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Supplementary Information for

Evolution of bi-directional costly mutualism from byproduct consumption
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This PDF file includes:

Figs. S1 to S3

Fig. S1.

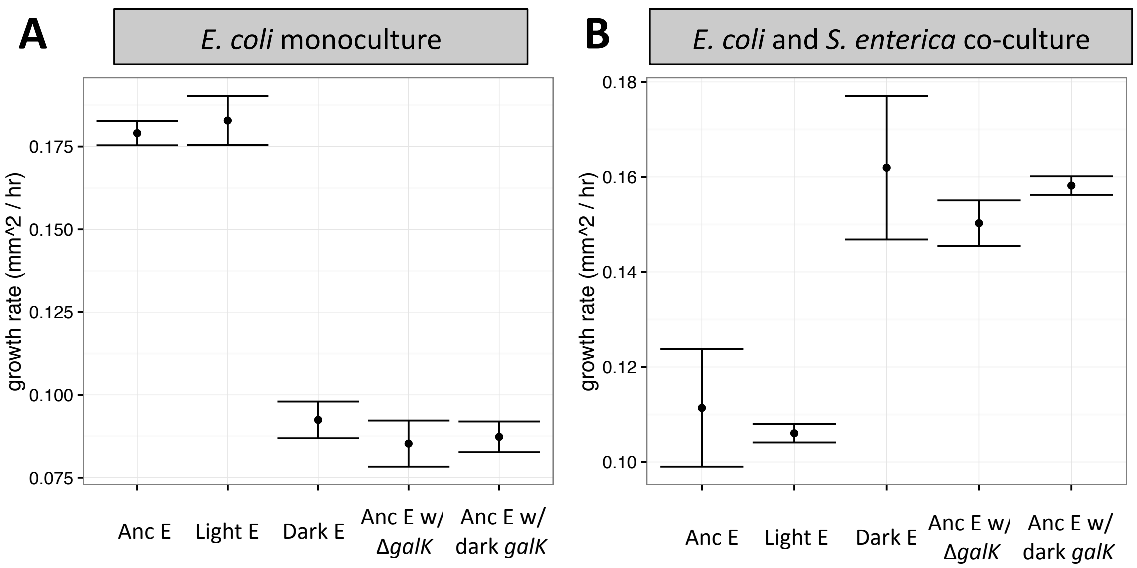

Fig. S1 - A *galK* frameshift or full deletion of *galK* are sufficient to recapitulate observed growth dynamics of evolved dark blue *E. coli.* A) Growth rates of *E. coli* monocultures, including the ancestral strain (Anc), were measured as the rate of expansion of spots on a lactose minimal medium plate with methionine. B) Growth rates of co-cultures were measured as the rate of expansion of spots inoculated with a 1:1 mix of *E. coli* and *S. enterica* on a lactose minimal medium plate. Means of 3 replicates with standard error bars.

Fig. S2.

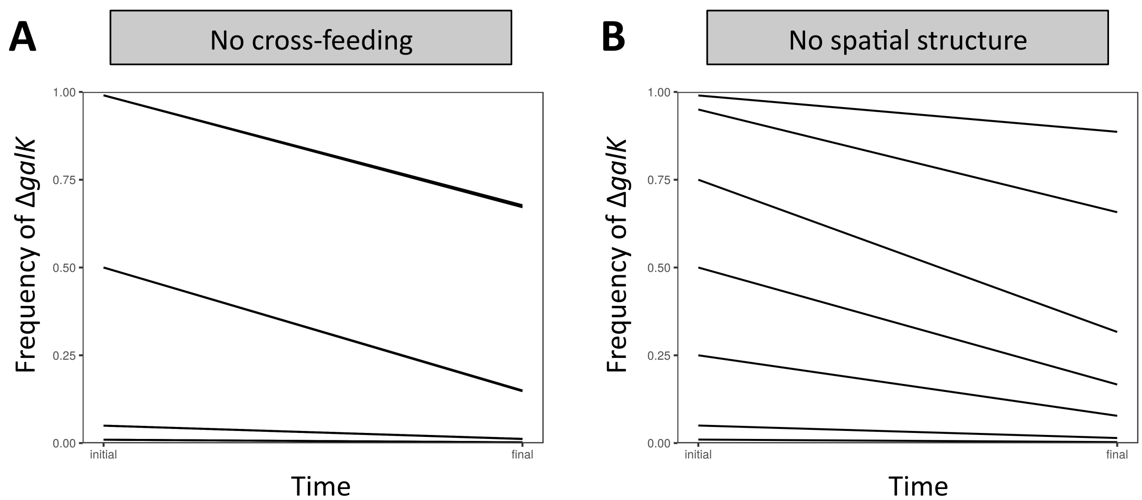

Fig. S2 - Genome-scale metabolic modeling showed that the dark Δ*galK* genotype is deleterious in the absence of cross-feeding or in the absence of spatial structure. The two *E. coli* genotypes were started at varying initial ratios and the change in frequency of dark versus ancestral *E. coli* was tracked through time and averaged for four independent simulations. In all cases the frequency of dark *E. coli* decreased. A) To test the absence of cross-feeding *E. coli* were randomly distributed in an environment that contained lactose and methionine. B) To test the absence of spatial structure *E. coli* genotypes were competed against each other in the presence of *S. enterica* but in a mass action environment in which all resources were equally accessible by all bacteria.

Fig. S3.

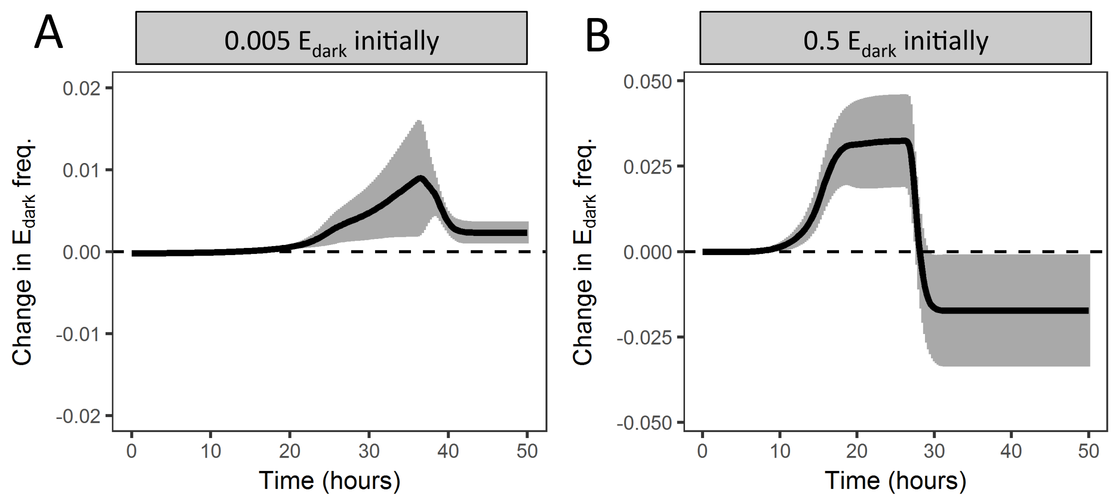

Fig S3 - Genome-scale metabolic modeling showed that dark Δ*galK* genotype experienced negative frequency dependent selection relative to ancestral *E. coli* when reliant on *S. enterica*. The two *E. coli* genotypes were started at varying initial ratios and randomly distributed in a lattice with *S. enterica*. The change in frequency of dark versus ancestral *E. coli* was tracked through time and averaged for four independent simulations. In all cases the frequency of dark *E. coli* initially increased, before settling to the final frequencies plotted in figure 3. The rapid decrease in the frequency of cooperative *E. coli* represents the point at which methionine became abundant enough that the costs of galactose excretion outweighed the benefits of increased access to the amino acid. A) Simulations started at 0.005 dark in the *E. coli* population. B) Simulations started at 0.5 dark in the *E. coli* population. The gray area represents standard deviation between four simulations that were started with biomass in different randomly assigned locations in the lattice.