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All essential endosymbionts of the ciliate *Euplotes* are cyclically replaced

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Symbiotic systems vary in the degree to which the partners are bound to each other¹. At one extreme, there are intracellular endosymbionts in mutually obligate relationships with their host, often interpreted as mutualistic. The symbiosis between the betaproteobacterium *Polynucleobacter* and the ciliate *Euplotes* (clade B) challenges this view²: although freshwater *Euplotes* species long ago became dependent on endosymbionts, the many extant *Polynucleobacter* lineages they harbour arose recently and in parallel from different free-living ancestors². The host requires the endosymbionts for reproduction and survival³, but each newly established symbiont is ultimately driven to extinction in a cycle of establishment, degeneration, and replacement. Similar replacement events have been observed in sap-feeding insects⁴⁻⁶, a model for bacteria-eukaryote symbioses⁷, but usually only affect a small subset of the host populations. Most insects retain an ancient coevolving symbiont, suggesting that long-term mutualism and permanent integration remains the rule and symbiont turnovers are mere evolutionary side-stories. Here we show that this is not the case for *Euplotes*. We examined all known essential *Euplotes* symbionts and found that none are ancient or coevolving; rather, all are recently established and continuously replaced over relatively short evolutionary time spans, making the symbiosis ancient for the host but not for any bacterial lineage.

In *Euplotes*, many symbionts other than *Polynucleobacter* have been described through ultrastructural and

molecular methods⁸. Non-essential bacteria are common, but only a small percentage of *Euplotes* populations in clade B lack *Polynucleobacter* and harbour instead one of two other essential symbionts⁸: “*Candidatus Protistobacter heckmanni*” or “*Candidatus Devosia symbiotica*”. Either might be argued to be the descendant of an ancestral symbiont still coevolving with its host, but replaced by *Polynucleobacter* strains in many instances. *Protistobacter* is exclusively symbiotic, whereas the genus *Devosia* comprises both free-living and symbiotic representatives,

including an essential symbiont of the marine *Euplotes magnicirratu*s⁸ (“*Candidatus Devosia euplotis*”), which would make an ancestral symbiosis with *Devosia* even older than the split between freshwater and marine *Euplotes*. To determine if these bacteria possess the telltale traits of ancient, integrated symbionts, we characterised the genomes of previously identified⁸ *Protistobacter* and *Devosia* strains (accession numbers: SAMN25125324-7) from all *Euplotes* species known to harbour either.

One apparently universal signature of obligate endosymbionts is genome

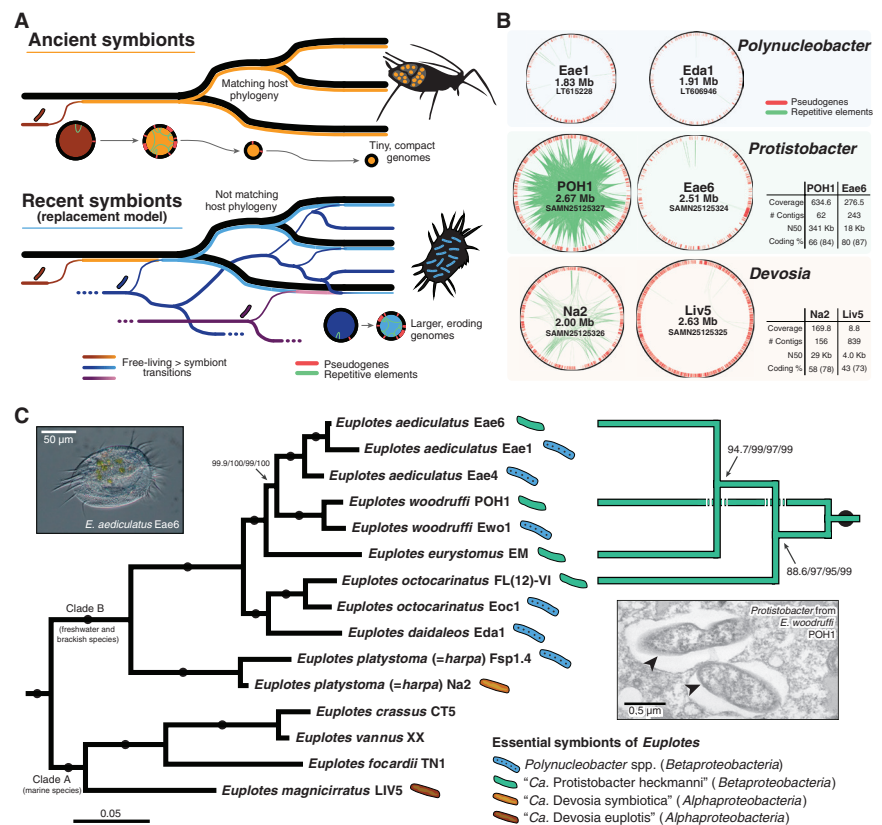


Figure 1. Symbiotic *Protistobacter* and *Devosia* strains were established recently and independently.

(A) Ancient, stable endosymbionts (for example, *Buchnera* in aphids) can be distinguished from recent ones (such as *Polynucleobacter* in *Euplotes*) based on genomic features and the extent of co-differentiation with their hosts. (B) The newly characterized genomes of *Protistobacter* and *Devosia* strains are slightly larger and show even more conspicuous signs of genome erosion than symbiotic *Polynucleobacter*. Curved green lines connect paralogous transposons. Coding density is shown both before (in parentheses) and after removing predicted pseudogenes from the estimate. (C) Phylogenomic branching order differs between hosts (tree on the left) and symbionts (cladogram on the right) in strains of *Protistobacter*-harbouring *Euplotes*. Numbers associated with nodes represent, from left to right: SH-aLRT values, ultrafast bootstrap support, non-parametric bootstrap support (1,000 pseudoreplicates), and ultrafast bootstrap support obtained with an alternative model (LG+C20+F+G4); black dots mark fully supported nodes. Inset micrographs show representative host (from *E. aediculatus* strain Eae6) and intracellular bacteria (*Protistobacter* inside vacuoles in the cytoplasm of *E. woodruffi* strain POH1, arrowheads).

erosion^{7,9}. Ancient symbionts possess tiny, compact genomes, whereas recently established symbionts have larger genomes, typically rich in pseudogenes and repetitive elements (Figure 1A). Two draft genomes each from *Protistobacter* and *Devosia* (from both freshwater and marine *Euplotes*) were all found to be large and enriched in pseudogenes and repetitive elements, even more so than those of recently established endosymbiotic *Polynucleobacter* strains (Figure 1B, Data S1). Moreover, different strains of *Protistobacter* and *Devosia* vary considerably in the number of mobile elements in their genomes, which argues against a long, shared evolutionary history as stable symbionts. Overall, these bacteria resemble neither free-living organisms (due to their many non-functional genes) nor ancient and streamlined symbionts with small, gene-rich genomes, but instead perfectly fit the prediction for recently established endosymbionts undergoing genome erosion.

Ancient obligate symbionts codiversify with their hosts, whereas independently established symbionts do not (Figure 1A). With only two available strains, the phylogeny of symbiotic *Devosia* cannot be assessed, but we could compare the phylogenetic relationships among multiple strains of *Protistobacter* because, in addition to the two almost complete genomes (strains POH1 and Eae6), we also generated partial draft genomes from two additional strains (EM and FL(12)-VI). The phylogenomic tree including seven out of nine *Euplotes* species with essential symbionts (Figure 1C) show that ciliate and bacteria phylogenies are incongruent. Approximately unbiased tests performed on both trees rejected alternative topologies constrained to match those of the symbiotic partners (*Euplotes*: $p < 10^{-8}$; bacteria: $p = 0.0037$). Therefore, we conclude that extant *Protistobacter*, like *Polynucleobacter*², have not coevolved with their hosts, and are the descendants of independently established symbioses.

Symbiont replacements in insects⁴⁻⁶ are exceptions that stand out against a background of long-lasting mutualism within a host lineage. In *Euplotes*, *Polynucleobacter* replacements already seemed to be the widespread norm², but *Protistobacter* and *Devosia*

remained realistic candidates for an ancient coevolving endosymbiont. Here we have shown instead that they too display all the hallmarks of recent establishment. New strains undergo genomic decay due to relaxed selection and drift until they are themselves replaced and driven to extinction, as it must have happened to any 'original' symbiont, which seems to have no extant descendent. The only alternative explanation is that an unknown bacterium, never observed in dozens of screened clade B *Euplotes*⁸, represents such an ancient lineage. This is highly unlikely, but such hypothetical bacterium would still constitute the 'sideshow' in a story of continuous replacements.

Our conclusion reinforces the fact that this mutually obligate symbiosis is not, and never was, a mutualism. *Euplotes* constantly require endocellular bacteria, but due to an ample available pool of potential symbionts, the recruited strains are always outcompeted and replaced by new ones with less eroded genomes. Why exactly *Euplotes* depends on symbionts³ remains unknown, but it must be something that all three bacteria can provide. Nutritional supplementation is a common mechanism^{1,7}, but seems unlikely in an omnivorous predator. A very different speculation following the constructive neutral evolution model¹⁰ is that this symbiosis requires no gain-of-function. If, for example, a ciliate host harboring non-essential bacteria would lose a universal metabolic pathway, the bacteria might compensate for that loss, changing an otherwise lethal mutation into a neutral one that might become fixed and locking the host in a relationship with any symbiont that can provide the lost function. More data, especially from host genomes, is required to test this hypothesis. Regardless of function, the *Euplotes* symbiotic system shows not only that repeated symbiont replacements take place frequently, but also that it is possible for none of the mutually obligate relationships to result in a permanent integration of the symbiotic partners. This may or may not be a frequent occurrence in nature; the default assumption of ancient mutualism needs to be tested on a more diverse set of systems.

SUPPLEMENTAL INFORMATION

Supplemental information includes experimental procedures, supplemental references, and one data file, and can be found online with this article at <https://doi.org/10.1016/j.cub.2022.06.052>.

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AUTHOR CONTRIBUTION

V.B., C.V., F.H., and P.J.K. conceived the project. P.J.K. is the senior author and provided supervision and funding. V.B., M.J.S., N.A.T.I., E.E.G., C.V., and F.H. performed lab work and analyses. V.B. wrote the original draft. All authors contributed to the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

- Moran, N.A. (2006). Symbiosis. *Curr. Biol.* 16, R866–R871.
- Boscaro, V., Kolisko, M., Felletti, M., Vannini, C., Lynn, D.H., and Keeling, P.J. (2017). Parallel genome reduction in symbionts descended from closely related free-living bacteria. *Nat. Ecol. Evol.* 1, 1160–1167.
- Heckmann, K., Ten Hagen, R., and Görtz, H.D. (1983). Freshwater *Euplotes* species with a 9 type 1 cirrus pattern depend upon endosymbionts. *J. Protozool.* 30, 284–289.
- Chong, R.A., and Moran, N.A. (2018). Evolutionary loss and replacement of *Buchnera*, the obligate endosymbiont of aphids. *ISME J.* 12, 898–908.
- Dial, D.T., Weglarz, K.M., Aremu, A.O., Havill, N.P., Pearson, T.A., Burke, G.R., and von Dohlen, C.D. (2021). Transitional genomes and nutritional role reversals identified for dual symbionts of adelgids. *ISME J.* 12, 898–908.
- Mao, M., and Bennett, G.M. (2020). Symbiont replacements reset the co-evolutionary relationship between insects and their heritable bacteria. *ISME J.* 14, 1384–1395.
- McCutcheon, J.P., Boyd, B.M., and Dale, C. (2019). The life of an insect endosymbiont from the cradle to the grave. *Curr. Biol.* 29, R485–R495.
- Boscaro, V., Husnik, F., Vannini, C., and Keeling, P.J. (2019). Symbionts of the ciliate *Euplotes*: diversity, patterns and potential as models for bacteria-eukaryote endosymbioses. *Proc. R. Soc. B.* 286, 20190693.
- McCutcheon, J.P. (2021). The genomics and cell biology of host-beneficial intracellular infections. *Annu. Rev. Cell. Develop. Biol.* 37, 115–142.
- Gray, M.W., Lukeš, J., Archibald, J.M., Keeling, P.J., and Doolittle, W.F. (2010). Irremediable complexity? *Science* 330, 920–921.

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