LETTERS

Retention of transcriptionally active cryptophyte nuclei by the ciliate *Myrionecta rubra*

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It is well documented that organelles can be retained and used by predatory organisms, but in most cases such sequestrations are limited to plastids of algal prey[1]. Furthermore, sequestrations of prey organelles are typically highly ephemeral[2] as a result of the inability of the organelle to remain functional in the absence of numerous nuclear-encoded genes involved in its regulation, division and function[3]. The marine photosynthetic ciliate *Myrionecta rubra* (Lohmann 1908) Jankowski 1976 (the same as *Mesodinium rubrum*)[4] is known to possess organelles of cryptophyte origin[5–9], which has led to debate concerning their status as permanent symbiotic or temporary sequestered fixtures[5–13]. Recently, *M. rubra* has been shown to steal plastids (that is, chloroplasts) from the cryptomonad, *Geminigera cryophila*, and prey nuclei were observed to accumulate after feeding[10]. Here we show that cryptophyte nuclei in *M. rubra* are retained for up to 30 days, are transcriptionally active and service plastids derived from multiple cryptophyte cells. Expression of a cryptophyte nuclear-encoded gene involved in plastid function declined in *M. rubra* as the sequestered nuclei disappeared from the population. Cytokinesis, plastid performance and their replication are dependent on recurrent stealing of cryptophyte nuclei. Karyoklepty (from Greek *karydi*, kernel; *kleftis*, thief) represents a previously unknown evolutionary strategy for acquiring biochemical potential.

We have previously shown that *M. rubra* ingests cryptophyte algae and retains organelles[10,12], leading to enhanced photosynthetic[10,11] and growth rates[10,11]. However, although cryptophyte nuclei have been observed in *M. rubra* after feeding[10,11], their viability or function has never been determined. The plastids of *M. rubra* are organized in numerous 'complexes'[5] that also contain cryptophyte mitochondria and cytoplasm, and they are packaged by a host membrane and two endoplasmic reticulum (ER) membranes[5,7–9]. When present, cryptophyte nuclei may be isolated in the cytosol (Fig. 1) or closely associated with one or more 'chloroplast–mitochondrial complexes' (Supplementary Fig. 1a) but are never found within them.

Geminigera cryophila cells are intact on ingestion (Fig. 1b), after which their membrane is compromised and organelles are sequestered. Newly ingested prey nuclei (3–4 μm) appear as they do in *G. cryophila* cells (Fig. 1a, inset); however, after 30 days 50% of cryptophyte nuclei in *M. rubra* increase in size to 7–10 μm (Fig. 1c, d, and Supplementary Fig. 2). When present, the double-membraned *G. cryophila* nucleus in *M. rubra* is surrounded by a single membrane, enclosing ribosome-rich cytoplasm and sometimes mitochondria, and closely associated with two ER membranes (Fig. 1c). Although the outer ER membrane surrounding nuclei and plastids in cryptophytes seems to be broken during the sequestration process, it is possible that the formation of ER connections between the two in *M. rubra* may facilitate protein secretion into organelle complexes. To verify that these nuclei were indeed from *G. cryophila* and to determine their fate, we applied a fluorescence *in situ* hybridization (FISH) probe for the cultured *G. cryophila* small-subunit (SSU) ribosomal RNA nuclear gene using techniques established previously[14]. The probe bound to RNA in the nucleolus of prey nuclei, to

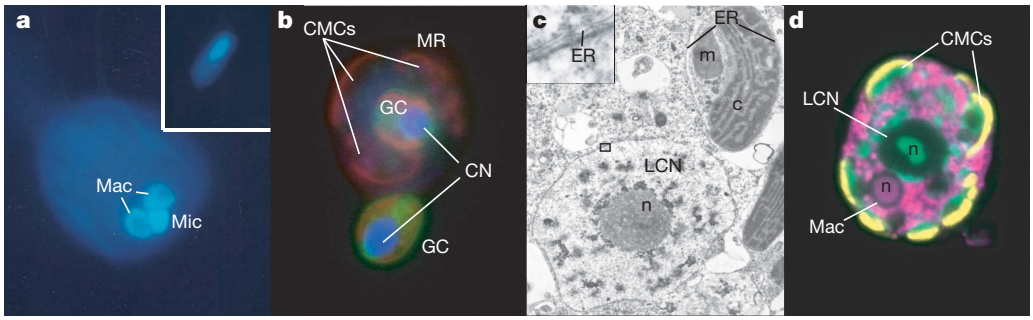

Figure 1 | Micrographs of *Myrionecta rubra* with *Geminigera cryophila* nuclei. **a**, Fluorescence micrograph of a DAPI-stained (blue) *Myrionecta rubra* cell without a cryptophyte nucleus and a free-living *Geminigera cryophila* cell (inset). Original magnification ×1,000. **b**, Layered fluorescence micrographs of a *M. rubra* cell with a newly ingested and free-living *G. cryophila* cell, hybridized with a FISH probe for *G. cryophila* SSU rRNA (green), stained with DAPI, and of endogenous plastid fluorescence (orange). Original magnification ×1,000. **c**, Transmission electron microscopy section of a *M. rubra* cell showing chloroplast–mitochondrial complexes and a cryptophyte nucleus, with detail of the surrounding membrane and ER (box and inset). Original magnification ×6,000. **d**, A layered three-channel (excitation at 488, 543 and 633 nm) confocal laser-scanning micrograph of a *M. rubra* cell dual-labelled with FISH probes for *G. cryophila* (green) and *M. rubra* (pink) SSU rRNA. Original magnification ×600. **c**, chloroplast; CMC, chloroplast–mitochondrial complex; CN, cryptophyte nucleus; GC, *G. cryophila* cell; LCN, large cryptophyte nucleus; m, mitochondrion; Mac, ciliate macronucleus; Mic, ciliate micronucleus; MR, *M. rubra* cell; n, nucleolus.

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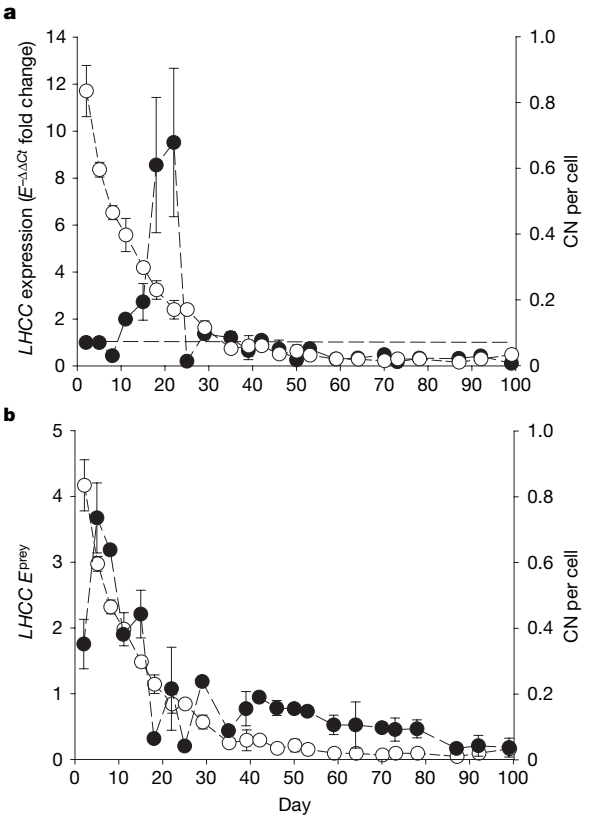

Figure 2 | Expression of the cryptophyte nuclear-encoded gene for the plastid-targeted protein *LHCC10* in *Myrionecta rubra*, and the presence of cryptophyte nuclei during starvation. **a**, Expression normalized to *M. rubra* β-tubulin gene expression ($E^{-\Delta\Delta Ct}$; filled circles)[15] shown with cryptophyte nuclei (CN) over time (open circles). The reference line across graph at 1 represents zero change from $t = 0$; below this line, expression has decreased from $t = 0$. **b**, Cryptophyte nuclei per cell (open circles) and *M. rubra LHCC* expression normalized to RNA standards of exponentially growing *G. cryophila* (E^{prey}), presented as equivalent *G. cryophila* cells per *M. rubra* cell (open circles). All results are means ± s.d.; $n = 2$.

cytoplasm enclosed within the ER surrounding the nucleus, to pockets of sequestered cytoplasm within the ciliate, and to the chloroplast–mitochondrial complexes (Fig. 1d). A FISH probe for the *M. rubra* nuclear SSU rRNA gene[14] was used in conjunction with the *G. cryophila* probe, and together they illustrate the mosaic nature of cryptophyte and endogenous cytoplasm in *M. rubra* (Fig. 1d; see also Supplementary Fig. 1a).

Gene expression of the sequestered nuclei in *M. rubra* includes plastid-targeted protein genes. Overexpression of the light-harvesting chloroplast complex protein gene (*LHCC10*)[15] was observed during the first 20–30 days after feeding (cultures fed 7 days before $t = 0$). When normalized to a *M. rubra* housekeeping gene[16], expression of *LHCC10* was enhanced up to tenfold in the first 20 days relative to $t = 0$ (Fig. 2a), and peaked when most sequestered nuclei had become unusually large (Supplementary Fig. 2a). Expression normalized to cryptophyte-only RNA standards was threefold to fourfold that of a cryptophyte cell during the same time and closely followed changes in cryptophyte nuclei per cell (Fig. 2b). The cryptophyte genes for the D1 protein (*psbA*, plastid) and the regulatory protein for ribulose bisphosphate carboxylase/oxygenase (RUBISCO) (*CbbX*, nucleomorph[17]) both revealed maximum expression during the first 2 weeks after the ingestion of cryptophyte prey (Supplementary Fig. 3). However, expression of the *CbbX* gene declined to relatively low expression levels after 3 weeks, whereas the plastid *psbA* gene maintained higher expression levels.

There was no evidence of net division of cryptophyte nuclei, although several sequestered nuclei were observed in karyokinesis (Supplementary Fig. 1b). Because the net retention time of plastids is greater than that for prey nuclei, an average *M. rubra* cell may have eight cryptophyte plastids per single prey nucleus. Thus, individual cryptophyte nuclei in *M. rubra* cells apparently service plastids originating from several *G. cryophila* cells. The decline in number of cryptophyte nuclei per cell followed an exponential decay curve ($r^2 = 0.983$) with a half-life of 9.53 days and a maximum retention time of 30 days (Supplementary Fig. 4). By day 35, cytokinesis of *M. rubra* decreased to half of levels at $t = 0$, approaching zero by the end of the experiment (Table 1). Loss of regulatory control over sequestered plastids lagged the loss of prey nuclei, with significant declines in plastid number per cell and photosynthetic quantum efficiency (F_v/F_m, where F_v is variable fluorescence and F_m is maximum fluorescence) after day 74 (Table 1).

These data show that sequestered cryptophyte nuclei are transcriptionally active in *M. rubra* and that cryptophyte organelles function 'symbiotically' during the period of nuclear retention. However, the destruction of cryptophyte prey cells precludes interpreting the relationship as symbiosis, and the process is best characterized as predation with farming of the prey organelles. Plastids in *M. rubra* do not seem to be permanently integrated cellular features. Prey nuclei are stolen and replaced by nearly constant feeding on cryptophyte algae; the nuclei seem to maintain the more stable plastids and mitochondria. Such a strategy may minimize predator investment in maintenance of the symbiont. Loss of prey nuclei results in the inability to divide plastids, leading to declines in organelle concentrations and biochemical potential (Table 1). Although the least stable aspect of this survival strategy seems to be the prey nucleus, the acquisition of new prey nuclei through feeding is potentially about 1 per day under natural conditions. The potential feeding rate is of the same order as cytokinesis rates and is much shorter than the observed half-life (about 10 days) of cryptophyte nuclei in the ciliate. Thus, in feeding *M. rubra* populations, retained prey nuclei could be present nearly continuously.

Whereas no organism has ever been described to sequester nuclei of another, red algal adelphoparasites are known to deliver a nucleus into their host cell cytoplasm, where it undergoes DNA synthesis and karyokinesis within the host's cytoplasm[18]. However, the relationship between *M. rubra* and *G. cryophila* is strikingly different from that between red algae and their adelphoparasites because of the distant phylogenetic relationships between host and prey. Foreign nuclei have also been observed transiently in some plastid-retaining dinoflagellates[19–21], but their role in the cells, if any, is unknown. Present-day observations of organelle retention, symbiosis and parasitism offer dynamic pictures of interspecies organellar and genomic interactions, and help in understanding the complex evolutionary history of eukaryotic cells. Although we cannot say whether nuclear retention

Table 1 | Physiological parameters for *Myrionecta rubra* during starvation

Period	Days	Growth per day	Plastid division per day	Nucleomorph genome per cell	Photosynthetic quantum efficiency
1	0–18	0.073 ± 0.019	0.075 ± 0.026	8.6 ± 1.4	0.61 ± 0.018
2	19–35	0.071 ± 0.016	0.061 ± 0.005	9.3 ± 1.2	0.61 ± 0.021
3	36–53	0.032 ± 0.002*	0.018 ± 0.009*	7.8 ± 1.2	0.60 ± 0.023
4	54–73	0.035 ± 0.001*	0.021 ± 0.001*	6.0 ± 1.0*	0.59 ± 0.038
5	74–99	0.015 ± 0.010*	0.009 ± 0.022*	3.9 ± 0.7*	0.49 ± 0.046*

All results are means ± s.d. ($n = 2$) over each period. Periods are defined by transfer to new medium. Nucleomorph genome per cell approximates to plastids per cell. Asterisk indicates a significant difference ($P < 0.05$) from period 1 values (one-tailed analysis of variance) with Tukey's Studentized range test.

is an evolutionary step towards the permanent establishment of a genetically integrated plastid, it offers a striking example of cellular chimaerism and has proved to be a successful ecological phenomenon. *M. rubra* is a highly successful and widespread member of the plankton, capable of forming dense red tides in coastal and upwelling waters[5,6]. Karyoklepty is a unique attribute, allowing temporary access to the genetic information and biochemical potential of another species.

METHODS

Experiment preparation. *Myrionecta rubra* (CCMP 2563) and *Geminigera cryophila* (CCMP 2564) cultures were maintained as described previously[11]. *G. cryophila* were added one week before $t = 0$ to semi-continuous batch cultures ($n = 2$) at a 4:1 ratio for the gene expression experiment. Sampling, measurement of growth rates, and nuclei counts by staining with 4′,6-diamidino-2-phenylindole (DAPI) were conducted with techniques described previously[10,11]. Transmission electron microscopy was performed, and F_v/F_m was measured, as described previously[12].

FISH. A FISH oligonucleotide probe, *TANU2*, labelled with fluorescein isothiocyanate (FITC) (Supplementary Table 1), for the *G. cryophila* SSU rRNA gene was designed by eye from DNA alignments with MacClade 4.05 (ref. 22). FISH probes for the *M. rubra* SSU rRNA gene (*MYR2*), labelled with 5-N,N′-diethyltetramethylindodicarbocyanine (Cy5)[14], positive control (uniC-FITC), and negative (anti-sense) control (uniR-FITC) probes[23] were also used. All techniques used were as described previously[14].

DNA extraction, polymerase chain reaction (PCR) and sequencing. DNA extraction, PCR amplification and gene sequencing for *M. rubra* genes were conducted with methods outlined previously[14]. The β-tubulin gene (*β-tub*) was isolated from *M. rubra* by using universal primers[24]. The light-harvesting complex protein (*LHCC10*) was isolated with primers (Supplementary Table 1) designed by eye from gene alignments in MacClade 4.05 (ref. 22).

RNA isolation, quantitative PCR and RT–PCR. RNA was isolated with the RNeasy Plant Mini Kit (Qiagen) and treated with DNase before use for RT–PCR (see Supplementary Information for details). Quantitative PCR was used to quantify nucleomorph (Nm) genome content in *M. rubra*, as a proxy for plastid number, with methods described previously[12]. Creation of complementary DNA and quantitative PCR for measurements of gene expression were conducted with TaqMan assays and a Cepheid Smart Cycler (see Supplementary Information for details).

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Author Information The sequences for the β-tubulin gene, *CbbX*, *LHCC10* and *psbA* are deposited in GenBank under accession numbers EF151014, EF151015, EF151016 and EF151017. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to M.D.J. (johnson@marine.rutgers.edu).