



Evolution of bacterial trade in a two-species community

Jennifer M. Farrell^a and Sam P. Brown^{a,1}

Our natural world is enriched by mutualisms, where organisms engage in a complex and often colorful trade of goods and services for their mutual benefit (Fig. 1D). An enduring question in evolutionary biology is how does this multispecies complexity emerge from simpler interactions? One-way beneficial relationships, such as those between a dung beetle and a mammal, have been proposed as a starting point for the elaboration of mutualisms (1–3) (Fig. 1A) and provide a vivid framing of the puzzle: When and how does it pay a dung beetle to help mammals make more dung? Harcombe et al. (4) took a microbial analog of a mammal–dung beetle relationship and used experimental evolution to probe the potential coevolutionary pathways. In their PNAS paper, Harcombe et al. report a striking result: the evolution of a costly bidirectional mutualism in the laboratory.

In an earlier study (3), the quest began with the design and experimental evolution of a two-species system with ingredients primed to favor the evolution of mutualisms (Fig. 1A). On one side was a strain of *Escherichia coli* engineered to be nutritionally deficient (a methionine auxotroph), and on the other a “dung feeder” (*Salmonella*) that was dependent on a waste product (acetate) produced by *E. coli*. Following ~10 generations of evolution in a spatially structured environment, Harcombe (3) reported an innovation in the dung-feeding *Salmonella*—the emergence of strains that overproduced methionine (Fig. 1B)—a costly investment in *E. coli* growth that returned more acetate to the new *Salmonella* strain.

Harcombe et al. (4) now report a striking innovation in their two-species system that marks a transition into a bidirectional costly mutualism. Following another 200+ generations of experimental evolution, the authors report the emergence of a novel *E. coli* strain that secretes a costly sugar, galactose, that can then be utilized by *Salmonella* (Fig. 1C). This sugar-secreting super-cooperative *E. coli* phenotype arose repeatedly across multiple replicate evolutionary lineages, in each case due to a different frameshift mutation in *galK* that blocked galactose metabolism. These

mutations are inevitably very costly when *E. coli* grows on lactose, as each lactose molecule yields one molecule of glucose and one of galactose, now available to support *Salmonella* growth. Harcombe et al. demonstrate that this “one for me, one for you” carbon sharing by *E. coli* leads to substantial costs when grown alone, but triggers net benefits to both partners when cultured together on an agar plate.

Despite enhancing the growth of both species in coculture, the sugar-producing *E. coli* mutants did not fix in any lineage. On first assessment, this could simply represent an intermediate observation along a transition toward fixation and accelerating mutualism. To test this hypothesis, Harcombe et al. (4) ran competition experiments between the ancestral and galactose-secreting *E. coli* at different frequencies (always in partnership with the methionine-secreting *Salmonella*) and instead found a signature of negative-frequency dependence: whichever *E. coli* strain was rare had a fitness advantage. This result, together with explicit metabolic simulations (4, 5), suggests that the *E. coli* polymorphism represents an ongoing intraspecific social tension between the individual cost of secreting half of their carbon source and the collective benefit of increased methionine, mediated by their *Salmonella* partner. From a social evolution perspective, these results map out a fascinating model system where each species plays an intraspecific “public goods” game, and is simultaneously a dynamical component in their partner species’ public good (6, 7).

What is next for these two entwined lineages? By reciprocating gifts of methionine and galactose that support each other’s growth, there is the theoretical possibility of an “orgy of mutual benefaction” (8), an ecological explosion in numbers that could open new evolutionary paths or environmentally mediated collapse. In the absence of immediate ecological destabilization, there are evolutionary threats to the stability of this mutualism. The clear social conflict within the *E. coli* population highlights the threat of cheats. The spatially structured environment limits the ability of cheats to out-compete the galactose-secreting cooperator (4), but an

^aSchool of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332

Author contributions: J.M.F. and S.P.B. wrote the paper.

The authors declare no conflict of interest.

Published under the PNAS license.

See companion article 10.1073/pnas.1810949115.

¹To whom correspondence should be addressed. Email: sam.brown@biology.gatech.edu.

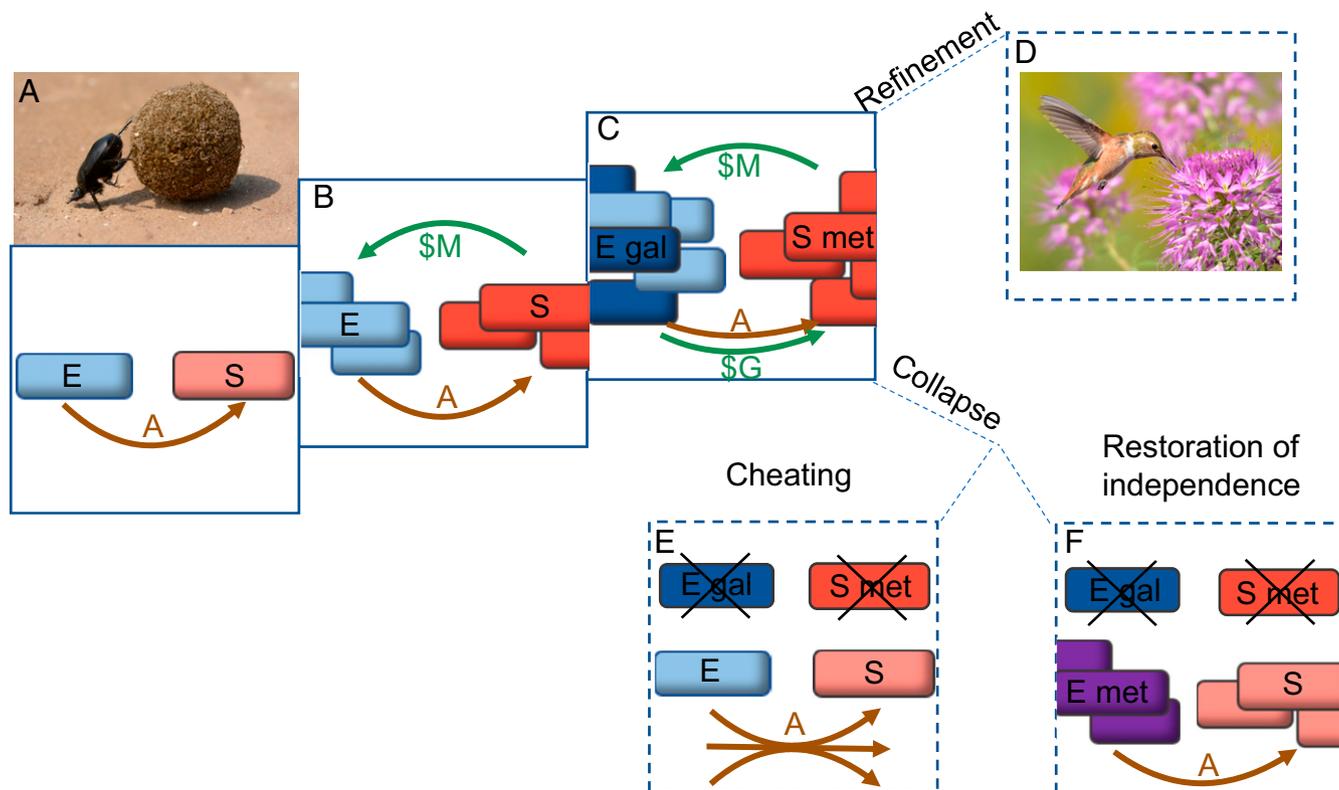


Fig. 1. Step-wise evolution of a costly bidirectional mutualism between *Salmonella enterica* and an *E. coli* methionine auxotroph. Costly products are indicated in green and costless products are indicated in brown. (A) The *E. coli* methionine auxotroph (light blue) produces acetate as a costless byproduct of lactose metabolism. *Salmonella* (light red) utilizes this acetate waste. This relationship is analogous to the dung beetle–mammal relationship. Image courtesy of Pixabay/Topi_Pigula. (B) Evolution of costly methionine secretion in *Salmonella* (dark red), enhancing the growth of both species (3). (C) Evolution of costly galactose secretion in *E. coli* (dark blue), enhancing growth of both species (4). (D–F) Future directions. (D) Interspecific cooperation becomes increasingly elaborate and codependent. Image courtesy of Flickr/Tom Koerner/USFWS. (E) *E. coli* galactose cheats take over in a well-mixed environment. (F) Restoration of independence via reversion of *E. coli* methionine auxotrophy to wild-type (purple).

environmental shift toward greater mixing would risk loss of the cooperator genotypes and therefore collapse of the mutualism (3, 9) (Fig. 1E). Finally, there is the threat of autonomy: if one species loses its dependency on the other, then the incentive to feed their partner is also gone. Autonomy could arise via simple environmental changes: for example, a rich growth medium

containing methionine and free carbon. Autonomy could also arise via gain-of-function mutations (10): for example, the restoration of methionine synthesis in *E. coli* would remove the incentive for trade (Fig. 1F). We encourage Harcombe et al. (4) to keep evolving their fascinating system, and we look forward to finding out what new surprises are in store.

1 Connor RC (1986) Pseudo-reciprocity: Investing in mutualism. *Anim Behav* 34:1562–1566.

2 Sachs JL, Mueller UG, Wilcox TP, Bull JJ (2004) The evolution of cooperation. *Q Rev Biol* 79:135–160.

3 Harcombe W (2010) Novel cooperation experimentally evolved between species. *Evolution* 64:2166–2172.

4 Harcombe WR, Chacón JM, Adamowicz EM, Chubiz LN, Marx CJ (2018) Evolution of bidirectional costly mutualism from byproduct consumption. *Proc Natl Acad Sci USA*, 10.1073/pnas.1810949115.

5 Harcombe WR, et al. (2014) Metabolic resource allocation in individual microbes determines ecosystem interactions and spatial dynamics. *Cell Reports* 7:1104–1115.

6 Brown SP, Taddei F (2007) The durability of public goods changes the dynamics and nature of social dilemmas. *PLoS One* 2:e593.

7 Weitz JS, Eksin C, Paarpor K, Brown SP, Ratcliff WC (2016) An oscillating tragedy of the commons in replicator dynamics with game–environment feedback. *Proc Natl Acad Sci USA* 113:E7518–E7525.

8 May R, McLean AR (2007) *Theoretical Ecology: Principles and Applications* (Oxford Univ Press, Oxford).

9 Foster KR, Wenseleers T (2006) A general model for the evolution of mutualisms. *J Evol Biol* 19:1283–1293.

10 Dragoš A, et al. (2018) Collapse of genetic division of labour and evolution of autonomy in pellicle biofilms. *Nat Microbiol*, 10.1038/s41564-018-0263-y.