Chlorella vulgaris and *C. sorokiniana* cannot be maintained in *Paramecium bursaria* cell

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The ciliated protozoan *Paramecium bursaria* contains several hundred unicellular algae which belong to the genus *Chlorella*. Three *Chlorella* species are known to be able to infect to *P. bursaria*: *C. vulgaris, C. sorokiniana* and *C. kessleri*. However, these species are so morphologically similar, it is difficult to tell whether they can co-exist in the same host cell. In this study, we used a monoclonal antibody specific for symbiotic *Chlorella* of *P. bursaria* to distinguish them, and observed fates of ingested *Chlorella* cells in two cases: aposymbiotic *P. bursaria* cells were fed simultaneously with symbiotic *C. vulgaris* and free-living *C. sorokiniana* cells, or the *C. vulgaris* cells were added to *P. bursaria* cells after establishment of endosymbiosis with *C. sorokiniana* cells. In both cases, the two *Chlorella* species successfully localized just beneath the cell membrane in the same *P. bursaria* cell for at least one week. In the former case, however, only *C. vulgaris* cells were eventually retained in the host cells. In the latter case, *P. bursaria* cells bearing *C. vulgaris* exclusively increased one week after the mixing with *C. vulgaris* cells. These results suggest that co-existence of two *Chlorella* species in the same host cell is a temporary phenomenon and only *C. vulgaris* cells are retained in the long term.