samples from the pelagic zones of three lakes (Spencer, Oneida and Cayuga). Periphyton and detritus were brushed from rocks, macrophytes and logs and pre-filtered through a 75-μm mesh to remove large invertebrates. Seston was pre-filtered through a 75 or 30 μm mesh. All periphyton and seston samples were then filtered onto pre-combusted glass fibre filters. Zooplankton were filtered from the water using a 150-μm mesh and visually inspected to remove particulate contaminants and predatory zooplankton. Each lake was sampled every two weeks from early June to late August (5–6 dates) in 1997 and 1998. Zooplankton were collected to provide the $\delta^{13}C_{base}$ of the pelagic food web because zooplankton are a better indicator of $\delta^{13}C_{base}$ for the pelagic food web than seston samples[28]. Snails and mussels were sampled in late August. In Spencer Lake we used unionid mussels (Unionacea) and in Cayuga and Oneida lakes we used zebra mussels (*Dreissena polymorpha*). In three other New York lakes (Champlain, Conesus and Keuka lakes), where unionid and zebra mussels occurred together, we found no difference in their $\delta^{13}C$ and $\delta^{15}N$ values (nested ANOVA with species nested in lake, d.f. = 3, $F = 2.85$, $P = 0.06$ for $\delta^{13}C$; d.f. = 3, $F = 1.79$, $P = 0.19$ for $\delta^{15}N$). Mussels and snails effectively captured the spatial variation and integrated the temporal variation in the $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ of pelagic and littoral food webs. Using lake-by-habitat combinations as replicates ($n = 6$ for both $\delta^{13}C$ and $\delta^{15}N$), we found no significant differences between the median $\delta^{13}C$ and $\delta^{15}N$ of each time series and the $\delta^{13}C$ and $\delta^{15}N$ of snails and mussels (paired *t*-test for means: $t = 2.29$, $P = 0.07$ for $\delta^{13}C$; $t = 2.19$, $P = 0.08$ for $\delta^{15}N$, where we subtracted 3.4‰ from the $\delta^{15}N$ of snails and mussels to remove the expected one trophic level of enrichment).

In each lake, we collected all fish species that were likely to feed at the top of the food web. Because trophic position can increase with fish length, we collected adult fish of each species and held length as constant as possible across the lake size gradient. The fish species collected and the lengths of fish analysed were: largemouth bass (*Micropterus salmoides*; 250–440 mm), smallmouth bass (*Micropterus dolomieu*; 250–450 mm), northern pike (*Esox lucius*; 450–840 mm), chain pickerel (*Esox niger*; 390–530 mm), walleye (*Stizostedion vitreum*; 300–700 mm), burbot (*Lota lota*; 580–740 mm), lake trout (*Salvelinus namaycush*; 460–730 mm), brook trout (*Salvelinus fontinalis*; 410 mm), chinook salmon (*Oncorhynchus tshawytscha*; 800–1000 mm), rainbow trout (*Oncorhynchus mykiss*; 340–480 mm), Atlantic salmon (*Salmo salar*; 490–770 mm) and brown trout (*Salmo trutta*; 310–610 mm). Snails and mussels were collected from each lake between late July and early September each year. Most fish were collected in late July to October, but for a few lakes we used fish collected in June. We used total phosphorus from integrated epilimnetic water samples taken in late July or August as an index of lake productivity. Our range of total phosphorus (2.6 to 230 $\mu g\,l^{-1}$) corresponds to a range of primary productivity of ~30–450 $g\,C\,m^{-2}\,yr^{-1}$ (ref. 29). We used previously documented volume estimates for 21 of our lakes. For the remaining four lakes, we estimated lake volume as a hyperbolic sinusoid: 0.43 × area × maximum depth.

We took a small section of muscle tissue from each fish for isotopic analysis. Snails and mussels were dissected and aggregated, particulate contaminants were removed and only soft tissue was used for isotopic analysis. Samples were dried at 40 °C for >48 h and ground to a fine powder. We then extracted lipids (using methanol-chloroform extraction) from all animal samples because lipids are depleted in ^{13}C compared with whole organisms[21,30] and lipid content in our tissue samples was variable (ranging from about 5% by mass in largemouth bass to >30% in some lake trout). Stable isotope analysis was performed using a Europa Geo 20/20 continuous flow isotope ratio mass spectrometer at the Cornell Laboratory for Stable Isotope Analysis. The standard error of the replicates of all our analyses were 0.05‰ for $\delta^{13}C$ and 0.18‰ for $\delta^{15}N$. All stable-isotope values are reported in the δ notation: $\delta^{15}N = ([(^{15}N_{sample}/^{14}N_{sample})/(^{15}N_{standard}/^{14}N_{standard})] - 1) \times 1{,}000$, where the global standard is atmospheric nitrogen, and $\delta^{13}C = ([(^{13}C_{sample}/^{12}C_{sample})/(^{13}C_{standard}/^{12}C_{standard})] - 1 \times 1{,}000$, where the global standard is PeeDee Belmnite[21].

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Cryptophyte algae are robbed of their organelles by the marine ciliate *Mesodinium rubrum*

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Mesodinium rubrum **(Lohmann 1908) Jankowski 1976 (= *Myrionecta rubra*)[1,2] is a common photosynthetic marine planktonic ciliate which can form coastal red-tides[3]. It may represent a 'species complex'[4,5] and since Darwin's voyage on the *Beagle*, it has been of great cytological, physiological and evolutionary interest[4]. It is considered to be functionally a phytoplankter because it was thought to have lost the capacity to feed and possesses a highly modified algal endosymbiont[5,6]. Whether *M. rubrum* is the result of a permanent endosymbiosis or a transient association between a ciliate and an alga is controversial[7]. We conducted 'feeding' experiments to determine how exposure to a cryptophyte alga affects *M. rubrum*. Here we show that although *M. rubrum* lacks a cytostome (oral cavity)[8], it ingests cryptophytes and steals their organelles, and may not maintain a permanent endosymbiont.**

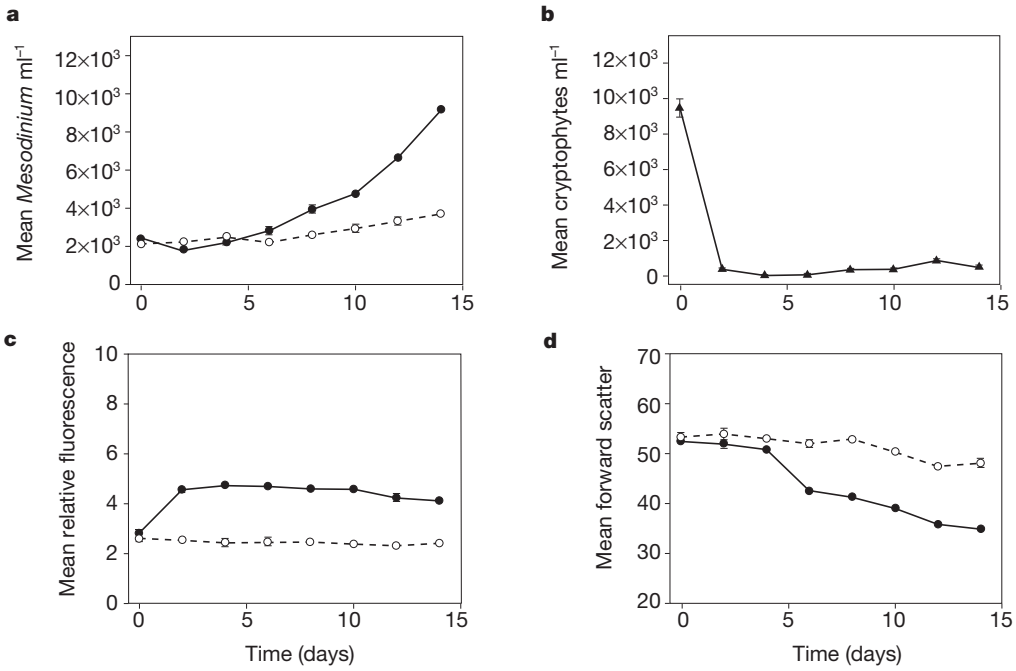

Figure 1 Changes in *Mesodinium rubrum* and cryptophyte populations measured by flow cytometry. Filled circles, cryptophyte-treated cultures; open circles, control cutures. **a**, Abundance of *M. rubrum* cells. **b**, Abundance of cryptophyte cells. **c**, Mean relative fluorescence of *M. rubrum*. **d**, Forward scatter of *M. rubrum*. (mean ± s.d., s.d. is not shown when it is no larger than the symbols).

***M. rubrum* does not fall into recognized cellular or functional categories, but may be a chimaera partially supported by organelle robbery.**

M. rubrum contains unusual 'incomplete symbionts' consisting of numerous functional chloroplasts associated with non-ciliate mitochrondria but apparently lacking nuclei[9]. Ultrastructural and pigment studies show that the chloroplasts are of cryptophycean origin[10]. The endosymbiont was thought to be highly modified, with little relationship to its free-living relations[6]. In contrast to the plastid-retaining ciliates[11,12], *M. rubrum* has a greatly reduced cytosome and no obvious digestive vacuoles[8]. It has never been observed to feed and there is no evidence of food particles inside the cell[13]. However, a related blue-pigmented *Mesodinium* sp. does capture cryptophytes[14].

Dense blooms of *M. rubrum* often result in non-toxic red-tides and have been associated with extremely high rates of primary production in estuaries, fjords and upwelling areas[5,15,16]. *M. rubrum* red-tides resulted in some of the highest recorded values for chlorophyll *a* and primary production in the marine environment[17]. There is good evidence for obligate phototrophy in *M. rubrum* from bloom studies, including high photosynthetic rates and uptake of inorganic nutrients[3,18,19]. This photosynthetic ciliate is often an important primary producer in coastal and upwelling environments even when it does not form red-tides[20–22].

M. rubrum is very fragile and difficult to culture, so previous studies have used field assemblages[23,24]. However, we obtained a culture of this ciliate from an enrichment of sea-ice/water collected in January 1996 from McMurdo Sound, Antarctica. We grew the isolate in algal growth media at 2–6 °C in the light. When grown in these conditions and supplied with a polar cryptophyte *Teleaulax acuta*, *M. rubrum* reaches densities of over 1.5×10^3 ml^{-1}, but it shows no sustained growth in the absence of algal 'prey'.

In the first experiment, we split a *M. rubrum* culture that had not been fed for 28 days into two; with (fed) and without (unfed, control) the addition of cryptophytes (10^4 ml^{-1}). The cultures were incubated for 14 days at 3 °C in 50–60 μmol photons m^{-2} s^{-1}PAR (photosynthetically active radiation, ~400–700 nm). The fed culture exhibited a higher sustained growth rate (0.19 divisions per day) than the control culture (0.09 divisions per day) (Fig. 1a). After addition of cryptophytes on day 1, *T. acuta* cells were reduced by 80% in 48 h (Fig. 1b). Red (chlorophyll *a*) and orange (phycoerythrin) fluorescence per *M. rubrum* cell increased by 1.5 times within 48 h and remained constant for 14 days (Fig. 1c). The forward scatter (an indicator of size) of *M. rubrum* cells decreased by 20% after 4–5 days since the cryptophyte addition and remained lower than in the control for the duration of the experiment (Fig. 1d).

Cultured *M. rubrum* cells are large (22–29 by 22–36 μm) with volume 1,400–4,900 μm^3 cell^{-1}. At addition, the average biovolumes were not significantly different between cultures (unfed 2,988 μm^3; fed 2,798 μm^3), whereas at day 6, there was a significant decrease (analysis of variance (ANOVA), $P < 0.05$) in the volume of the cryptophyte-treated cells (unfed 3,142 μm^3; fed 2,166 μm^3). By the end of the experiment, *M. rubrum* cells in the control culture were significantly larger (ANOVA, $P < 0.05$) than in the fed culture (2,716 μm^3 and 1,896 μm^3, respectively). Although *M. rubrum* can sustain a greater cell volume in the absence of cryptophytes, exposure to cryptophytes promotes cell division, resulting in a decrease in average cell size, but an increase in population size and biomass.

By day 6 there was a dramatic colour difference between the treatments; the fed culture was bright pink (similar to the cryptophyte) whereas the control culture was colourless. We examined 50 *M. rubrum* cells with epifluorescence microscopy at 0.5, 1.0, 4.0, 12, 24 h and 14 days after feeding. There were no fluorescence-blocking chlorophyll degradation bodies, evidence of digestion of algal 'prey', although these are observed in the digestive vacuoles of mixotrophic oligotrichous ciliates (D.K.S., personal observation).

In a second experiment, the percentage of *M. rubrum* with cryptophyte nuclei increased dramatically over the first hour after exposure to about 10^4 *T. acuta* ml^{-1} after not being fed for 14 days (Fig. 2a). The *M. rubrum* cells lacked cryptophyte nuclei at the beginning of the incubation. Within 5 min, about 50% of the *M. rubrum* cells had one cryptophyte nucleus and by 60 min, about 20% of the ciliates had three or more cryptophyte nuclei (Fig. 2a). Within a few minutes of exposure to cryptophytes, 40% of

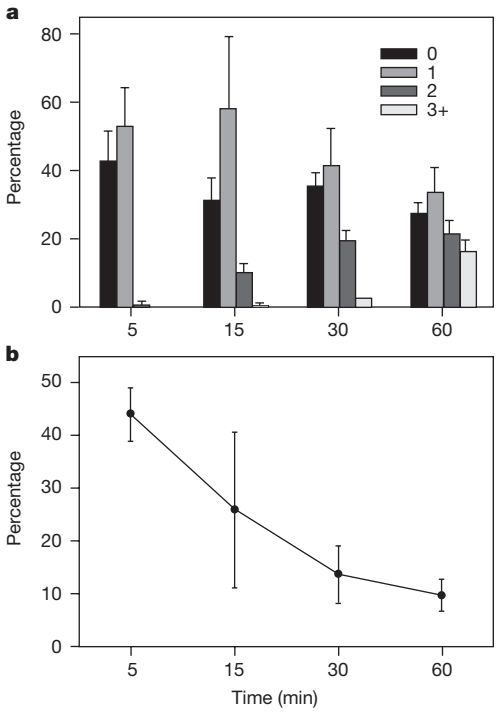

Figure 2 Ingestion of cryptophytes by *M. rubrum* when exposed to about 10^4 cryptophytes ml^{-1} after not being fed for 14 days. **a**, Percentage of *M. rubrum* containing cryptophyte nuclei. 0, no cryptophyte nuclei; 1, one cryptophyte nucleus; 2, two cryptophyte nuclei; 3+, three to seven cryptophyte nuclei present in one *M. rubrum* cell. **b**, Percentage of *M. rubrum* in the process of ingesting cryptophytes (mean ± s.d.).

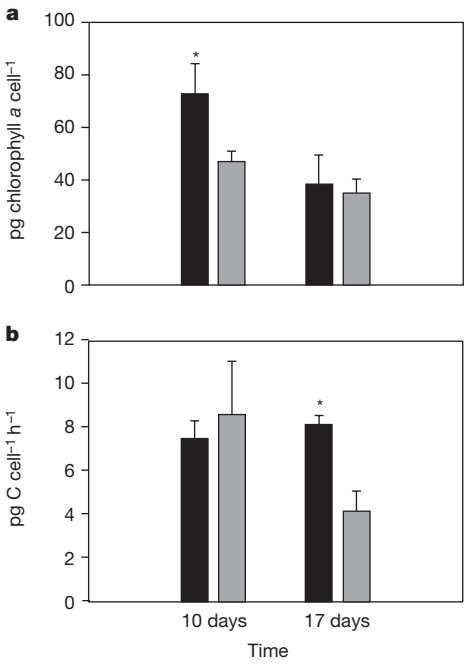

Figure 3 Chlorophyll content per cell and photosynthetic performance of *M. rubrum* at 10 and 17 days after addition of cryptophytes. Black bars, cryptophyte-treated culture; grey bars, control (untreated) culture. **a**, The chlorophyll *a* content. **b**, Photosynthetic performance per cell at 28–33 μmol photons m^{-2} s^{-1} (mean ± s.d.). Asterisk, significant difference between the two cultures ($P < 0.05$).

the ciliates were in the process of ingesting cryptophytes, a percentage which decreased over time (Fig. 2b). During the first hour, the *M. rubrum* exhibited an ingestion rate of 1.3 ± 0.03 cryptophytes $cell^{-1}$ h^{-1} (mean ± s.d.) and a clearance of approximately 128 nl $cell^{-1}$ h^{-1}.

In a third experiment, we added *T. acuta* to an *M. rubrum* culture that had not been fed for 14 days. Photosynthetic parameters were measured for fed and control cultures at day 10 and 17 of the incubation. Average chlorophyll content of *M. rubrum* was significantly higher in the fed culture than in the control on day 10 but not on day 17 (Fig. 3a). The *M. rubrum* chlorophyll *a* increased between day 10 and 17 from 223 to 394 ng ml^{-1} in the control and from 461 to 686 ng ml^{-1} in the fed culture. The average photosynthetic rate was not different between the fed and control cultures on day 10, but on day 17 the rate of photosynthesis per *M. rubrum* cell was greater in the fed culture than in the control (Fig. 3b). On day 17, the average chlorophyll-specific rates of photosynthesis of *M. rubrum* from the fed culture was significantly higher, 0.22 ± 0.03 pg C (pg chlorophyll *a*)$^{-1}$ h^{-1} than in the control, 0.12 ± 0.03 pg C (pg chlorophyll *a*)$^{-1}$ h^{-1} ($P < 0.05$).

Our results show that *M. rubrum* ingests free-living algae. Uptake of cryptophyte organelles is necessary for the sustained rapid growth of our isolate. Thus, the availability of cryptophyte 'prey' may trigger *M. rubrum* blooms and blooms may be partially limited by the availability of cryptophytes as a source of new organelles or nutrition. Whole cryptophytes may be present within the ciliate for a while after ingestion, but plastids are preferentially retained over cryptophyte nuclei. We did not observe chlorophyll degradation bodies, an indicator for digestion of algal prey, and digestive vacuoles have not been reported in investigations of this species with TEM (transmission electron microscopy)[7–9]. *M. rubrum*'s physiological ecology is very different from that of plastid-retaining ciliates, which are obligate mixotrophs and regularly need to ingest and digest prey to survive[25,26].

Increase in chlorophyll content in cultures deprived of prey for more than 16 days indicates that chlorophyll *a* can be synthesized within the ciliate. However, the uptake of cryptophyte plastids and the observed decrease in photosynthesis and growth of cells which had not recently been fed indicates that *M. rubrum* may need to feed periodically to replace ageing chloroplasts or chloroplasts diluted out by cell division. Although *M. rubrum* is photosynthetic and can synthesize chlorophyll *a*, it may not be an example of permanent endosymbiosis between an alga and a ciliate. It may be possible for the 'endosymbiont' to undergo degradation after ingestion by the ciliate and for the chloroplast and other organelles to persist for relatively long periods[27]. Thus, *M. rubrum* may be a cell chimaera dependent on periodic ingestion of cryptophyte algae. From our data it is not possible to determine if endosymbiont or plastid reproduction occurs in *M. rubrum*. It is possible that when stolen organelles become non-functional, they are disposed of through egestion or digestion, although there is no direct evidence for either. In contrast to plastid-retaining ciliates, but similarly to algae, *M. rubrum* is able to use inorganic nutrients from the water column[19]. Thus, our isolate of *M. rubrum* does not fall into recognized categories for functional types of planktonic organisms.

Methods

Feeding experiments

We used triplicate incubation bottles for each treatment. We collected and preserved samples in 2% glutaraldehyde. We quantified cell numbers and cell attributes by using Coulter EPICS Profile II and Becton Dickinson FACSCalibur flow cytometers. For microscopy, we stained subsamples with the DNA specific stain, 4,6-diamidino-2-phenylindole (DAPI), and examined them with Zeiss blue 450–490 nm filter set for observation of plastids and degradation bodies and with Zeiss UV 365nm filter set for observation of nuclei.

^{14}C techniques

We used triplicate incubation bottles for both the fed and control treatments. We added ^{14}C-bicarbonate (final activity about 1.0 μCi ml^{-1}) to subsamples from each replicate. At the time of subsampling, there were 140 free cryptophytes ml^{-1}. After adding ^{14}C, we took aliquots to determine total activity and split the replicates into dark (wrapped with

aluminum foil) and light bottles (28–33 μmol photons $m^{-2} s^{-1}$) and incubated them for 24 h at 3 °C. We isolated individual ciliates from each light and dark replicate, washed them three times with sterile media (10 ml) and transferred ten washed cells into a scintillation vial. During the 2–3 h isolation period we maintained the samples in the dark on ice. We prepared the samples for liquid scintillation counting as described[28]. We calculated rates of photosynthesis by subtracting average ^{14}C fixation in the dark from fixation in the light. For the determination of *M. rubrum* chlorophyll, we isolated and washed ten cells before transferring them into vials containing cold 90% acetone and incubating them at −20 °C overnight for extraction. We measured chlorophyll *a* using a 10-AU Turner fluorometer.

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Language trees support the express-train sequence of Austronesian expansion

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Languages, like molecules, document evolutionary history. Darwin[1] observed that evolutionary change in languages greatly resembled the processes of biological evolution: inheritance from a common ancestor and convergent evolution operate in both. Despite many suggestions[2–4], few attempts have been made to apply the phylogenetic methods used in biology to linguistic data. Here we report a parsimony analysis of a large language data set. We use this analysis to test competing hypotheses—the "express-train"[5] and the "entangled-bank"[6,7] models—for the colonization of the Pacific by Austronesian-speaking peoples. The parsimony analysis of a matrix of 77 Austronesian languages with 5,185 lexical items produced a single most-parsimonious tree. The express-train model was converted into an ordered geographical character and mapped onto the language tree. We found that the topology of the language tree was highly compatible with the express-train model.

There are many parallels between the processes of biological and linguistic evolution and the methods used to analyse them[4]. Despite these parallels, however, historical linguists have not used the quantitative phylogenetic methods that have revolutionized evolutionary biology in the past 20 years[8]. So, although linguists routinely use the "comparative method"[9] to construct language family trees from discrete lexical, morphological and phonological data, they do not use an explicit optimality criterion to select the best tree, nor do they typically use an efficient computer algorithm to search for the best tree from the discrete data. This is surprising given that the task of finding the best tree is inherently a combinatorial optimization problem of considerable computational difficulty[10]. One potential problem with a quantitative phylogenetic approach to linguistic evolution arises from the more reticulate nature of cultural evolution. Some authors[11,12] have claimed that reticulate processes in linguistic evolution overshadow those of descent, leading them to reject the appropriateness of the family-tree model. We believe that this is an empirical claim, which can be evaluated using phylogenetic methods. If the data fit well on the tree and there is little systematic conflicting signal, then the family-tree model is supported. If the data fit poorly, then alternative phylogenetic methods that do not assume a tree model, such as spectral analysis or split decomposition, should be investigated. A critical part of phylogenetic inference involves testing for congruence between independent lines of evidence. Here we test a model of the colonization of the Pacific that is derived from predominantly archaeological data by quantitatively examining its fit with a parsimony tree of Austronesian languages.

Prehistoric human colonization in the Pacific happened in two phases. Initially, Pleistocene hunter–gatherer expansions from Island Southeast Asia through New Guinea reached the Bismarck archipelago by 33,000 BP and the Papuan-speaking descendants of these people are dispersed throughout New Guinea and parts of Island Melanesia[13]. The second colonization wave of Austronesian language speakers involved a diaspora of Neolithic farming peoples out of south China and Taiwan around 6,000 BP[13–15]. According to the 'express train to Polynesia' model, the Austronesian expansion from Taiwan was extremely rapid, taking roughly 2,100 years to reach the edges of western Polynesia—a distance of 10,000 kilometres.