998) and by Bayesian analysis using MRBAYES 3.0 (Huelsenbeck & Ronquist 2001). For the analyses of alveolates and cryptomonads, the general-time reversible + gamma + I and Tamura-Nei + gamma + I models, respectively, were selected using Modeltest version 3.04 (Posada & Crandall 1998). Parameter values for the likelihood analysis were estimated from a test tree obtained using PAUP*. For each maximum likelihood analysis, the best tree was found using 20 random additions and tree bisection-reconstruction (TBR) branch-swapping, and a 200 replicate bootstrap analysis was performed. Posterior probabilities of phylogenetic trees under the Tamura-Nei + gamma + I and general-time reversible + gamma + I models were estimated using MRBAYES 3.0 (Huelsenbeck & Ronquist 2001). Four simultaneous Markov Chain Monte Carlo (MCMC) chains were run for 1 000 000 generations, and were sampled every 500 generations (burn-in 200 000 generations).

RESULTS AND DISCUSSION

Phylogenetic analysis of temperate *Myrionecta rubra* strain MR-MAL01

The size of the 18S rRNA gene (1552 bp) in *Myrionecta rubra* MR-MAL01 was similar to that in *M. rubra* GenBank Accession No. AY587129 (1548 bp) and *Mesodinium pulex* (1543 bp), but much shorter than those in other alveolates (average 1712 bp, data not shown). The 18S rRNA genes of strains *M. rubra* MR-MAL01, *M. rubra* AY587129 and *M. pulex* shared the identical oligomer sequence (i.e. 5′-TTGGACCGGACGAAGAC-3′) that is found solely in the 18S rRNA genes of *M. rubra* (AY587129) and *M. pulex* (Johnson et al. 2004). The 18S rRNA gene sequence of *M. rubra* MR-MAL01 was extremely similar (99.6%) to that of *M. rubra* AY587129 (data not shown). In the phylogenetic tree (Fig. 1), the *M. rubra* strain MR-MAL01 was placed within the Mesodiniidae, with high bootstrap support (maximum likelihood: 100%) and posterior probability of 1, and formed a clade with *M. rubra* AY587129 with high bootstrap support (98%) and posterior probability of 1. Our strain consistently conformed to the behavioral and morphological characteristics of *M. rubra* (Lynn & Small 2002).

Phylogenetic analysis of six experimental cryptomonad strains

Cryptomonads, including the 6 experimental strains, were divided into 7 major lineages in a phylogenetic tree with moderately strong bootstrap support (maximum likelihood 71%) or high posterior probability (0.95, Fig. 2), including 2 lineages comprising the monospecific genera *Falcomonas* and *Proteomonas*, as previously reported for the phylogenetic tree of crytomonads (Clay & Kugrens 1999, Hoef-Emden et al. 2002). In addition, phagotrophic *Goniomonas truncata* demonstrated the highest divergence of all sequences in the phylogenetic tree, and represented a basal taxon within the crytomonads (Clay & Kugrens 1999, Hoef-Emden et al. 2002). Phylogenetic analysis of the aligned 18S rRNA sequences suggested that our 6 strains differed from each other, and they did not fall

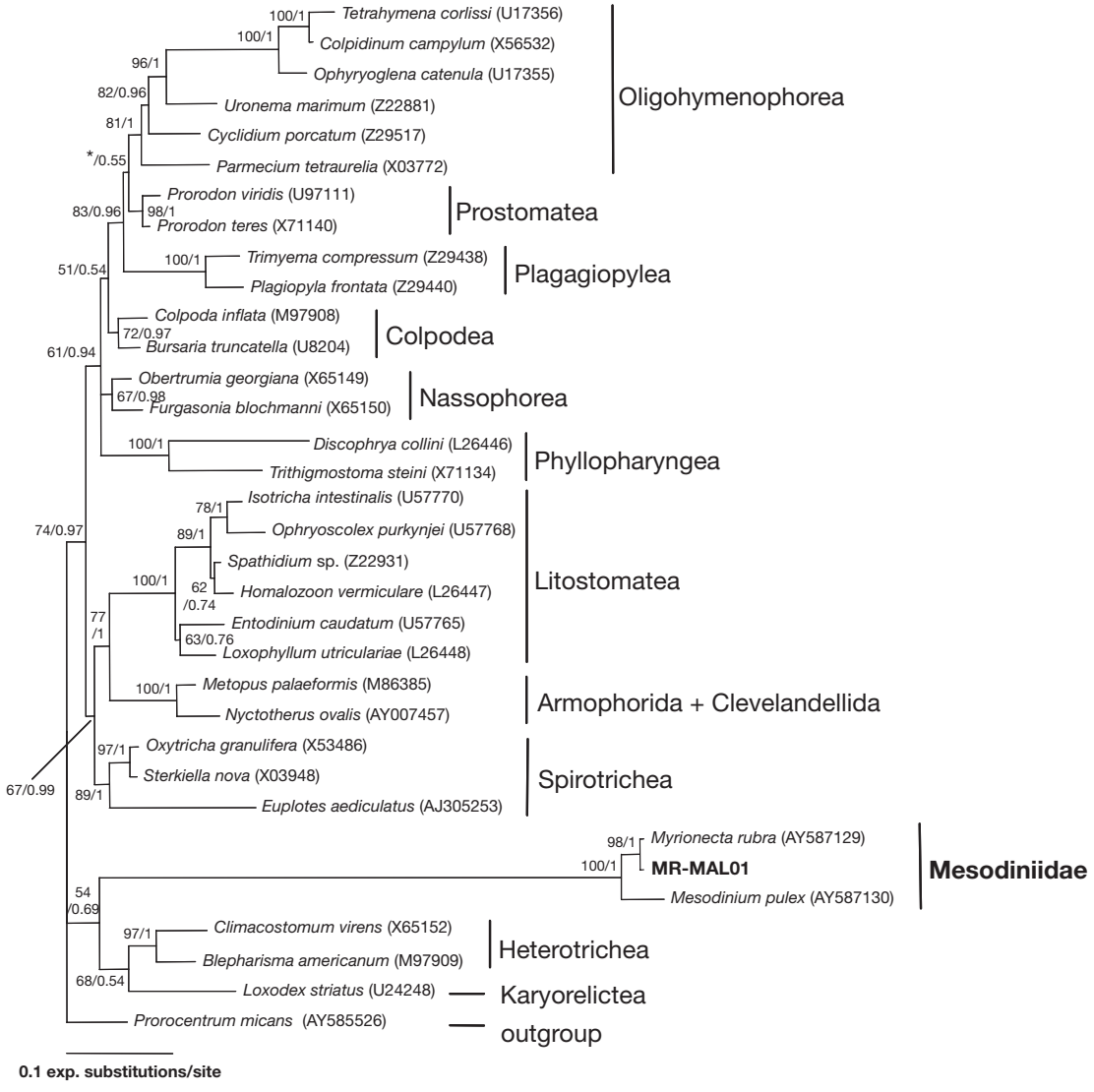

Fig. 1. 18S rRNA gene tree showing the phylogenetic position of *Myrionecta rubra* strain MR-MAL01 relative to 32 other alveolates. *Prorocentrum micans* was used as the outgroup. Bootstrap values (>50%) from the maximum likelihood method (ML; 200 replicates) and Bayesian posterior probability (MB) are indicated at nodes (presented in the order ML/MB). Accession numbers of each taxon presented in parentheses. *: bootstrap value of <50% in ML analysis

within any established species of the cryptomonad order Pyrenomonadales (Fig. 2). The strains CR-MAL01, CR-MAL02, CR-MAL04, and CR-MAL05 were included in cryptomonad Clade 2, while the strains CR-MAL06 and CR-MAL03 were included in cryptomonad Clade 3 (Fig. 2). Cryptomonad Clade 2 is divided into the following 4 lineages with high bootstrap support (98%) or a posterior probability of 1: (1) CR-MAL01, CR-MAL02, *Teleaulax amphioxeia*, CR-MAL05, *Plagioselmis prolonga*, (2) CR-MAL04, (3) *Teleaulax acuta*, and (4) *Germinigera cryophila*. Cryptomonad Clade 3 is divided into the following 2 lineages with high bootstrap support (98%) or a posterior probability of 1: (1) *Pyrenomonas salina*, CR-MAL06, *Rhodomonas abbreviata*, *Rhodomonas* sp. M1480, *Rhinomonas pauca*, CR-MAL03, and (2) *Storeatula major* (Fig. 2). Our cryptomonad strains were included in cryptomonad Clades 2 and 3, which are known to be widely distributed in marine ecosystems (Hoef-Emden et al. 2002).

Growth response of *Myrionecta rubra* to different cryptomonad strains

The 20 d starved *Myrionecta rubra* MR-MAL01 exhibited exponential growth in the bispecies culture bottles for 6 d in response to all but one (i.e. CR-

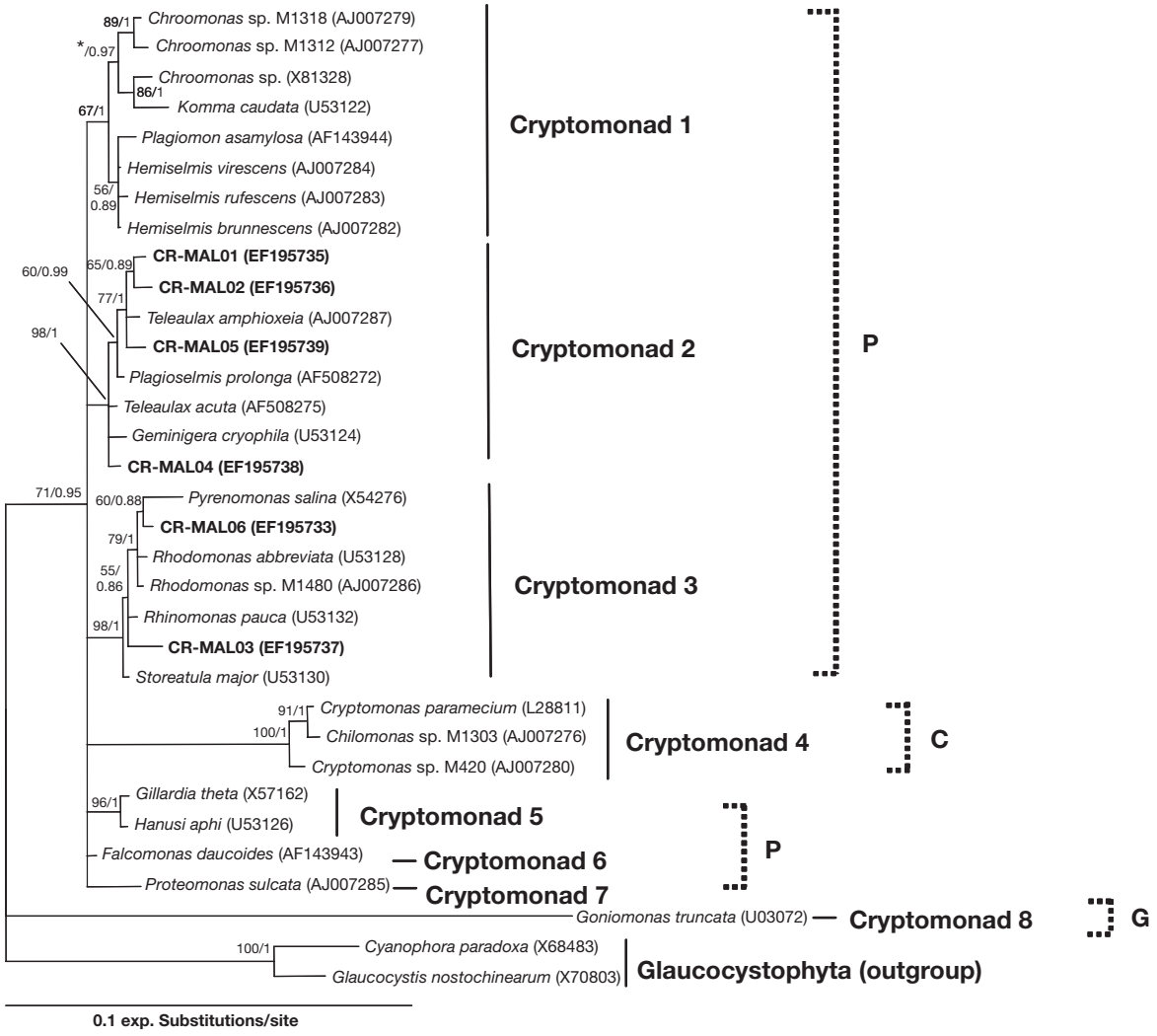

Fig. 2. 18S rRNA gene tree showing the phylogenetic position of 6 cryptomonad strains (CR-MAL01, CR-MAL02, CR-MAL03, CR-MAL04, CR-MAL05, and CR-MAL06) relative to 25 other cryptomonads. Glaucocystophyta was used as the outgroup. Bootstrap values (>50%) from maximum likelihood (ML; 200 replicates) and Bayesian posterior probability (MB) are indicated at nodes (presented in order ML/MB). Accession numbers of each taxon presented in parentheses. P: Pyrenomonadales; C: Cryptomonadales; G: Goniomonadida; *: bootstrap value of <50% in ML analysis

MAL06) of the cryptomonad prey strains (Fig. 3). The growth rates of *M. rubra* MR-MAL01 gradually decreased in the presence of different cryptomonad prey as follows: CR-MAL05 (0.40 d^{-1}), CR-MAL02 (0.27 d^{-1}), CR-MAL01 (0.20 d^{-1}), CR-MAL04 (0.17 d^{-1}), and CR-MAL03 (0.16 d^{-1}) (Table 1). Simultaneously, the prey species exhibited considerable intrinsic growth (i.e. μ d^{-1} of 0.31 to 0.85), which confirms the prey-replete condition of the experimental ciliate during the 6 d period (Table 1; Yih et al. 2004a).

Recently, Gustafson et al. (2000) found that 28 d starved *Myrionecta rubra* AY587129 obtained from Antarctic seawater grew better in the presence of *Geminigera* cf. *cryophila* (formerly *Teleaulax acuta*, Johnson et al. 2006) than in its absence (0.13 vs. 0.06 d^{-1}). In addition, Yih et al. (2004a) demonstrated that the growth rate of 14 d starved *M. rubra* MR-MAL01 (0.52 d^{-1}) was 1.4 times faster following the addition of cryptomonad prey strain CR-MAL01 than that in its absence (0.36 d^{-1}). Likewise, our experiments consistently showed that the 20 d starved *M. rubra* strain MR-MAL01 obtained from temperate coastal seawater grew better in the presence of cryptomonad strains CR-MAL01, CR-MAL02, CR-MAL04, and CR-MAL05 (0.17 to 0.40 d^{-1}) than in the absence of a cryptomonad strain (0.011 d^{-1}, Fig. 3, Table 1). Thus, it seems that the greater the pre-starvation period of *M. rubra* MR-MAL01, the slower its growth rate, even

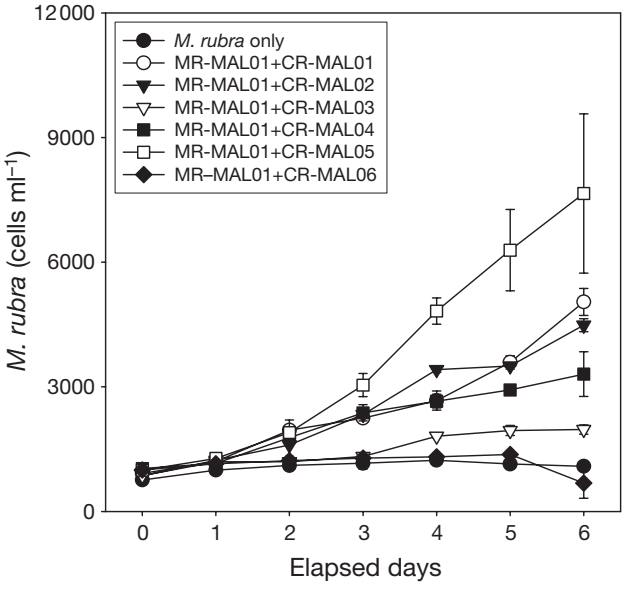

Fig. 3. *Myrionecta rubra*. Change in abundances of strain MR-MAL01, starved for 20 d then incubated in the presence of CR-MAL01, CR-MAL02, CR-MAL03, CR-MAL04, CR-MAL05, and CR-MAL06, and in the absence of any cryptomonad strain. Error bars = ±1 SD

when resupplied with cryptomonad prey (Johnson & Stoecker 2005).

Preferential growth of *Myrionecta rubra* (MR-MAL01) according to cryptomonad strain

Interestingly, the growth rate of *Myrionecta rubra* MR-MAL01 in the presence of cryptomonad strain CR-MAL06 (mean ± SD = 0.039 ± 0.034 d^{-1}) was indistinguishable from that in the absence of cryptomonads (mean ± SD = 0.011 ± 0.014 d^{-1}, *t*-test, $p > 0.05$), essentially indicating that the ciliate growth was not enhanced. Similarly, *M. rubra* MR-MAL01 demonstrated only a slightly elevated level of growth in the presence of cryptomonad strain CR-MAL03 (mean ± SD = 0.162 ± 0.031 d^{-1}) compared with the control (no addition of cryptomonad, Fig. 3, Table 1). Our hypothesis is that the starved *M. rubra* MR-MAL01 favored cryptomonads in Clade 3 to a lesser extent than cryptomonads in Clade 2. Further comparisons of the growth rates of *M. rubra* MR-MAL01 revealed that the growth rates gradually decreased in the presence of Clade 2 cryptomonad prey in the following order: CR-MAL05 (0.40 d^{-1}), CR-MAL02 (0.27 d^{-1}), CR-MAL01 (0.20 d^{-1}), and CR-MAL04 (0.17 d^{-1}) (Table 1), even though incubation conditions were identical (i.e. starvation time and initial density of MR-MAL01, temperature, salinity, light intensity, and the ratio of MR-MAL01 to prey). Therefore, our results indicate that *M. rubra* MR-MAL01 prefers some cryptomonad prey over others, and the growth of *M. rubra* MR-MAL01 is partially influenced by the availability of the different cryptomonad prey type. In laboratory cultures, sustained growth of *M. rubra* over generations requires that the ciliate ingest *Geminigera* cf. *cryophila* (Johnson & Stoecker 2005, Johnson et al. 2006), *Teleaulax* sp. (Hansen & Fenchel 2006), or 5 different cryptomonad strains (this study, Yih et al. 2004a) mostly belonging to cryptomonad Clade 2 of our phylogenetic tree. Therefore, one of the reasons for the failure of many earlier attempts to culture *M. rubra* might have been the choice of inappropriate cryptophyte prey; many culture collections comprise a majority of cryptomonad Clade 3 strains.

Finally, no relationship was found between the growth rates of *Myrionecta rubra* MR-MAL01 and the intrinsic growth rates (0.31 to 0.85 d^{-1}, Table 1) or biovolumes (82 to 294 μm^3, Table 1) of the cryptomonad prey (data not shown). It is possible that the preferential growth of the starved *M. rubra* MR-MAL01 could be controlled by other factor(s). After reviewing the

Table 1. *Myrionecta rubra*. Growth rates with and without the addition of cryptomonad strains, intrinsic growth rates, estimated biovolume, and sources of cryptomonad strains. na: not available

Strain	Growth rate of *M. rubra* (mean ± SD, d^{-1})	Growth rate of cryptomonads (mean ± SD, d^{-1})	Biovolume of cryptomonads[a] (μm^3)	*In situ* temp. (°C)	*In situ* salinity (psu)	Latitude, longitude	Source
CR-MAL01	0.20 ± 0.000	0.63 ± 0.096	111	7.8	30.1	35° 35′ N, 126° 36′ E	Gomso Bay, Korea
CR-MAL02	0.27 ± 0.055	0.79 ± 0.069	106	12	30.3	35° 35′ N, 126° 36′ E	Gomso Bay, Korea
CR-MAL03	0.16 ± 0.031	0.61 ± 0.006	82	10.5	30.8	36° 49′ N, 126° 10′ E	Taean, Korea
CR-MAL04	0.17 ± 0.001	0.31 ± 0.003	294	7.5	31.6	35° 05′ N, 126° 09′ E	Shinan, Korea
CR-MAL05	0.40 ± 0.002	0.85 ± 0.038	102	5.4	20.6	35° 58′ N, 126° 35′ E	Gunsan, Korea
CR-MAL06	0.04 ± 0.034	0.75 ± 0.067	215	na	na	na	na
No addition	0.01 ± 0.014	na	na	na	na	na	na

[a]Estimated biovolume of cryptomonads calculated as described by Hillebrand et al. (1999)

present study, Johnson et al. (2007) reported that plastid division of the cryptomonad *Geminigera cryophila* in *M. rubra* AY587129 was induced by the sequestered cryptomonad nuclei. Likewise, loss of the cryptomonad nuclei leads to decreases in the growth rates of *M. rubra* AY587129. Thus, it is possible that the retention time of the nuclei in *M. rubra* MR-MAL01 may differ according to the donor prey species. The retention times of the sequestered nuclei of various cryptomonad species are currently unknown; however, this merits further examination, particularly since the fate of the *Geminigera cryophila* nuclei in *M. rubra* AY587129 has been uniquely examined thus far.

Implications of kleptoplastidy and the feeding preferences of *Myrionecta rubra*

It is still highly controversial whether *Myrionecta rubra* ingests cryptophyte cells for the nutritional supply or for kleptoplastidy (Hansen & Fenchel 2006, Park et al. 2006). The plastids of cryptophyte origin in *M. rubra* were believed to be cryptophyte symbionts for a long time (Lohmann 1908, Fonds & Eisma 1967, Ryther 1967), probably owing to repeated failed culture attempts. However, by using monoxenic cultures, it was recently shown that the ciliate actually ingests cryptophyte cells (Yih et al. 2004a, Hansen & Fenchel 2006) in order to retain the photosynthetic capacity of plastids derived from the prey cryptophytes, which may last up to 30 d (Johnson & Stoecker 2005, Johnson et al. 2007).

If *Myrionecta rubra* is kleptoplastidic, then the successful acquisition of plastids should be important for its sustained growth (Yih et al. 2004a). In that case, the rapidly growing free-living cells of *M. rubra* MR-MAL01 require the following criteria for their growth: (1) cryptophyte prey, (2) that available cryptophyte members include CR-MAL05, CR-MAL02, and CR-MAL01 rather than the other cryptomonads of the present study (Table 1), and finally, (3) the maintenance of high photosynthetic rates, supported by the retained plastids. Moreover, it is likely that the preferential consumption of certain cryptophyte prey potentially leads to changes in cryptophyte communities in diverse marine environments, particularly during the *M. rubra* blooming period.

In conclusion, we demonstrated that the growth rate of *Myrionecta rubra* MR-MAL01 differed markedly when provided with various cryptomonad prey strains (i.e. CR-MAL01, CR-MAL02, CR-MAL03, CR-MAL04, and CR-MAL05). The 5 prey strains were grouped into 2 cryptomonad clades, which represent a much wider range of prey than previously thought. Furthermore, the growth rate of *M. rubra* MR-MAL01 may be influenced by the availability of specific prey types.

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