or are they resolved? Both the ants and the termites cultivate their fungal crops in monocultures. This is remarkable, because there is ample genetic variation of fungal strains across colonies so that horizontal transmission should at least occasionally (in the ants) or regularly (in most termites) establish genetically variable fungus gardens. In the ants, monocultures are actively enforced because fungal incompatibility compounds hitchhike through the ant guts to be expressed in the feces that fertilize new implants of somatic fungal fragments [14]. The termites, however, propagate their symbionts within colonies by asexual spores that they embed in newly deposited fecal substrate. This system is therefore expected to produce symbiont monocultures by a combination of genetic drift and selection for rapid spore formation, rather than by active competition via incompatibility compounds [11,12].

Can we learn something from the sustainable farming practices of insect societies? The farming insect societies had tens of millions of years of natural selection to solve many of the challenges that are also well known to human farmers. They have conveyor belt substrate processing, produce their own pesticides and antibiotics, and practice active waste management [1]. Neither the ants, nor the termites, however, have been able to overcome the fundamental laws of host–symbiont conflicts, which imply that only monoculture farming is evolutionarily stable. Our own farming practices evolved culturally by frequent exchange of crops, learning and copying innovative practices. The problem is that, on the larger scale that we apply today, many of these practices are unlikely to be sustainable, even on an ecological time scale. It may be, therefore, that further research on the long-term evolutionary stable farming systems of the ants and termites may provide useful lessons for our own future food production.

References

1. Mueller, U.G., Gerardo, N.M., Aanen, D.K., Six, D.L., and Schultz, T.R. (2005). The evolution of agriculture in insects. Annu. Rev. Ecol. Evol. Syst. *36*, 563–595.
2. Aanen, D.K., Eggleton, P., Rouland-Lefevre, C., Guldberg-Froslev, T., Rosendahl, S., and Boomsma, J.J. (2002). The evolution of fungus-growing termites and their mutualistic fungal symbionts. Proc. Natl. Acad. Sci. USA *99*, 14887.
3. Bourke, A.F.G. (2005) Genetics, relatedness and social behaviour in insect societies. In *Insect Evolutionary Ecology*, M.D.E. Fellowes, G.J. Holloway and J. Rolff, eds. (Wallingford: CABI Publishing).
4. Foster, K.R., and Wenseleers, T. (2006). A general model for the evolution of mutualisms. J. Evol. Biol. *19*, 1283–1293.
5. Kiers, E.T., Rousseau, R.A., West, S.A., and Denison, R.F. (2003). Host sanctions and the legume-rhizobium mutualism. Nature *425*, 78–81.
6. Currie, C.R., Scott, J.A., Summerbell, R.C., and Malloch, D. (1999). Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. Nature *398*, 701–704.
7. Currie, C.R., Wong, B., Stuart, A.E., Schultz, T.R., Rehner, S.A., Mueller, U.G., Sung, G.H., Spatafora, J.W., and Straus, N.A. (2003). Ancient tripartite coevolution in the attine ant-microbe symbiosis. Science *299*, 386–388.
8. Currie, C.R., Poulsen, M., Mendenhall, J., Boomsma, J.J., and Billen, J. (2006). Coevolved Crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. Science *311*, 81–83.
9. Green, A.M., Mueller, U.G., and Adams, R.M.M. (2002). Extensive exchange of fungal cultivars between sympatric species of fungus-growing ants. Mol. Ecol. *11*, 191–195.
10. Mikheyev, A.S., Mueller, U.G., and Abbot, P. (2006). Cryptic sex and many-to-one coevolution in the fungus-growing ant symbiosis. Proc. Natl. Acad. Sci. USA *103*, 10702–10706.
11. Aanen, D.K. (2006). As you reap, so shall you sow: coupling of inoculating and harvesting stabilizes the mutualism between termites and fungi. Biol. Lett. *2*, 209–212.
12. Aanen, D.K., and Boomsma, J.J. (2006). The evolutionary origin and maintenance of the mutualism between termites and fungi. In *Insect Symbiosis II*, K. Bourtzis and T.A. Miller, eds. (Boca Raton: CRC Press).
13. Mueller, U.G. (2002). Ant versus Fungus versus Mutualism: Ant-cultivar conflict and the deconstruction of the attine ant-fungus symbiosis. Am. Nat. *160*, S67–S98.
14. Poulsen, M., and Boomsma, J.J. (2005). Mutualistic fungi control crop diversity in fungus-growing ants. Science *307*, 741–744.
15. Frank, S.A. (1996). Host-symbiont conflict over the mixing of symbiotic lineages. Proc. R. Soc. Lond. B *263*, 339–344.

[1]Laboratory of Genetics, Wageningen University and Research Center, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands. [2]Department of Population Biology, Institute of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark.
E-mail: duur.aanen@wur.nl

Correspondences

The difference between organelles and endosymbionts

Ursula Theissen and William Martin*

Three recent contributions in *Current Biology* [1–3] have addressed new findings on the classical cyanobacterial endosymbiont of *Paulinella chromatophora*, but refer to the endosymbiont as a 'plastid'. Yoon *et al.* [2] even opine that *Paulinella "has the honor of being the only known case of an independent primary (cyanobacterial) plastid acquisition."* Others have called the *Paulinella* endosymbiont a "*photosynthetic organelle*" [4] instead.

Endosymbionts are organisms that live within other organisms. Many endosymbionts are obligate — they cannot live outside their hosts [5] — as also reported for *Paulinella chromatophora* [2]. And many obligate endosymbionts are essential for their hosts as well [5], for example *Buchnera aphidicola*, which supplies amino acids for its aphid host [6].

Plastids, such as mitochondria, are not endosymbionts; they are organelles. They once were endosymbionts, but they now are double membrane-bounded organelles, compartments of eukaryotic cells.

All of the functional proteins in the cytosol of an endosymbiont are encoded by its own genome. By contrast, only a very small fraction of the proteins that function in organelles are encoded by organellar DNA. The majority of organellar proteins are encoded by the nuclear DNA, translated on cytosolic ribosomes and imported into the organelle with the help of a protein import apparatus [7,8].

This evolutionarily and functionally sharp distinction between organelles and endosymbionts — protein import, or not — was crisply articulated by Cavalier-Smith and Lee [9]. It has proven to be exquisitely robust.

Unless the *Paulinella* endosymbiont can be shown to possess a protein import apparatus, it is just another member in a long list of known cases of endosymbionts: the proteobacterial endosymbionts of insects such as *Buchnera*, *Wigglesworthia*, and *Wolbachia* [5,6,10], the methanogenic endosymbionts of anaerobic ciliates [11], the nitrogen-fixing symbionts in the diatom *Rhopalodia* [12], the chemosynthetic endosymbiont consortia of gutless tubeworms [13], the cyanobacterial endosymbionts of sponges [14], and endosymbionts that live within other prokaryotes [15] — to name just very few examples.

The rate-limiting step in the transition from endosymbionts to organelles would appear to be the origin of the protein import machinery itself [9]: the TIM and TOM complexes of mitochondria [7] and the TIC and TOC complexes of plastids [8].

The origin of those complexes allowed each organelle to specifically import proteins synthesized in the host's cytosol, thereby allowing the endosymbionts to relinquish their prokaryotic genes without relinquishing their prokaryotic biochemistry.

Calling the *Paulinella* endosymbiont a plastid or an organelle might make a story more exciting, but at the cost of scientific accuracy. Some proteobacterial endosymbionts of aphids have genomes smaller than those of some plastids [16]. Would anyone call those endosymbionts 'mitochondria'? Hardly.

For the same reasons, we should not call the *Paulinella* endosymbionts 'plastids' any more than we should say that sponges [14] have 'plastids'. There is a difference between endosymbionts and organelles.

References

1. Archibald, J.M. (2006). Endosymbiosis: Double-take on plastid origins. Curr. Biol. *16*, R690–R692.
2. Yoon, H.S., Reyes-Prieto, A., Melkonian, M., Bhattacharya, D. (2006). Minimal plastid evolution in the *Paulinella* endosymbiont. Curr. Biol. *16*, R670–R672.
3. Rodriguez-Ezpeleta, N., and Philippe, H. (2006). Plastid origin: Replaying the tape. Curr. Biol. *16*, R54–R56.
4. Marin B, Nowack E.C.M., Melkonian, M. (2005). A plastid in the making: Evidence for a second primary endosymbiosis. Protist, Vol. *156*, 425–432.
5. Zientz, E., Dandekar, T., and Gross, R. (2004). Metabolic interdependence of obligate intracellular bacteria and their insect hosts. Microbiol. Mol. Biol. Rev. *68*, 745–770.
6. Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y., and Ishikawa, H. (2000). Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera sp*. APS. Nature *407*, 81–86.
7. Dolezal, P., Likic, V., Tachezy, J. and Lithgow, T. (2006). Evolution of the molecular machines for protein import into mitochondria. Science *313*, 314-318.
8. Soll, J. and Schleiff, E. (2004). Protein import into chloroplasts. Nat. Rev. Mol. Cell Biol. *5*, 198–208.
9. Cavalier-Smith, T., and Lee, J.J. (1985). Protozoa as hosts for endosymbioses and the conversion of symbionts into organelles. J. Protozool. *32*, 376–379.
10. Wu, M., Sun, L., Vamathevan, J., Riegler, M., Deboy, R., Brownlie, J., McGraw, E., Mohamoud, Y., Lee, P., Berry, K. *et al.* (2004). The genome sequence and evolution of the reproductive parasite *Wolbachia pipientis* wMel: a streamlined -Proteobacterium massively infected with mobile genetic elements. PLoS Biol. *2*, 327–341.
11. Embley, T.M. and Finlay, B.J. (1993). Systematic and morphological diversity of endosymbiotic methanogens in anaerobic ciliates. Antonie Van Leeuwenhoek *64*, 261–271.
12. Prechtl, J., Kneip, C., Lockhart, P., Wenderoth, K. and Maier, U.G. (2004). Intracellular spheroid bodies of *Rhopalodia gibba* have nitrogen-fixing apparatus of cyanobacterial origin. Mol. Biol. Evol. *21*, 1477–1481.
13. Woyke, T., Teeling, H., Ivanova, N.N., Huntemann, M., Richter, M., Gloeckner, F.O., Boffelli, D., Anderson, I.J., Barry, K.W., Shapiro, *et al.* (2006). Symbiosis insights through metagenomic analysis of a microbial consortium. Nature *443*, 950–955.
14. Thacker, R.W. (2005). Impacts of shading on sponge-Cyanobacteria symbioses: A comparison between host-specific and generalist associations. Integr. Comp. Biol. *45*, 369–376.
15. Wujek, D.E. (1979). Intracellular bacteria in the blue-green-alga *Pleurocapsa minor*. Trans. Am. Micros. Soc. *98*, 143–145.
16. Nakabachi, A., Yamashita, A., Toh, H., Ishikawa, H., Dunbar, H.E., Moran, N.A., Hattori M. (2006). The 160-kilobase genome of the bacterial endosymbiont *Carsonella*. Science *314*, 267.

Institute of Botany III, University of Düsseldorf, D-40225 Düsseldorf, Germany.
*E-mail: w.martin@uni-duesseldorf.de

Response to Theissen and Martin

Debashish Bhattacharya[1] and John M. Archibald[2]

Theissen and Martin [1] question the use of the term organelle — and, by extension, plastid — as applied to the photosynthetic inclusions of the filose amoeba *Paulinella chromatophora*. We suggest that the apparent degree of biochemical and cellular integration of host and 'endosymbiont' in this unicellular eukaryote distinguishes it from other examples of prokaryotic endosymbionts, warranting use of the term 'plastid'.

The question is as previously stated: *"to what extent can the* P. chromatophora *endosymbiont be considered a bona fide organelle?"* [2]. The answer depends on what future studies reveal about the biology of *Paulinella*. It also depends on one's definition of organelle. Theissen and Martin [1] argue that the difference between endosymbionts and organelles is protein import: all of the cytosolic proteins in an endosymbiont are encoded in its own genome, whereas most organellar proteins are encoded by nuclear DNA, translated in the host cytosol and targeted to the organelle using a protein import apparatus, as in mitochondria and plastids [3,4]. It will indeed be important to determine whether a rudimentary protein import apparatus is necessary in *Paulinella* and, if so, in which form it exists. Clearly it would look nothing like the TIC/TOC import apparatus that evolved once in canonical plastids [4].

Does this matter? How complex would such an import apparatus have to be to justify use of the terms 'organelle' and 'plastid'? For example, would the targeting of host- or endosymbiont-derived, nucleus-encoded proteins to the endosymbiont via the secretory pathway, as recently shown for carbonic anhydrase