# Bacterial cross-feeding can promote gene retention by lowering gene expression costs

Short title: Cross-feeding can promote gene retention

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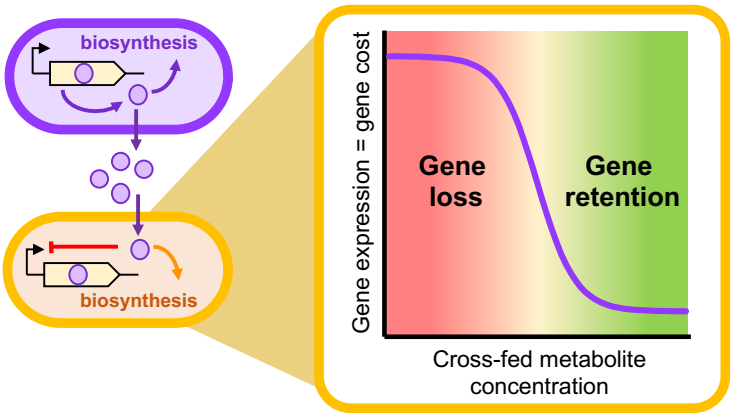
## ABSTRACT

Gene loss is expected in microbial communities when the benefit of obtaining a biosynthetic precursor from a neighbor via cross-feeding outweighs the cost of retaining a biosynthetic gene. However, gene cost primarily comes from expression, and many biosynthetic genes are only expressed when needed. Thus, one can conversely expect cross-feeding to repress biosynthetic gene expression and promote gene retention by lowering gene cost. Here we examined long-term bacterial cocultures pairing *Escherichia coli* and *Rhodopseudomonas palustris* for evidence of gene loss or retention in response to cross-feeding of non-essential adenine. Although *R. palustris* continued to externalize adenine in long-term cultures, *E. coli* did not accumulate mutations in purine synthesis genes, even after 700 generations. *E. coli* purine synthesis gene expression was low in coculture, suggesting that gene repression removed selective pressure for gene loss. In support of this explanation, *R. palustris* also had low transcript levels for iron-scavenging siderophore genes in coculture, likely because *E. coli* facilitated iron acquisition by *R. palustris*. *R. palustris* siderophore gene mutations were correspondingly rare in long-term cocultures but were prevalent in monocultures where transcript levels were high. Our data suggests that cross-feeding does not always drive gene loss, but can instead promote gene retention by repressing costly expression.


**Graphical abstract**



## INTRODUCTION

Changes to the genetic inventory of a given microbe can be influenced by living in a community. An example of this influence is described in the Black Queen Hypothesis (BQH). Based on the card game Hearts, where players avoid holding the costly Queen of Spades, the BQH posits that gene loss (herein we do not distinguish loss-of-function mutations from gene deletions for convenience) will occur when acquiring a resource from neighbor is less costly than producing that resource (1). The BQH involves a producer that generates a public resource, and a beneficiary that uses the resource and is thus subject to beneficial gene loss. The BQH is analogous to the emergence of cheaters that lose genes and benefit by exploiting a public resource. However, unlike a cheater, a beneficiary is less likely to be the same species as the producer and is less likely to harm the producer (1, 2). The BQH, or analogous processes, are now commonly used to explain the prevalence of auxotrophs, which cannot synthesize one or more essential nutrients (3, 4). Indeed, several groups have observed that auxotrophs carrying biosynthetic gene deletions have a fitness advantage over wild-type cells when corresponding biosynthetic precursors are provided (5-9).

A confounding aspect of BQH prediction of gene loss is that nutrient availability can also repress gene expression and thus lower the cost of a gene (3, 10, 11); most of a gene's cost comes from its expression (12-16), and thus repressing gene expression could have similar cost-savings as a loss-of-function mutation. Thus, one can arrive at an opposite prediction wherein cross-feeding could promote gene retention by repressing costly gene expression. This prediction is well-documented from studies of cheaters. For example, cheaters that had lost genes for iron-scavenging siderophores emerged when iron was scare and siderophore genes were expressed, but gene loss was not observed when iron was plentiful and siderophore genes were repressed (11, 17, 18). Thus, nutrient availability alone might not be a good predictor for gene loss and the emergence of beneficiaries.

Previously, we developed cocultures pairing fermentative *Escherichia coli* and phototrophic *Rhodopseudomonas palustris*, wherein each species is dependent on the other for essential nutrients; *E. coli* ferments glucose into organic acids that provide essential carbon to *R. palustris* while *R. palustris* converts $N_2$ gas into $NH_4^+$, providing essential nitrogen to *E. coli* (19). More recently, we discovered that *R. palustris* also externalizes adenine at levels that can support an *E. coli* purine auxotroph (20, 21). There is also suggestive evidence that *E. coli* facilitates *R. palustris* iron acquisition, perhaps through cross-feeding of *E. coli* siderophores (22). Based on the BQH alone, one might predict that the public availability of adenine and siderophores would enrich for *E. coli* adenine auxotrophs and *R. palustris* siderophore mutants over time.

Here we examined long-term monocultures and cocultures for evidence of gene loss due to the availability of non-essential adenine and possible siderophores from a partner. In each case gene retention was correlated with low expression, suggesting that repression of gene expression can decrease the frequency of gene loss.

## RESULTS

**Gene loss is not beneficial when adenine is provided to *E. coli* monocultures.** In anaerobic cocultures where *R. palustris* provided essential nitrogen ($NH_4^+$) to *E. coli* and *E. coli* provided essential carbon (organic acids) to *R. palustris* (19), we learned that *R. palustris* also externalizes enough adenine to support an *E. coli ΔpurH* purine auxotroph (20, 21). Herein, we recognized adenine availability as an opportunity to look for evidence of the BQH; de novo purine synthesis costs more ATP than the purine salvage pathway, and thus could lead to purine synthesis gene loss.

Fitness benefits have been observed for auxotrophic mutants, when the corresponding nutrient is available either as a supplement or via cross-feeding (5-8). Thus, we first tested whether loss of purine biosynthesis would be advantageous in the presence of adenine by comparing the growth of *E. coli ΔpurH* versus wild-type (WT) PFM2 parent cultures. We focused on *E. coli* PFM2 instead of the MG1655 (used previously by our lab) because PFM2 does not carry the *rph* mutation that leads to slower growth in the presence of adenine (23, 24), which can be alleviated by a *ΔpurH* mutant (Chuang and McKinlay in prep). Despite the availability of adenine, the *ΔpurH* mutant did not grow faster than the PFM2 parent in the presence of adenine (Fig 1A, B).

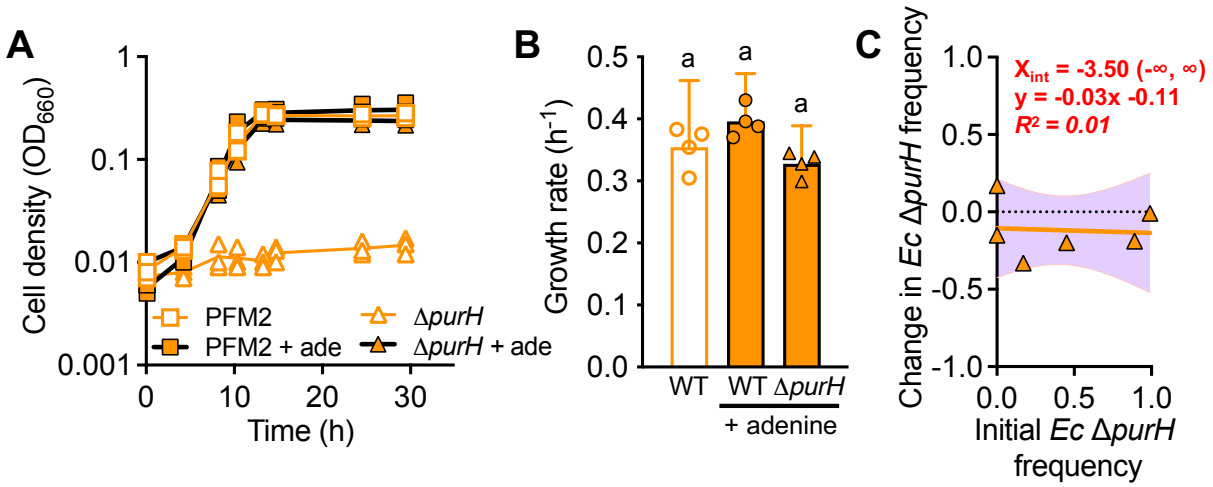



**Figure 1. Adenine availability does not provide a fitness advantage to an engineered *E. coli* PFM2 purine auxotroph. A.** Growth of WT *E. coli* PFM2 and its *ΔpurH* mutant in monoculture with and without 35 μM adenine (ade)**. B.** Monoculture growth rates for WT PFM2 vs a corresponding *ΔpurH* mutant with and without 35 μM adenine. 'a,' indicates statistically similar comparisons ($p > 0.01$) as determined using a one-way ANOVA with Tukey's multiple comparisons test. **C.** Invasion-from-rare assay competing a *ΔpurH* mutant against its PFM2 parent in coculture with *R. palustris* CGA676 under $N_2$-fixing conditions where *R. palustris* excretes $NH_4^+$ and adenine. The orange line is the best fit from a linear regression analysis with 95% CI shaded in purple. Corresponding equations and x-intercepts (upper, lower bounds for 95% CI) are shown. Change in frequency = (*E. coli* Δ*purH* / (*E. coli* WT + *E. coli* Δ*purH*))$_{final}$ – (*E. coli* Δ*purH* / (*E. coli* WT + *E. coli* Δ*purH*))$_{initial}$. **A-C.** Each data point represents a measurement from a single biological replicate.

We also assessed the fitness impact of a *ΔpurH* mutation by competing the mutant against the PFM2 parent in coculture with $NH_4^+$- and adenine-excreting *R. palustris* NifA* (CGA676). We used a range of initial frequencies so that we could simultaneously test for coexistence by mutual invasibility, where an equilibrium frequency can be extrapolated from the x-intercept by linear regression analysis (25-27). However, there was poor linear correlation in the invasion-from-rare assay (Fig 1C). The same was true when a *ΔpurH*::km$^R$ mutant was used (Fig S1). However, the *ΔpurH* mutant tended to decrease in frequency relative to parent in cocultures, where adenine is available (Fig 1C and S1). Taken together, our results suggest that a *ΔpurH* mutant does not have a competitive advantage over PFM2 in the presence of adenine. Thus, at least in this engineered context, we do not expect that adenine availability to benefit *E. coli* PFM2 purine auxotrophs.

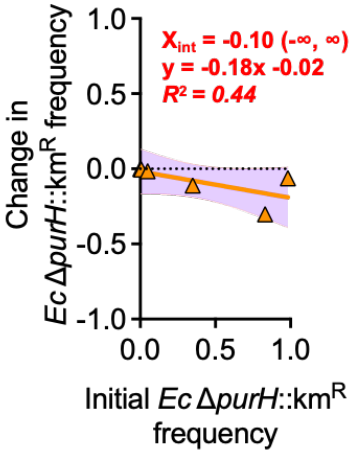



**Fig S1. Invasion-from-rare assay competing *E. coli ΔpurH*::km$^R$ against its PFM2 parent in coculture with *R. palustris* CGA676.** The orange line is the best fit from a linear regression analysis with 95% CI shaded in purple. Corresponding equations and x-intercepts (upper, lower bounds for 95% CI) are shown. Change in frequency = (*E. coli* Δ*purH* / (*E. coli* WT + *E. coli* Δ*purH*))$_{final}$ – (*E. coli* Δ*purH* / (*E. coli* WT + *E. coli* Δ*purH*))$_{initial}$. Each data point represents a measurement from a single biological replicate.

**Adenine auxotrophs are rare in long-term cocultures.** Although the engineered *ΔpurH* mutant did not have an obvious fitness advantage, it is possible that (i) there was a subtle difference in growth rate that could be enriched over many generations and (ii) other kinds of purine auxotrophy mutations could lead to a fitness advantage. To account for these possibilities, we looked for the evidence of purine auxotrophs in long-term monocultures and cocultures of *E. coli* PFM2 and $NH_4^+$- and adenine-excreting *R. palustris* (CGA676; NifA*) that we had maintained for other reasons (Fig 2A; Table S1-5). Adenine is amply available even in evolved cocultures (Chuang and McKinlay in prep). Focusing on the latest common timepoint between monoculture vs coculture treatments (generation 650), we did not observe obvious mutations in purine synthesis genes in PFM2 in cocultures or monocultures (Fig 2B; Fig S2). When considering other time points, only one purine biosynthesis gene mutation across was observed in one monoculture line at generation 204 (*purT* at 54.3%, line 15; Table S2).

To account for non-obvious mutations that might lead to purine auxotrophy. We also screened four *E. coli* isolates from each monoculture and coculture line (40 monoculture isolates and 40 coculture isolates) for adenine auxotrophy in liquid cultures in 96-well plates (Fig 2C). Although several monoculture isolates could not grow in minimal media, suggesting that dependencies might have developed between *E. coli* subpopulations, only two monoculture isolates were possibly adenine auxotrophs (line 20; Fig 2C). We suspect that the other auxotrophs are due to mutations in *rpoC*, which were

conditionally enriched in monoculture (Fig S2A). Certain mutations in *E. coli rpoC* are known to generate polyauxotrophies (28, 29).

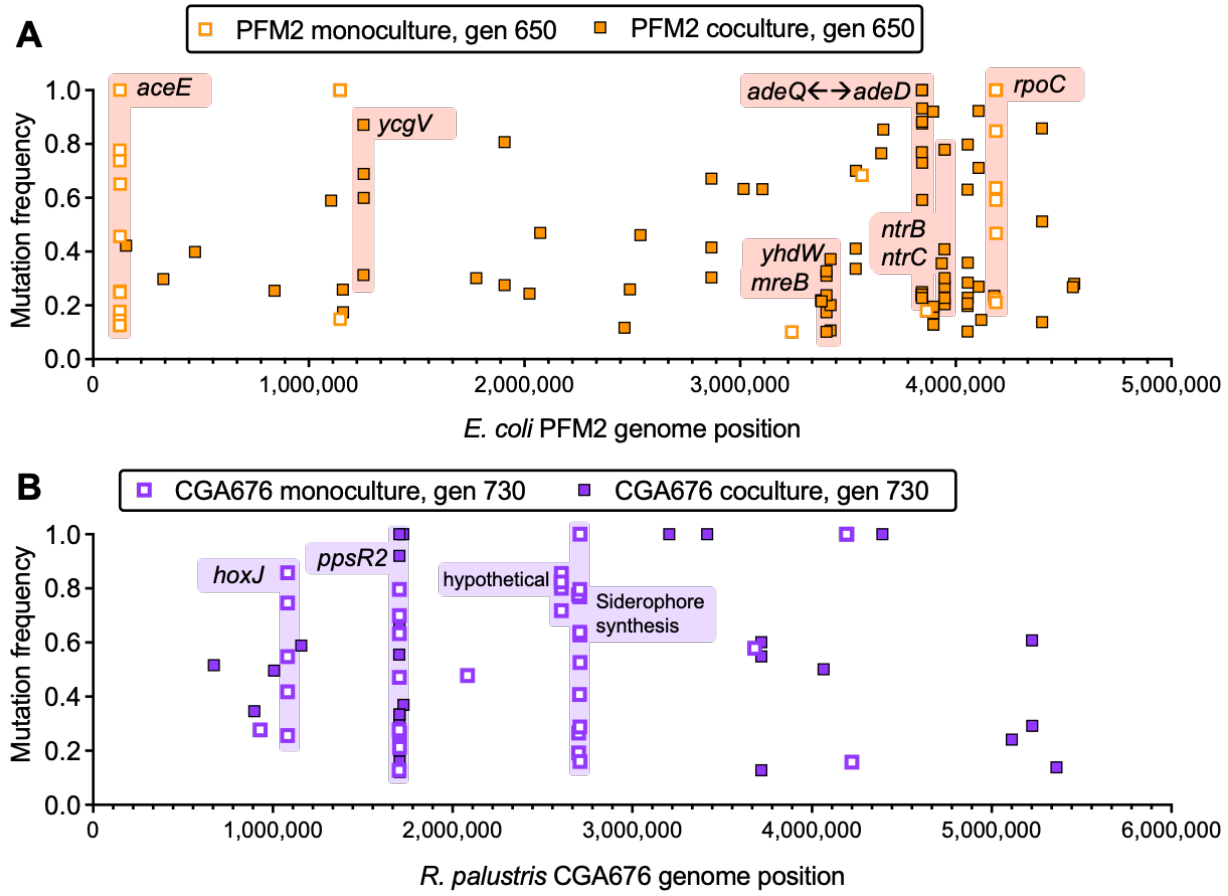



**Fig S2. Mutations enriched in *E. coli* PFM2 (A) and *R. palustris* CGA676 (B) in long-term monocultures and cocultures.** Each data point represents a mutation observed at a frequency >0.1 in an experimental line. Generation: gen.

No adenine auxotrophs were isolated from cocultures and all coculture isolates grew in defined media, suggesting that our coculture conditions might not favor emergence of other dependencies between subpopulations. Overall, we conclude that *E. coli* adenine auxotrophs are not a major subpopulation in any long term coculture despite the availability of adenine.

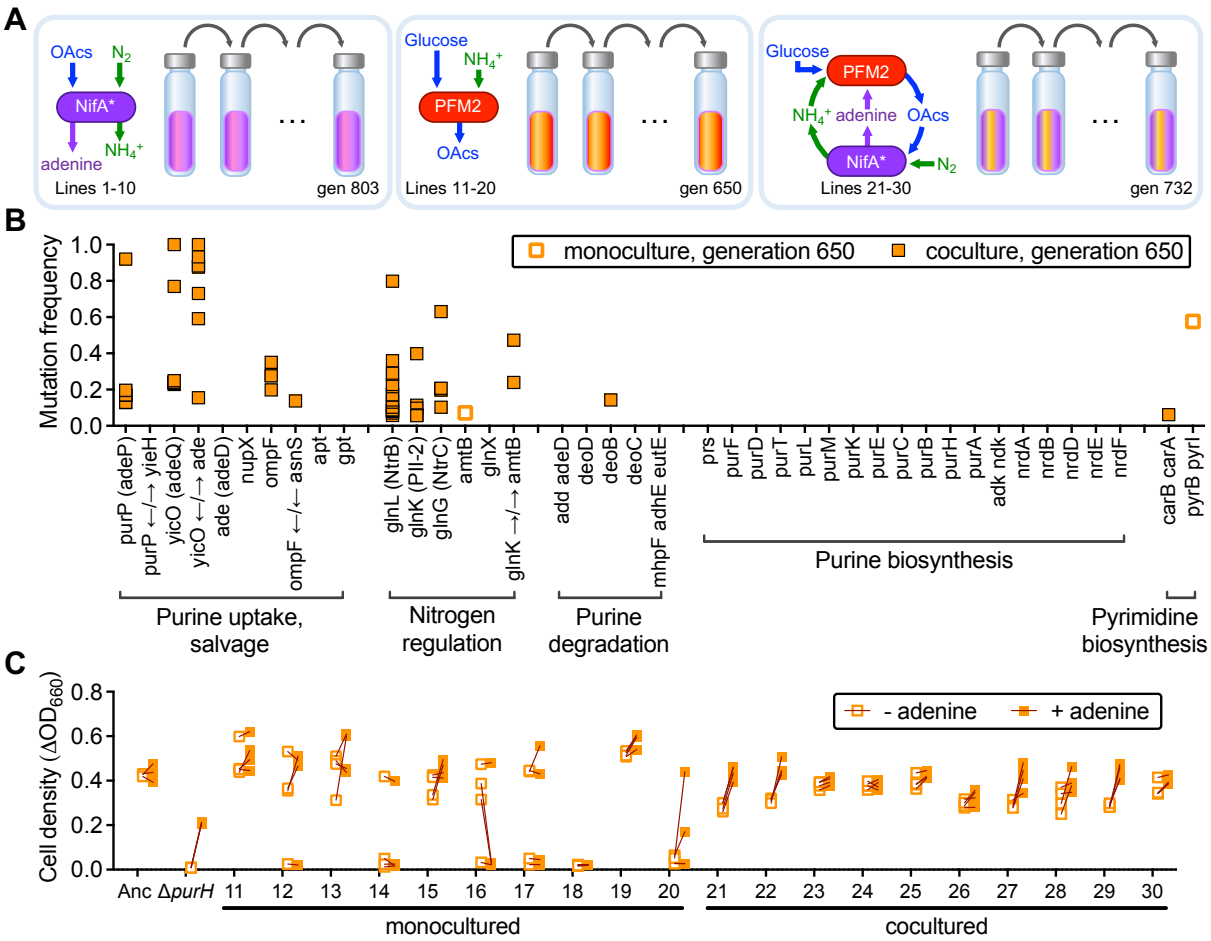
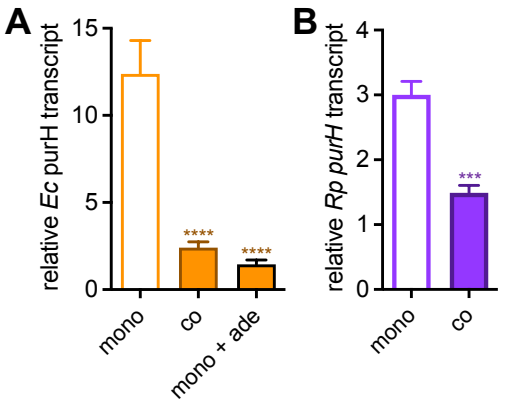



**Fig 2. *E. coli* adenine auxotrophs are not prevalent in long-term cocultures. A.** Long-term monoculture and coculture conditions. OAcs, organic acids. **B.** Enriched *E. coli* mutations in genes for nucleobase metabolism and regulation of nitrogen metabolism for long-term monocultures and cocultures. Each point represents a mutation frequency in a given gene for a given evolutionary line. **C.** Random evolved *E. coli* isolates (generation 650; n=4) were screened for adenine auxotrophy in liquid monoculture conditions with and without 50 μM adenine. Ancestral strain (Anc) and *ΔpurH* (ΔH) mutant values are also included (n=3). Each point represents a single measurement for a single isolate (replicate measurements were not made for any isolate). Each line connects measurements for the same isolate grown with and without adenine.

**Cross-fed adenine represses purine synthesis gene expression.** One possible explanation for why *E. coli* purine auxotrophs did not emerge in coculture is because adenine availability repressed gene expression, thus lowering the purine biosynthesis gene costs. In support of this notion, we previously saw that *E. coli* MG1655 down-regulated several purine synthesis genes in coculture versus monoculture (22). To determine if the same is true for PFM2, we quantified *purH* transcripts by RT-qPCR. Coculture PFM2 *purH* levels were 20% of those in monoculture (Fig 3A). Adding adenine to monocultures resulted in a *purH* transcript level that was 12% of that

220 observed without adenine, suggesting that the low expression in coculture was due to
221 adenine availability (Fig 3A).
222
223 *R. palustris* CGA676 also showed less *purH* transcript in coculture, 50% of that
224 observed in monoculture (Fig 3B). It is unclear how *E. coli* would influence repression of
225 *R. palustris purH* expression. However, the lower expression supports previous findings
226 that *E. coli* does not stimulate *R. palustris* adenine production (20). Adenine availability
227 and expression of purine biosynthesis genes curiously did not lead to purine
228 biosynthesis gene mutations in *R. palustris* monocultures. It is possible that *R. palustris*
229 lacks effective adenine uptake mechanisms. In a separate study, we found that adenine
230 can be toxic to *R. palustris*, and purine externalizing strains, including the parent of
231 CGA676 are more resistant to exogenous adenine (Chuang and McKinlay, in prep).
232 Thus, CGA676 might be pre-dispositioned for adenine externalization rather than
233 uptake.
234
235 **Fig 3. Purine biosynthesis genes are down-**
236 **regulated in coculture.** RT-qPCR
237 quantification of *purH* transcript levels in *E. coli*
238 PFM2 (**A**; relative to *hcaT*) and *R. palustris*
239 CGA676 (**B**; relative to *fixJ*). Adenine (+ ade)
240 was added to a final concentration of 0.1 mM to
241 ensure that it was not used up during culture
242 growth. Values are the mean ± SD, n = 3-4.
243 Statistically significant differences from the
244 monoculture condition for each strain were
245 determined using an unpaired two-tail t-test;
246 ***, $p < 0.001$; ****, $p < 0.0001$.




247
248 ***R. palustris* siderophore gene loss is correlated with expression.** We looked for
249 other correlations of gene retention and low gene expression in our long-term cultures.
250 A standout was *R. palustris* siderophore genes which accumulated mutations in
251 monoculture but not coculture (Fig 4A, B; Fig S2). We previously saw that *R. palustris*
252 down-regulated siderophore synthesis genes in coculture with *E. coli* MG1655, relative
253 to monocultures (22). We verified that this trend was also true for *R. palustris* in
254 coculture with PFM2; RT-qPCR analysis showed that the transcript levels for the
255 siderophore synthesis gene RPA2390 was 20% in coculture compared to monoculture
256 (Fig 4C). Adding soluble iron (ammonium ferric citrate) to monocultures decreased
257 RPA2390 expression to 3% of that observed without added iron, suggesting that the low
258 expression in coculture was a response to enhanced iron availability in coculture (Fig
259 4C).
260
261 *R. palustris* might down-regulate siderophore production in coculture if *E. coli* facilitates
262 iron acquisition. Others have speculated that *R. palustris* can use siderophores from
263 other bacteria, because its genome encodes multiple transporters but only one
264 siderophore synthesis cluster for petrobactin-like siderophores (30, 31); *E. coli* produces
265 enterobactin siderophores (32). In agreement with this speculation, *E. coli* siderophore

gene expression seemed to respond to iron loss to *R. palustris*; the *entF* transcript level was 3.3-fold higher in coculture than in monoculture (Fig. 4D). These observations support a notion that *R. palustris* can use *E. coli* siderophores, however the actual molecular nature of this relationship is pending a thorough investigation.

Despite the increase in *E. coli entF* expression in coculture, *E. coli* siderophore mutants were rare and inconsistent across coculture lines and were not observed in long-term monocultures (Table S2, S4). It is possible that this expression range is not enough to pose a fitness cost, perhaps due to a low iron requirement under fermentative conditions (e.g., little use for iron-containing cytochromes). In agreement with this notion, transposon insertions in *E. coli* MG1655 siderophore genes had mainly neutral effects on fitness in monocultures and cocultures (21).

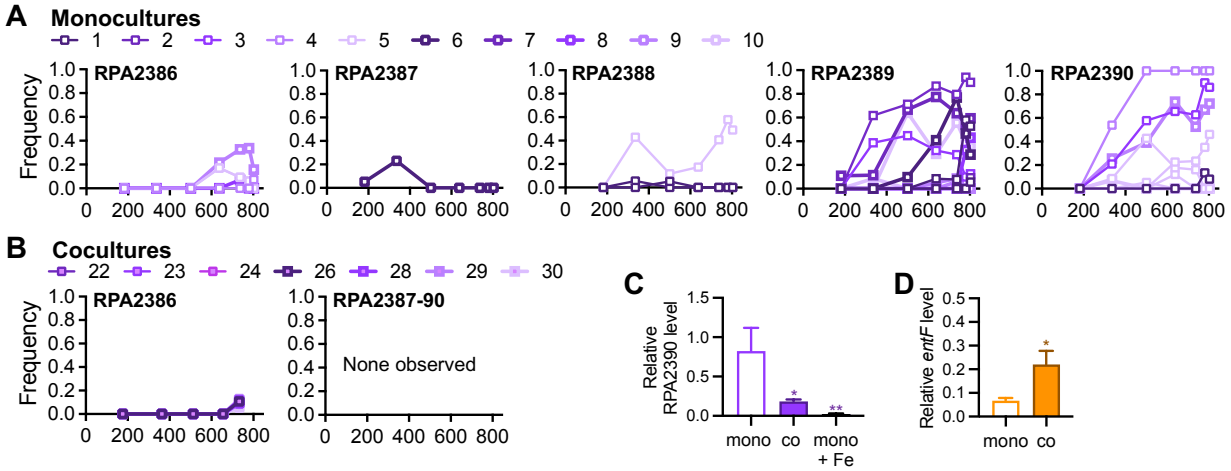



**Fig 4. Siderophore gene loss is prevalent in *R. palustris* monocultures where gene expression is high. A, B.** *R. palustris* CGA676 mutation frequencies in siderophore synthesis genes for each monoculture (**A**) or coculture line (**B**) where mutations were observed (no mutations were observed in lines 21, 25, and 27). Repeat colors in a given graph indicate different mutations in the same gene. **C, D.** RT-qPCR quantification of siderophore synthesis genes in *R. palustris* (**C**; RPA2390 relative to *fixJ*) and in *E. coli* (**D**; *entF* relative to *hcaT*). Values are the mean ± SD, n = 3. Statistically significant differences from the monoculture (mono) condition for each strain were determined using an unpaired two-tail t-test; *, $p < 0.05$; **, $p < 0.01$; co, coculture, Fe; ammonium ferric citrate.

## Discussion

In long-term cocultures, the availability of cross-fed adenine, and possibly siderophores, did not result in corresponding gene loss in a recipient. Our data suggest that the externalized resource repressed recipient gene expression, thereby lowering gene cost and selective pressure for gene loss. Conversely, siderophore loss-of-function mutations accumulated in *R. palustris* monocultures where gene expression was high. Although there are likely situations where cross-feeding promotes gene loss, there are

also likely situations where cross-feeding instead promotes gene retention through repressing gene expression.

Our results do not counter the BQH, which posits that gene loss will occur if the benefit of acquiring resources from a neighbor outweighs the cost of gene retention (1). If the cost of gene retention is low, then gene loss would only have neutral fitness effects, rather than beneficial effects. Others have suggested that low gene expression is not enough to promote gene retention because the maintenance and activity of sensor and regulatory proteins can still drive of gene loss (3). However, the fitness benefits of mutants lacking biosynthetic genes suggests that benefits can be realized while regulatory circuits remain intact (5-9). The association between gene cost and gene expression is well-documented (10-16). Our data, and data from others, suggest that there are cases where resource availability can lower gene cost simply by repressing gene expression. For example, repression of an antibiotic resistance gene in *E. coli* eliminated the fitness cost of that gene (10). In another example closer to the conditions of the current study, iron-limitation that prompted high siderophore production by *P. aeruginosa* led to a higher frequency of siderophore-deficient mutants compared to iron-rich conditions where siderophore production was low (11). The *P. aeruginosa* results were presented in the context of cheating, where gene loss is associated with exploitation that harms the producer, unlike BQH beneficiaries that should not harm the producer (1). Yet, the conditions leading to the emergence of cheaters and BQH beneficiaries are analogous (1). As such, many lessons from literature on cheaters can apply to BQH scenarios.

Insights into BQH gene-loss can also come from resource-rich monocultures, with the caveat that nutrient access might be well above that from a cross-feeding partner. For example, repression of gene expression in long-term nutrient-rich *E. coli* monocultures could explain why the loss of some catabolic genes was not observed in parallel lines and occurred without fitness effects (33). Repression of gene expression will shift the cost of a gene towards neutral. Gene loss with neutral fitness effects can still occur, but enrichment of those mutations would require hitchhiking with a beneficial mutation or passage through a severe population bottleneck. Cost-neutral gene loss, as opposed to beneficial gene loss, might also require time, and possibly spatial structure, that were not part of our experimental design. Should cost-neutral auxotrophic mutants emerge in nature, the chances of rescue via cross-feeding from a neighbor could be high (34, 35).

The extent to which gene repression can promote gene retention will also depend on the level of expression strength of repression; as noted above, relatively low expression might explain the lack of *E. coli* siderophore gene mutations. Gene expression can be noisy, and that noise can be costly and subject to selective optimization in a stable nutrient regime (36, 37). Noisy gene expression might explain why some engineered auxotrophs have greater fitness over a prototrophic parent (5-9). In long-term *E. coli* monocultures, loss-of-function mutations for maltose catabolism were arguably associated with weak gene repression in the presence of glucose (38). During growth on glucose, ancestral expression of the maltoporin was half that compared to during growth on maltose. Several evolved isolates carried mutations that prevented growth on

345 maltose. However, in an evolved isolate that retained the ability to grow on maltose,
346 maltoporin expression with glucose was 5% of that with maltose (38), suggesting that
347 stronger repression contributed to gene retention.
348
349 The level of nutrient availability also likely affects the level of gene repression. In other
350 words, there might be a resource concentration range over which BQH gene loss would
351 occur. For example, *Acinetobacter baylyi* histidine autotrophs had a fitness advantage
352 over a prototrophic parent when there was $\leq$ 50 μM histidine, but the advantage was
353 reversed as histidine levels reached 100-200 μM (6), possibly due to stronger gene
354 repression in the parent, though this is speculation on our part. One might also consider
355 that the responses of a prototrophic strain versus an otherwise clonal auxotroph to an
356 extracellular nutrient can vary widely; whereas an auxotroph would benefit from the
357 nutrient, the prototroph might experience beneficial, neutral or even detrimental fitness
358 effects (6). The extent to which loss of a biosynthetic gene can have compounding
359 fitness effects by affecting expression of other genes deserves investigation.
360
361 Our work presents a caveat to the popular expectation of cross-feeding as a driver of
362 gene loss. Cross-feeding can instead promote gene retention when resource availability
363 is high enough to repress gene expression and thus lower the cost of a gene. The
364 likelihood of gene loss then becomes the same as any other gene with a neutral cost.
365
366 **MATERIALS AND METHODS**

367 **Bacterial strains.** *R. palustris* CGA676 is derived from CGA0092 (39) and carries a
368 *nifA** mutation that causes $NH_4^+$ excretion under $N_2$-fixing conditions (19, 40). *E. coli*
369 PFM2 (41, 42) Δ*purH*::$km^R$ was made via lambda Red recombination (43) using
370 constructs amplified from KEIO mutants (44) using primers YCC29 (GCG CAA ACG
371 TTT TCG TTA CAA TGC) and YCC30 (TGC ATT ACC CGG AGC AAC). FLP-mediated
372 excision was used to remove the kanamycin resistance cassette ($km^R$) to generate the
373 Δ*purH* strain (43).
374
375 **Growth conditions.** Anoxic media in test tubes were prepared by bubbling $N_2$ through
376 10 ml of media in 27-ml anaerobic test tubes, then sealing with rubber stoppers and
377 aluminum crimps prior to autoclaving. Monocultures and cocultures were grown
378 horizontally at 30°C with light from a 45 W halogen bulb (430 lumens) and shaking at
379 150 rpm in minimal M9-derived coculture medium (MDC) (19) with either (i) *E. coli*
380 monocultures: 25 mM glucose, 10 mM $NH_4Cl$, and cation solution (100X stock: 100 mM
381 $MgSO_4$ and 10 mM $CaCl_2$); *R. palustris* monocultures: 20 mM sodium acetate and 10
382 mM $NH_4Cl$; or cocultures: 25 mM glucose and cation solution. Cultures with plasmid-
383 carrying strains were also supplemented with 100 μg/ml gentamycin or 25 μg/ml
384 chloramphenicol as appropriate. Starter cultures were inoculated with single colonies.
385 *R. palustris* starter cultures were grown in MDC with 20 mM acetate and 10 mM $NH_4Cl$.
386 *E. coli* starter cultures were grown aerobically in lysogeny broth, with 30 μg/ml
387 kanamycin (km) when appropriate. *E. coli* starter cultures were washed twice in 1 ml
388 MDC prior to inoculating test cultures or bioassays. Cocultures were inoculated with 0.1
389 ml each of *R. palustris* and *E. coli* to an initial optical density ($OD_{660}$) of ~0.003 each.
390

391 Cultures in 96-well plates were treated similarly except that oxic stock solutions were
392 used to prepare 0.2 ml volumes in each well. Anoxic conditions were achieved by
393 sealing plates inside a BD GasPak EZ large incubation container with 2 anaerobic
394 sachets.
395
396 **Experimental evolution.** Founder monocultures of *E. coli* PFM2 and *R. palustris* NifA*
397 CGA676 were grown from a single colony in anoxic MDC with either 25 mM glucose,
398 cation solution, and 3 mM $NH_4Cl$ for PFM2 or 20 mM sodium acetate for CGA676. A
399 single founder monoculture was then used to inoculate 10 monocultures and 10
400 cocultures. All cultures were grown horizontally without shaking at 30°C with light in
401 MDC. PFM2 monocultures were supplemented with 10 mM glucose, cation solution, 25
402 mM NaCl, and 2.3 mM $NH_4Cl$. CGA676 monocultures were supplemented with 25 mM
403 glucose, cation solution, 8 mM disodium succinate, 7.3 mM sodium acetate, 0.25 mM
404 sodium formate, 1.4 mM sodium lactate, and 6.3 mM ethanol. Cocultures were
405 supplemented with 25 mM glucose, cation solution, and 25 mM NaCl. Every 7 days,
406 cultures were vortexed and 0.25 ml was transferred to fresh medium. About every 5
407 transfers, stocks were frozen in 25% glycerol at -80$^{o}$C and separate cell pellets from 1
408 ml samples were frozen for gDNA extraction.
409
410 **Analytical procedures.** Cell densities were measured via turbidity ($OD_{660}$) using a
411 Genesys 20 visible spectrophotometer (Thermo-Fisher).
412
413 **Competition assays.** Competition assays were conducted in an invasion-from-rare
414 format to consider coexistence by the mutual invasion criterion, where each population
415 can increase when rare (25, 27). Cocultures were started from various initial
416 frequencies (targeting 0.01 – 0.99) of each *E. coli* strain (WT vs Δ*purH* or WT vs
417 Δ*purH*::km$^{R}$) for a total initial cell density of ~$10^6$ colony forming units (CFU) / ml. *R.*
418 *palustris* CGA676 was inoculated to an initial density of ~$10^6$ CFU / ml. Frequencies
419 were determined upon inoculation and after 5 days. WT and Δ*purH* mutants were
420 distinguished by counting CFUs on M9 agar with cations and 25 mM glucose and on LB
421 agar and then determining Δ*purH* populations from the difference. When Δ*purH*::km$^{R}$
422 mutants were used LB agar included km to allow for direct determination of population
423 size. Change in frequency = (*E. coli* Δ*purH* / (*E. coli* WT + *E. coli* Δ*purH*))$_{final}$ – (*E. coli*
424 Δ*purH* / (*E. coli* WT + *E. coli* Δ*purH*))$_{initial}$ (26).
425
426 **Genome sequencing and mutation analysis.** gDNA was purified from cells using a
427 Qiagen DNeasy Blood and Tissue kit following the manufacturer's instructions. Lysis
428 was facilitated after resuspension by adding proteinase K (50 µg/ml final), and
429 incubating at 56°C for 10 min. RNaseA (4 µl, Promega) was then added and the lysate
430 was incubated for 2 min before proceeding.
431
432 DNA fragment libraries were made using a NextFlex Bioo Rapid DNA kit and libraries
433 were sequenced using Illumina NextSeq 500 150x150 paired-end runs by the IU Center
434 for Genomics and Bioinformatics. Paired-end reads were pre-processed for quality with
435 cutadapt 3.4 (45) with the following options: -a AGATCGGAAGAGC -A
436 AGATCGGAAGAGC ; -q 15,10; -u 6. Mutations were called using breseq v. 0.32.0 on

437 polymorphism mode (46). *E. coli* monoculture population sequences were mapped to
438 the MG1655 genome (accession NC_000913); *R. palustris* monoculture population
439 sequences were mapped to a concatenated reference genome consisting of the
440 CGA009 chromosome (accession BX571963), and its plasmid pRPA (accession
441 BX571964). Co-culture sequences were mapped to a concatenation of the *E. coli* and
442 *R. palustris* reference genomes. Polymorphisms that co-occurred in both the
443 monoculture and co-culture datasets were filtered and maintained as a subset to enrich
444 for the most informative variants representing treatment differences (Table S1-4). This
445 filtering step also removed sequence differences between the reference sequences and
446 those of the experimental strains used. Variants were prioritized as mutations of interest
447 if they were detectable at the final two sequencing timepoints and co-occurred across
448 multiple populations in the same locus. All mutations can be found in Tables S1-4.
449 Locus tag conversions can be found in Table S5.
450
451 **Reverse transcription quantitative real-time PCR (RT-qPCR).** Cultures received 100
452 μM adenine or ammonium ferric [iron(III)] citrate as indicated. Cultures were harvested
453 in exponential phase at 0.6-0.8 $OD_{660}$ except for *E. coli* monocultures (+/- adenine
454 experiment), which were harvested at 0.3-0.4 $OD_{660}$ to avoid adenine depletion.
455 Cultures were chilled on ice and pelleted by centrifugation. Lysis, RNA purification, and
456 cDNA generation were performed exactly as described (20). Standard curves were
457 generated using gDNA. Transcripts were quantified as described (20) using the
458 appropriate primers (Table S6) with a Mastercycler ep *realplex* real-time PCR system
459 (Eppendorf). Data was analyzed by *realplex* software using Noiseband. Specificities
460 were validated by melting curves and by the presence of a single band on an agarose
461 gel.
462
463 **Statistical analyses.** Statistical analyses of growth and RT-qPCR data were performed
464 using Graphpad Prism (v10).
465
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478
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592

593 **Table S6. Primers used in this study**

594 ?

| Primer | Sequence (5'-3') | Description |
|---|---|---|
| YCC29 | GCG CAA ACG TTT TCG TTA CAA TGC G | 5' of Δ*purH*::*Km* in JW3970 |
| YCC30 | TGC ATT ACC CGG AGC AAC | 3' of Δ*purH*::*Km* in JW3970 |
| YCC80 | TAA GGG AAC CGT GCA TGT G | Forward qPCR primer for *Rp fixJ* |
| YCC81 | GGA TTC GTA CAG CTT GAC CTC | Reverse qPCR primer for *Rp fixJ* |
| YCC97 | ACG TCG TCG TGG TTC TTG | Forward qPCR primer for *Rp purH* |
| YCC98 | CGA AGC CAC CGT CGA TAA A | Reverse qPCR primer for *Rp purH* |
| YCC99 | GCA ACA CGT TCT GCT GAT G | Forward qPCR primer for *Rp RPA2390* |
| YCC100 | CAT TGG TTC TCG GCC TAT CT | Reverse qPCR primer for *Rp RPA2390* |
| YCC91 | AAC CGC ATG GCC CTT ATT | Forward qPCR primer for *Ec entF* |
| YCC92 | GTA TCC AGC AAG CCA AGA AAT G | Reverse qPCR primer for *Ec entF* |
| YCC93 | GTG TTC GAA GGC TTT GAT GG | Forward qPCR primer for *Ec purH* |
| YCC94 | GTG AAG AGC AGC GAC TAT GA | Reverse qPCR primer for *Ec purH* |
| YCC95 | GTC GCA CTT TGC CGT AAT C | Forward qPCR primer for *Ec hcaT* |
| YCC96 | GCT GAT GCT GGT GAT GAT TG | Reverse qPCR primer for *Ec hcaT* |

595