Report

Current Biology

Shining a Light on Exploitative Host Control in a Photosynthetic Endosymbiosis

Highlights

- For protozoa and their algal symbionts, fitness in symbiosis is context dependent
- Symbiosis changes from costly to beneficial for hosts with increasing light
- Symbiosis is always costly for algae whose interests do not align with the host's
- Evolutionary stability of the symbiosis results from exploitation not mutualism

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In Brief

Lowe et al. show that a photosynthetic symbiosis between an algal symbiont and a protist host is based on exploitation of symbionts by the host, not mutual benefit. Symbiosis becomes more beneficial for hosts with increasing light, but more costly for symbionts, such that the fitness interests of the interacting species do not align.

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Shining a Light on Exploitative Host Control in a Photosynthetic Endosymbiosis

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SUMMARY

Endosymbiosis allows hosts to acquire new functional traits such that the combined host and endosymbiont can exploit vacant ecological niches and occupy novel environments [1, 2]; consequently, endosymbiosis affects the structure and function of ecosystems [3, 4]. However, for many endosymbioses, it is unknown whether their evolutionary basis is mutualism or exploitation [5–9]. We estimated the fitness consequences of symbiosis using the interaction between the protist host *Paramecium bursaria* and the algal symbiont *Chlorella* sp. [10]. Host fitness was strongly context dependent: whereas hosts benefited from symbiosis at high light intensity, carrying endosymbionts was costly to hosts in the dark and conferred no benefit over growing autonomously at intermediate light levels. Autonomous *Chlorella* densities increased monotonically with light intensity, whereas per-host symbiont load and symbiont abundance peaked at intermediate light levels and were lowest at high light intensity. This suggests that hosts controlled the costs of symbiosis by manipulating symbiont load according to light intensity. Photosynthetic efficiency was consistently lower for symbiotic compared to autonomous algae, suggesting nutritional constraints upon algae in symbiosis. At intermediate light levels, we observed the establishment of small populations of free-living algae alongside the hosts with endosymbionts, suggesting that symbionts could escape symbiosis, but only under conditions where hosts didn't benefit from symbiosis. Together, these data suggest that hosts exerted strong control over endosymbionts and that there were no conditions where this nutritional symbiosis was mutually beneficial. Our findings support theoretical predictions (e.g., [5, 9]) that controlled exploitation is an important evolutionary pathway toward stable endosymbiosis.

RESULTS AND DISCUSSION

Endosymbiosis is an intimate association in which one species lives inside another. Understanding the mechanisms of evolutionary stability in endosymbiosis, i.e., whether it is founded upon mutualism or exploitation [5–9], requires quantification of the fitness effects of symbiosis relative to autonomy for both hosts and endosymbionts [5, 11]. Quantification of autonomous growth of species is often challenging in extant endosymbioses, and consequently very few empirical tests exist [11]. Here we exploit a highly tractable microbial endosymbiosis between a heterotrophic ciliate (*Paramecium bursaria*) and a green alga (*Chlorella* sp.) that engage in a facultative photosymbiosis [10]. The *P. bursaria-Chlorella* symbiosis is widespread in shallow freshwater habitats and is based primarily upon the exchange of metabolites between the host and the endosymbiont [12–14]. Specifically, hosts provide endosymbionts with nitrogen compound(s) derived from heterotrophy, whereas endosymbionts provide hosts with maltose and oxygen derived from photosynthesis [13, 15, 16]. In this study, we estimated the fitness effects of symbiosis by comparing intrinsic growth rate or abundance in symbiotic and autonomous states. To test the context dependence of the fitness effects of symbiosis, we independently manipulated the supplies of light (affecting symbiont photosynthesis) and bacterial food (affecting host nutrients via heterotrophy) within ecologically realistic ranges [17–19].

For hosts, growth rate increased with increased food concentration irrespective of symbiosis or irradiance (Figures 1 and S1). Autonomous host growth rate was invariant with light; in contrast, hosts with endosymbionts suffered net mortality in the dark and achieved highest positive growth rates at highest irradiances. Consequently, the net effect of symbiosis on host growth rate (in terms of the ratio of symbiotic to autonomous growth) was context dependent, shifting from net costly to net beneficial with increasing irradiance (Figure 1). The highest net benefit of symbiosis occurred at high light and low food provision (Figure 1), an observation supporting the common assertion that photosymbiosis allows hosts with endosymbionts to exploit oligotrophic (i.e., nutrient limited) aquatic habitats [14, 20].

A potential mechanism by which hosts may manipulate the cost or benefit of symbiosis is via adjustment of symbiont load (i.e., the average number of symbionts per host). Although

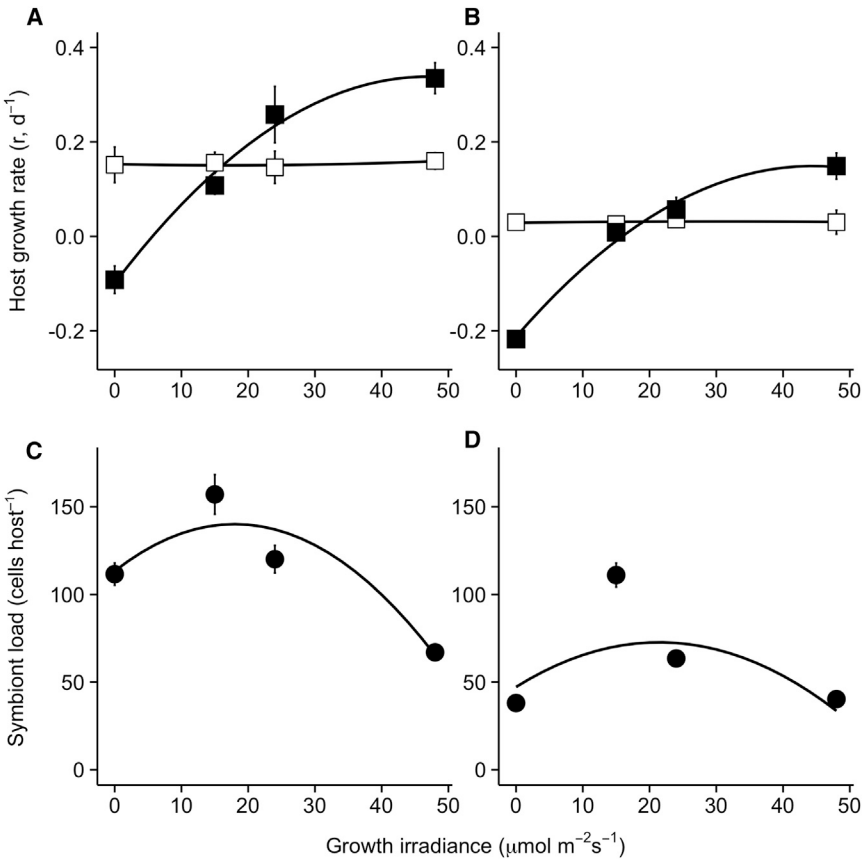

Figure 1. Host Growth Rate and Symbiont Loads in Response to Light

(A and B) Mean growth rate (n = 3 replicates; ±SE) responses to light of *Paramecium bursaria* in symbiosis (filled symbols) and in autonomy (open symbols). Assays were conducted under food-replete (i.e., 4.6 × 10^7 colony-forming units [CFU] per ml of *S. marcescens*; A) and food-limited (2.8 × 10^6 CFU per ml of *S. marcescens*; B) conditions. Responses were compared by analysis of covariance using quadratic functions. The effect of light on growth was dependent on the presence/absence of symbionts ($F_{2,\ 36}$ = 58.81, $p < 0.001$), and there was a significant main effect of food concentration on growth (i.e., growth rate was higher in the food-replete treatment independent of light and symbiont presence; $F_{11,36}$ = 31.62, $p < 0.001$). The ratio of autonomous to symbiotic host growth was greatest at high light and low food concentration (ratios at high light were 5.0 and 2.1 for hosts grown under food-limiting and food-replete conditions, respectively).

(C and D) The associated change in mean symbiont load (±SE, estimated from 25 host cells per replicate) at high (C) and low (D) food concentrations. Both light and food concentration had significant main effects on symbiont abundance within hosts ($F_{5,194}$ = 47.12, $p < 0.001$).

See also Figure S1 and Table S1.

the volume of the host cell places a clear upper limit on symbiont load, hosts individually encapsulate symbionts, potentially providing the basis of a regulation mechanism and preventing competition between symbionts for host-derived nutrients. Although precise mechanisms remain unknown, it is thought that hosts could regulate symbionts in a number of ways, including limiting metabolite exchange and therefore restraining algal cell division, coordinating algal cytokinesis, digesting non-photosynthesizing symbionts, and acquiring new symbionts from the environment [3, 15, 21–24]. Consequently, in parallel to host growth, we quantified symbiont load in response to light (Figure 1). Symbiont load displayed a high degree of plasticity with light level and followed similar trajectories in both food treatments: symbiont load peaked at low light (15 μmol irradiance), decreased in the dark, and was lowest at highest irradiance.

Reduction in symbiont load in the dark could result from host digestion, as previously described [21], or through a combination of the proposed host regulation mechanisms [3, 15, 21–24]. In light, the inverse relationship between symbiont load and light is also consistent with host control: hosts should downregulate symbiont load at higher irradiances because host energetic requirements can be met by fewer symbionts (since per-symbiont photosynthetic output increases with light) [25, 26]. For photosymbiotic interactions more broadly, the relationship between ambient irradiance and symbiont load is highly variable; most data concern natural coral-zooxanthallae populations, in which symbionts loads are invariant or decrease with increasing light (see [3] and references therein). In these studies, although host control is thought to be the key mechanism of plasticity in symbiont load, it has also been suggested that light inhibition, whereby reduced algal growth could result from photo-inhibition at elevated irradiance, may play a role [27, 28].

To distinguish effects of host control versus light inhibition on symbiont load, test the capacity of the algae for autonomous growth, and estimate the fitness effect of symbiosis for the algae, we isolated *Chlorella* from hosts to perform assays. Algae were established in conditioned protozoan growth medium, and the growth response to light quantified under precisely the same conditions as for hosts. *Chlorella* was capable of autonomous growth and consistently increased in abundance in response to increasing irradiance (Figure 2). There was no evidence of decreased abundance for autonomous algae at high light, supporting host control as the most likely mechanism of decreased symbiont load at high light. The capacity of *Chlorella* to grow autonomously in the same environment as its host allows a meaningful estimate of the fitness effect of symbiosis based upon comparison of the abundance of *Chlorella* in symbiosis (i.e., symbiont load multiplied by host density) versus the abundance in autonomy (Figure 2). The abundance of symbiotic and autonomous *Chlorella* diverged at high light. Whereas the abundance of symbiotic *Chlorella* decreased at high light, the autonomous algal abundance increased, indicating a clear light-dependent cost of symbiosis for *Chlorella*. In symbiotic populations, free-living populations of algal cells that had

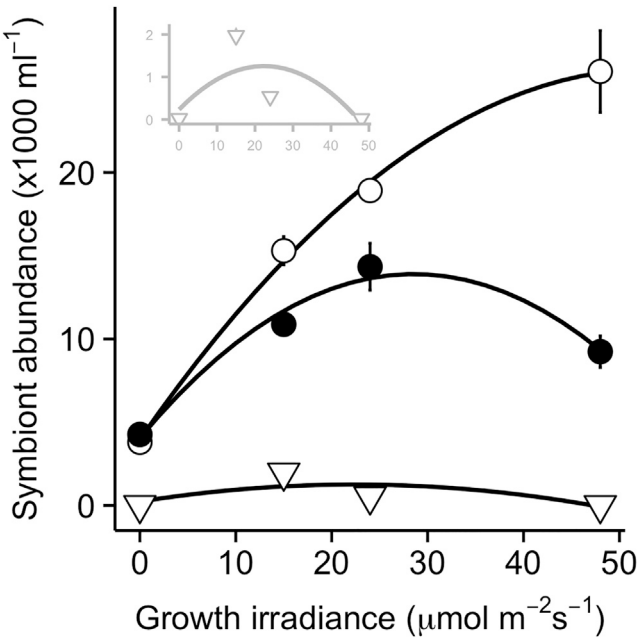

Figure 2. Algal Abundances in Autonomous, Symbiotic, and Free-Living States

The abundance of *Chlorella* sp. in response to a gradient in light differed depending on state (i.e., autonomous, free living, or symbiotic; $F_{5,30} = 50.19$, $p < 0.001$). Filled circles indicate the density of symbiotic algae (determined as the product of mean symbiont load and mean host density) within hosts grown under food repletion. Open circles indicate cell densities for *Chlorella* grown autonomously (i.e., cultured independently of hosts) in conditioned protozoan culture medium. Open triangles indicate mean density of free-living *Chlorella* within host-symbiont cultures (i.e., the abundance of "free" algal cells occurring in co-culture with hosts); the response is also provided in the inset figure at a magnified scale. Autonomous cell cultures were established at cell densities equivalent to those present in host-symbiont cultures at zero light. All responses presented as the mean (n = 3) ±SE. See also Figure S2 and Table S1.

escaped from symbiosis were also observed, the density of which—in parallel to symbiont load—peaked at low light, where hosts gained no benefit of symbiosis (Figure 2, inset). Interestingly, free-living algal populations were not observed in high light, where hosts gain the most benefit of symbiosis and, conversely, where the algae would most benefit from autonomy. This pattern suggests that hosts exert particularly tight control over their symbionts at high light, preventing their escape to free living.

It is thought that the major benefit gained by *Chlorella* in symbiosis is provision of nitrogen, the supply of which the host appears to directly regulate [3, 13, 16, 29]. Experiments using isolated symbiotic *Chlorella* show that algal growth and extracellular photosynthate release is regulated by the concentration of a variety of nitrogen compounds [29], and host supply of nitrogen is thought to play a role in coordination and progression of cytokinesis [30–32], suggesting an evolved mechanism of reciprocal metabolite exchange. The precise mechanisms underlying this are unknown as direct measurement of host-symbiont metabolite flux in microbial endosymbioses is beyond the resolution of current imaging mass spectrometry technologies. However, because of high nitrogen demand for the biosynthesis of chlorophyll and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) [33], photosynthesis is highly sensitive to nutrient stress. Therefore, we hypothesized that host limitation of the nitrogen supply could reduce photosynthetic efficiency in symbiosis relative to autonomy. To test this, we compared the response of algal photosynthesis to light in symbiosis versus autonomy by measuring key indicators of photosynthetic performance: the photochemical efficiency of photosystem II (PSII; i.e., F_v/F_m and Φ_{PSII}) and chlorophyll composition (i.e., the ratio of chlorophyll a and b). F_v/F_m is a measure of the maximum potential efficiency of PSII (i.e., in a state where all reaction centers are open), whereas Φ_{PSII} estimates the proportion of the total light absorbed by PSII that is actually used in photochemistry; both are sensitive to nutrient stress [34–36]. The ratio of chlorophyll a/b (Figure 3B) measures the proportions of light-harvesting complexes versus reaction centers, which typically increases in response to growth at high light [35]. Both symbiotic and autonomous algae showed a typical pattern of photo-acclimation [34–36] in that maximum photochemical efficiency (F_v/F_m) peaked in cells grown at low light, and the steady-state quantum yield (Φ_{PSII}) response to actinic light and the chlorophyll a/b ratio were elevated as a result of growth at high light (Figure 3). However, F_v/F_m and Φ_{PSII} were higher at all light levels in autonomous algae as compared to endosymbionts (Figure 3 and Table S1), indicating an overall reduction in photosynthetic efficiency in symbiosis. These patterns imply that symbiotic algae experienced higher nutrient stress than autonomous algae, suggesting that hosts restricted nitrogen supply to algae in symbiosis, which is consistent with the hypothesis that this symbiotic association is exploitative on the part of the host.

Endosymbiotic mutualisms have commonly been seen in terms of mutual exchange of costly resources, and the conceptual challenge has been to understand how such interactions remain stable despite the apparent selective advantage gained from cheating (e.g., failing to reciprocate in nutrient exchange) [5, 6, 38]. We provide experimental support for the growing body of theory predicting that stable symbiotic interactions can result from exploitation, rather than mutualism (e.g., [5, 9, 38]). Hosts controlled the cost of symbiosis by manipulating their symbiont load according to the light level. The observed plasticity in symbiont load with light is consistent with a recent mathematical model of protist-algal photosymbiosis, which predicts that this plastic response is characteristic of host control [26]: as light increases, hosts exert stronger suppression on symbiont densities due to increasing per symbiont photosynthetic output, allowing hosts to reduce their total investment in nitrogen provisioning and optimize the nutrient exchange in their favor [26]. We also show that algae could escape from exploitative symbiosis to establish free-living populations alongside hosts with symbionts, potentially via host death or egestion but that, interestingly, this only occurred in light environments where hosts gained no benefit of symbiosis, i.e., in low light (Figure 2).

We show that the fitness consequences of symbiosis for both hosts and symbionts are strongly context dependent and, critically, that the fitness interests of species in symbiosis may become de-coupled across environmental gradients (for other

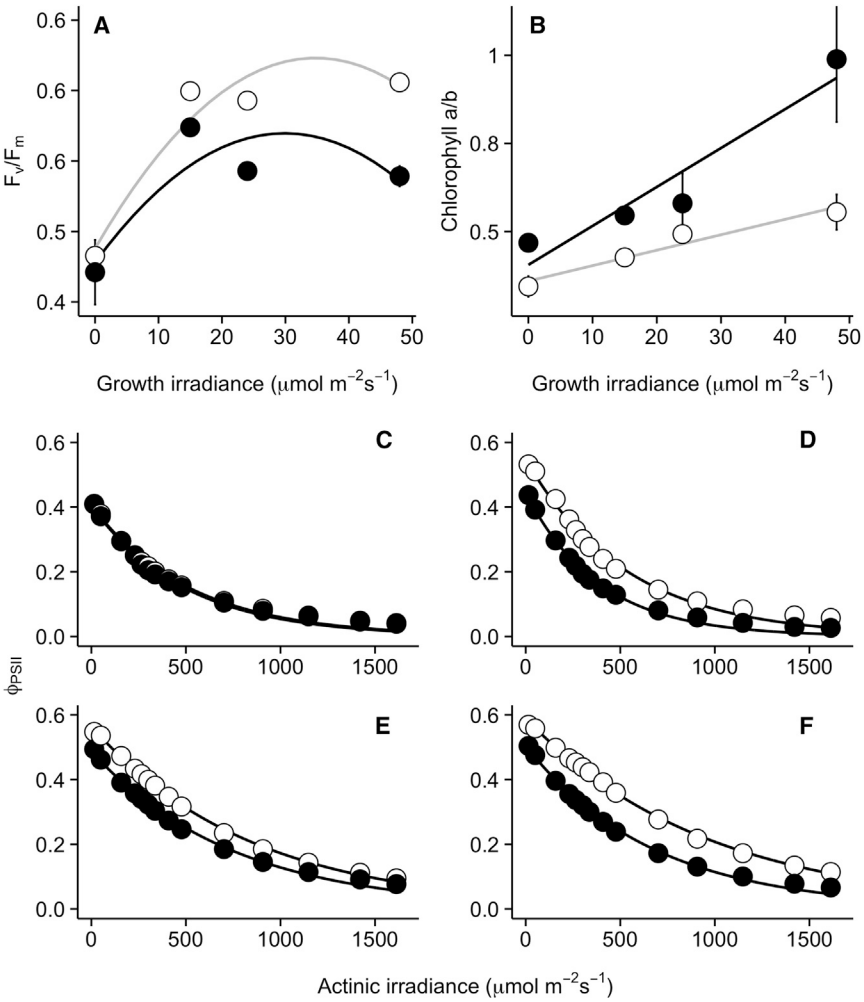

Figure 3. Photosynthetic Efficiency Parameters for Autonomous and Symbiotic Algae

(A) Estimates of maximum quantum yield of photosystem II (F_v/F_m). There was a significant effect of light on F_v/F_m ($F_{2,18} = 35.26$, $p < 0.001$), and F_v/F_m was higher in autonomous versus symbiotic algae ($F_{1,18} = 16.05$, $p < 0.001$).

(B) Chlorophyll a/b ratio for autonomous and symbiotic algae grown across a gradient in light. Chlorophyll a/b ratio increased with light, and this response was more pronounced for symbiotic versus autonomous algae ($F_{1,20} = 5.22$, $p = 0.034$).

(C–F) Light-adapted quantum yield of photosystem II (Φ_{PSII}) for algae grown in autonomy (open circles) and symbiosis (filled circles); (C)–(F) correspond to growth irradiances of 0, 15, 24, and 48 μmol photons m^{-2} s^{-1}, respectively. Lines represent exponential decay models of the form $y = a \cdot e^{(light * b)}$ fit using the nlme package in R. Replicates within treatments were treated as random effects; growth irradiance and state were treated as fixed effects. The intercept of Φ_{PSII} responses to light were significantly greater in autonomous versus symbiotic algae, but the rate of change of Φ_{PSII} in response to actinic light did not consistently differ between autonomous and symbiotic algae (see Table S1 for full statistical output). Chlorophyll fluorescence parameters were measured using a FastAct fast repetition rate fluorometer [37].

For all panels, responses are presented as the mean (n = 3) ±SE. See also Figure S3 and Table S1.

recent examples, see [39, 40]). Whereas the net benefit of symbiosis increased with light for hosts, symbionts experienced increasing costs of symbiosis from low to high light levels, such that the interaction was never mutually beneficial in any of the experimental conditions. The light and food supply regimes used here are well within the ranges experienced in natural environments [17–19], suggesting that similar dynamics are likely to occur in nature. Indeed, surveys of natural populations, albeit limited in number and scale, report variation in symbiont load and the occurrence of symbiont-free *P. bursaria* (e.g., [41, 42]). Moreover symbiotic and free-living *Chlorella* form polyphyletic groups (e.g., [43, 44]), suggesting repeated transitions from autonomy to symbiosis. Although none of the environmental conditions used here resulted in mutual benefits of nutrient exchange, it is possible that other environmental factors, like parasitism or predation [13], may enhance the benefits of symbiosis to *Chlorella* in more complex natural environments, and this will be a focus of future experiments. Host-symbiont conflicts arising from exploitative host control and strongly context-dependent fitness effects of symbiosis are likely to favor retention of the capacity for living autonomously and impede evolutionary transitions from facultative to obligate symbiosis.

ACCESSION NUMBERS

Data are available via the University of Exeter's open access data repository (https://ore.exeter.ac.uk/repository/) catalogued under the title of the manuscript.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.11.052.

AUTHOR CONTRIBUTIONS

C.D.L., M.A.B., and E.J.M. conceived and designed the experiment. C.D.L. and D.D.C. conducted experimental work and analyzed the data. All authors wrote the manuscript.

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REFERENCES

1. Wernegreen, J.J. (2012). Endosymbiosis. Curr. Biol. *22*, R555–R561.
2. Archibald, J.M. (2009). The puzzle of plastid evolution. Curr. Biol. *19*, R81–R88.
3. Davy, S.K., Allemand, D., and Weis, V.M. (2012). Cell biology of cnidarian-dinoflagellate symbiosis. Microbiol. Mol. Biol. Rev. *76*, 229–261.
4. Wooldridge, S.A. (2010). Is the coral-algae symbiosis really 'mutually beneficial' for the partners? BioEssays *32*, 615–625.
5. Law, R., and Dieckmann, U. (1998). Symbiosis through exploitation and the merger of lineages in evolution. Proc. R. Soc. B Biol. Sci. *265*, 1245–1253.
6. Wilkinson, D., and Sherratt, T.N. (2001). Horizontally acquired mutualisms, an unsolved problem in ecology? Oikos *92*, 377–384.
7. Wooldridge, S.A. (2009). A new conceptual model for the warm-water breakdown of the coral – algae endosymbiosis. Mar. Freshw. Res. *60*, 483–496.
8. Lesser, M.P., Stat, M., and Gates, R.D. (2013). The endosymbiotic dinoflagellates (*Symbiodinium* sp.) of corals are parasites and mutualists. Coral Reefs *32*, 603–611.
9. Frank, S.A. (1997). Models of symbiosis. Am. Nat. *150* (*Suppl 1*), S80–S99.
10. Fujishima, M., and Kodama, Y. (2012). Endosymbionts in *paramecium*. Eur. J. Protistol. *48*, 124–137.
11. Garcia, J.R., and Gerardo, N.M. (2014). The symbiont side of symbiosis: do microbes really benefit? Front Microbiol *5*, 510.
12. Karakashian, S.J. (1963). Growth of Paramecium bursaria as influenced by the presence of algal symbionts. Physiol. Zool. *36*, 52–68.
13. Esteban, G.F., Fenchel, T., and Finlay, B.J. (2010). Mixotrophy in ciliates. Protist *161*, 621–641.
14. Johnson, M.D. (2011). The acquisition of phototrophy: adaptive strategies of hosting endosymbionts and organelles. Photosynth. Res. *107*, 117–132.
15. Reisser, W. (1987). Studies on the ecophysiology of endocytobiotic associations of ciliates and algae. II potential features of adaptation of symbiotic and free-living *Chlorella* spp to the endocytobiotic habitat formed by *Paramecium bursaria*. Endocytobiotic Cell Res. *4*, 317–329.
16. Reisser, W. (1976). [The metabolic interactions between Paramecium bursaria Ehrbg. and Chlorella spec. in the Paramecium bursaria-symbiosis. II. Symbiosis-specific properties of the physiology and the cytology of the symbiotic unit and their regulation (author's transl)]. Arch. Microbiol. *111*, 161–170.
17. Liboriussen, L., and Jeppesen, E. (2003). Temporal dynamics in epipelic, pelagic and epiphytic algal production in a clear and a turbid shallow lake. Freshw. Biol. *48*, 418–431.
18. Liboriussen, L., and Jeppesen, E. (2006). Structure, biomass, production and depth distribution of periphyton on artificial substratum in shallow lakes with contrasting nutrient concentrations. Freshw. Biol. *51*, 95–109.
19. Christoffersen, K., Andersen, N., Søndergaard, M., Liboriussen, L., and Jeppesen, E. (2006). Implications of climate-enforced temperature increases on freshwater pico- and nanoplankton populations studied in artificial ponds during 16 months. Hydrobiologia *560*, 259–266.
20. Stoecker, D., Johnson, M., DeVargas, C., and Not, F. (2009). Acquired phototrophy in aquatic protists. Aquat. Microb. Ecol. *57*, 279–310.
21. Kodama, Y., and Fujishima, M. (2008). Cycloheximide induces synchronous swelling of perialgal vacuoles enclosing symbiotic Chlorella vulgaris and digestion of the algae in the ciliate *Paramecium bursaria*. Protist *159*, 483–494.
22. Kadono, T., Kawano, T., Hosoya, H., and Kosaka, T. (2004). Flow cytometric studies of the host-regulated cell cycle in algae symbiotic with green *paramecium*. Protoplasma *223*, 133–141.
23. Takahashi, T., Shirai, Y., Kosaka, T., and Hosoya, H. (2007). Arrest of cytoplasmic streaming induces algal proliferation in green *paramecia*. PLoS ONE *2*, e1352.
24. Kodama, Y., and Fujishima, M. (2012). Cell division and density of symbiotic Chlorella variabilis of the ciliate Paramecium bursaria is controlled by the host's nutritional conditions during early infection process. Environ. Microbiol. *14*, 2800–2811.
25. Hoogenboom, M., Beraud, E., and Ferrier-Pagès, C. (2009). Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral *Cladocora caespitosa*. Coral Reefs *29*, 21–29.
26. Dean, A., Minter, E., Sørensen, M., Lowe, C., Cameron, D., Brockhurst, M., and Jamie, A. (2015). Nutrient trading and host control in a photosynthetic symbiosis. arXiv, arXiv:1512.01595. http://arxiv.org/abs/1512.01595.
27. Iglesias-Prieto, R., and Trench, R. (1994). Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. Mar. Ecol. Prog. Ser. *113*, 163–175.
28. Iglesias-Prieto, R., and Trench, R.K. (1997). Acclimation and adaptation to irradiance in symbiotic dinoflagellates. II. Response of chlorophyll-protein complexes to different photon-flux densities. Mar. Biol. *130*, 23–33.
29. Kato, Y., Ueno, S., and Imamura, N. (2006). Studies on the nitrogen utilization of endosymbiotic algae isolated from Japanese *Paramecium bursaria*. Plant Sci. *170*, 481–486.
30. McAuley, P.J., and Muscatine, L. (1986). The cell cycle of symbiotic *Chlorella*. IV. DNA content of algae slowly increases during host starvation of green hydra. J. Cell Sci. *85*, 73–84.
31. McAuley, P.J. (1992). The effect of maltose release on growth and nitrogen metabolism of symbiotic *Chlorella*. Br. Phycol. J *27*, 417–422.
32. McAuley, P.J. (1987). Nitrogen limitation and amino-acid metabolism of Chlorella symbiotic with green hydra. Planta *171*, 532–538.
33. Irving, L.J., and Robinson, D. (2006). A dynamic model of Rubisco turnover in cereal leaves. New Phytol. *169*, 493–504.
34. Kitajima, K., and Hogan, K.P. (2003). Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. Plant Cell Environ. *26*, 857–865.
35. Walters, R.G. (2005). Towards an understanding of photosynthetic acclimation. J. Exp. Bot. *56*, 435–447.
36. Kargul, J., and Barber, J. (2008). Photosynthetic acclimation: structural reorganisation of light harvesting antenna–role of redox-dependent phosphorylation of major and minor chlorophyll a/b binding proteins. FEBS J. *275*, 1056–1068.
37. Oxborough, K., Moore, C.M., Suggett, D.J., Lawson, T., Chan, H.G., and Geider, R.J. (2012). Direct estimation of functional PSII reaction center concentration and PSII electron flux on a volume basis: a new approach to the analysis of Fast Repetition Rate fluorometry (FRRf) data. Limnol. Oceanogr. Methods *10*, 142–154.
38. Antonovics, J., Bergmann, J., Hempel, S., Verbruggen, E., Veresoglou, S., and Rillig, M. (2015). The evolution of mutualism from reciprocal parasitism: more ecological clothes for the Prisoner's Dilemma. Evol. Ecol. *29*, 627–641.
39. DiSalvo, S., Haselkorn, T.S., Bashir, U., Jimenez, D., Brock, D.A., Queller, D.C., and Strassmann, J.E. (2015). Burkholderia bacteria infectiously induce the proto-farming symbiosis of Dictyostelium amoebae and food bacteria. Proc. Natl. Acad. Sci. USA *112*, E5029–E5037.
40. Regus, J.U., Gano, K.A., Hollowell, A.C., Sofish, V., and Sachs, J.L. (2015). Lotus hosts delimit the mutualism-parasitism continuum of *Bradyrhizobium*. J. Evol. Biol. *28*, 447–456.
41. Tonooka, Y., and Watanabe, T. (2002). A natural strain of *Paramecium bursaria* lacking symbiotic algae. Eur. J. Protistol. *38*, 55–58.
42. Tonooka, Y., and Watanabe, T. (2007). Genetics of the relationship between the ciliate *Paramecium bursaria* and its symbiotic algae. Invertebr. Biol. *126*, 287–294.
43. Hoshina, R., and Imamura, N. (2008). Multiple origins of the symbioses in *Paramecium bursaria*. Protist *159*, 53–63.
44. Summerer, M., Sonntag, B., and Sommaruga, R. (2008). Ciliate-symbiont specificity of freshwater endosymbiotic *Chlorella* (Trebouxiophyceae, Chlorophyta). J. Phycol. *44*, 77–84.