



**HAL**  
open science

## Proteome allocation and the evolution of metabolic cross-feeding

Florian Labourel, Vincent Daubin, Frédéric Menu, Etienne Rajon

► **To cite this version:**

Florian Labourel, Vincent Daubin, Frédéric Menu, Etienne Rajon. Proteome allocation and the evolution of metabolic cross-feeding. *Evolution - International Journal of Organic Evolution*, 2024, 10.1093/evolut/qpae008 . hal-04525995

**HAL Id: hal-04525995**

**<https://univ-lyon1.hal.science/hal-04525995>**

Submitted on 18 Jun 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# 1 Proteome allocation and the 2 evolution of metabolic cross-feeding

3 Florian J. F. Labourel<sup>1,2,3✉</sup>, Vincent Daubin<sup>1</sup>, Frédéric Menu<sup>1</sup>, Etienne Rajon<sup>1</sup>

4 <sup>1</sup>Univ Lyon, Université Lyon 1, CNRS, Laboratoire de Biométrie et Biologie Evolutive  
5 UMR5558, Villeurbanne, France; <sup>2</sup>Milner Centre for Evolution, University of Bath, Bath  
6 BA2 7AY, United Kingdom; <sup>3</sup>Department of Life Sciences, University of Bath, Bath BA2  
7 7AY, United Kingdom

**Key words:** Metabolism |  
Specialisation | Proteome  
optimisation | Gene  
expression | Mechanistic  
fitness

**Significance statement:** Can  
two species thrive on a single  
energetic resource? While the  
competitive exclusion  
principle predicts that one in  
the pair should go extinct, it  
may occur that an organism  
releases partly metabolised  
molecules in the environment,  
securing an ecological niche  
for a second organism in a  
specialisation process called  
metabolic cross-feeding. Here  
we investigate how evolution  
may favor the waste of a  
useful resource using a model  
that considers how a cell  
packed with proteins may be  
less efficient, hence favoring a  
shortening of metabolic  
pathways in order to reduce  
cell packing. Our model  
indicates that such  
specialisation only occurs  
under restricted conditions.  
Incidentally, this makes the  
signatures of cross-feeding,  
such as which metabolites are  
preferentially involved, quite  
predictable.

✉ **For correspondence:**  
fjfl20@bath.ac.uk

**Data availability:** All the  
scripts generated for this  
project are available at:  
<https://github.com/FloTuzoLab/Scripts-Evolution-CF-proteome-allocation>.

**Competing interests:** The  
authors declare no competing  
interests.

## 9 Abstract

10 Metabolic cross-feeding (MCF) is a widespread type of ecological interaction where organisms  
11 share nutrients. In a common instance of MCF, an organism incompletely metabolises sugars and  
12 releases metabolites that are used by another as a carbon source to produce energy. Why would  
13 the former waste edible food, and why does this preferentially occur at specific locations in the  
14 sugar metabolic pathway (acetate and glycerol are preferentially exchanged) have challenged evo-  
15 lutionary theory for decades. Addressing these questions requires to model the cellular features in-  
16 volved; to this end, we built an explicit model of metabolic reactions, including their enzyme-driven  
17 catalysis and the cellular constraints acting on the proteome that may incur a cost to expressing  
18 all enzymes along a pathway. After showing that cells should in principle prioritise upstream reac-  
19 tions when metabolites are restrained inside the cell, we investigate how the diffusivity of these  
20 metabolites may trigger the emergence of MCF in a population. We find that the occurrence of  
21 MCF is rare and requires that an intermediate metabolite be extremely diffusive: indeed, up to  
22 high membrane permeability coefficients, the expected evolutionary outcome is not a diversifica-  
23 tion that resembles MCF but a single genotype that instead overexpresses downstream enzymes.  
24 Only at very high levels of membrane permeability and under distinctive sets of parameters should  
25 the population diversify and MCF evolve. These results help understand the origins of simple mi-  
26 crobial communities, and may later be extended to investigate how evolution has progressively  
27 built up today's extremely diverse communities.

## 29 Introduction

30 Genetic diversification [1, 2] may occur when different ecological niches are encountered [3–5], for  
31 instance when different carbon sources are available in the environment [6, 7]. What may at first  
32 glance sound puzzling – why not using all the available nutrients? – finds an explanation in physi-  
33 ological and environmental constraints or even absolute incompatibilities that make specialists of  
34 each resource outperform generalists [8–13]. Even more bewildering is the observation that diver-  
35 sification occurs in the homogeneous presence of a single energetic resource [14–17]. One finds a  
36 clear example in chemostats or controlled experimental systems in which glucose is continuously  
37 injected, where glucose consumers may evolve that release metabolites for others to use as carbon  
38 sources [14, 18, 19]. This unidirectional by-product process is a form of metabolite cross-feeding  
39 [20, 21], and its evolutionary underpinnings are still blurry [20, 22].

40 In particular, the reasons why specific metabolites are more likely involved in cross-feeding  
41 remain unclear. Indeed, a large number of metabolites produced by a glucose-reliant organism  
42 may constitute viable single carbon sources for others [for example, a glucose-reliant strain of *Es-*

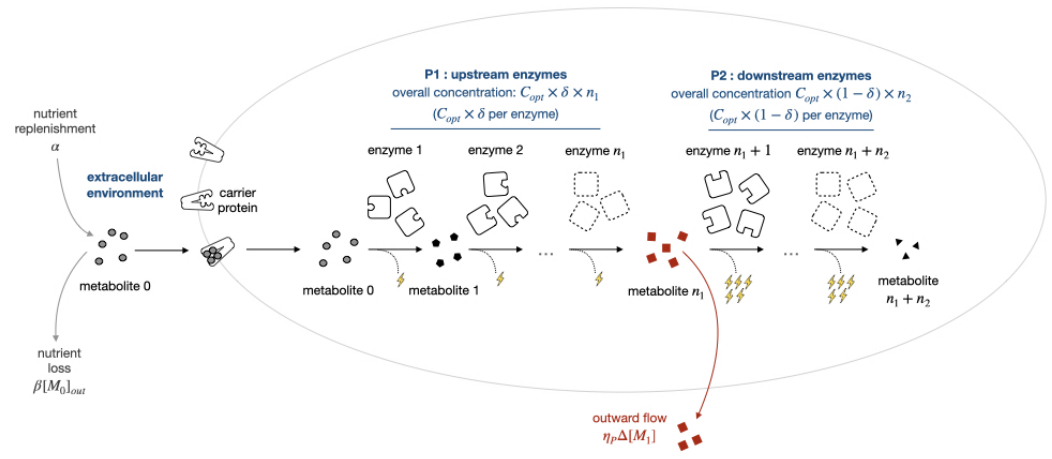
43 *cherichia coli* can theoretically produce up to 58 useful metabolites, 22]. Yet only two metabolites  
44 are commonly reported – from experiments – as being traded in such cross-feeding interactions,  
45 namely acetate and glycerol [14, 19, 23, 24]. In line with the fact that some of these lineages may  
46 have been predisposed to use these metabolites [23], San-Roman and Wagner [25] have hypothe-  
47 sized that this preferential evolution could be due to shorter mutational paths. Accordingly, mod-  
48 ifying the metabolic network to produce these interacting strains would require fewer and less  
49 destabilising mutations and could thence arise more readily. But their conclusion is that acetate  
50 or glycerol trades are no more likely than others to appear through mutation, making the mystery  
51 about their involvement – and potential predispositions of strains – in the evolution of metabolic  
52 cross-feeding even deeper.

53  
54 Adaptation is often incomprehensible without considering the ability of an organism to per-  
55 form a task as being dependent on internal constraints [26–29]. For example, fully expressing  
56 all enzymes along a metabolic pathway may incur a fitness cost [30, 31], to such an extent that  
57 sacrificing a part of a pathway becomes beneficial [32, 33]. Cells whose cytoplasm gets crowded  
58 with proteins actually pay a two-fold cost [34]. First, producing enzymes incurs a direct energetic  
59 expense, approximately proportional to the sum of enzyme concentrations in the cell [35–37]. Sec-  
60 ond, cell packing eventually compromises the diffusion of proteins, thereby hindering metabolic  
61 efficiency [38, 39].

62 In a previous study, we have shown that the evolution of enzyme kinetic parameters and con-  
63 centrations is contingent on their competition with other processes for their substrate [34]. One of  
64 these competing processes may be leakage through the cell membrane, such that highly diffusive  
65 metabolites should be processed by more efficient or concentrated enzymes. The combination  
66 of this requirement for high concentrations, and the cost of an abundant proteome, could make  
67 these metabolites the preferential breakpoints in a metabolic pathway. Very few metabolites can  
68 diffuse through membranes, either because of their size or due to their electronic properties [40].  
69 Such diffusion may be direct, as is the case for glycerol, or indirect when a non-diffusive metabolite  
70 can spontaneously transform into a diffusive one, as is the case with acetate [41, 42].

71  
72 In this work – see Figure 1 for an overview of the metabolic model – we first determine how cells  
73 should allocate their proteome when metabolite diffusion is limited. We find that upstream reac-  
74 tions should be favored when selective pressures are similar along the pathway. We then assess  
75 the hypothesis that cross-feeding evolves in response to the high diffusion rate of an intermediate  
76 metabolite, due to the incurred selection on downstream enzymes. Indeed, a genotype produc-  
77 ing the diffusive metabolite must also be very efficient at metabolising it to prevent its loss, and  
78 thus pays a high cost over-expressing downstream enzymes that, at some point, may become in-  
79 surmountable. Interestingly, a second genotype feeding on the intermediate metabolite and only  
80 expressing downstream enzymes would thrive in this context where the metabolite has become  
81 a resource. This is because, on top of saving on the expression of upstream enzymes, extensive  
82 over-expression downstream is no longer a requirement as the high permeability coefficient of  
83 the metabolite actually helps its uptake. Yet, the overall evolutionary process must be continuous,  
84 instead of the schematic two-steps sequence presented here, making it difficult to predict when  
85 the evolution of cross-feeding should occur.

86 In order to embrace this continuity, we use Adaptive Dynamics [43, 44] to model the evolu-  
87 tion of the pattern of enzyme expression along the pathway. This framework is particularly suited  
88 to model the complex ecological interactions that may arise as the genotype(s) composing the  
89 population shape their environment [32, 45], by controlling the equilibrium frequencies of both  
90 the nutrient and the diffusing metabolite. We find that MCF sometimes evolves, characterised by  
91 an evolutionary diversification giving rise to the coexistence between a genotype only expressing  
92 the enzymes upstream the diffusing metabolite, and another one expressing the enzymes down-  
93 stream. This occurs at very high membrane permeability levels of the diffusing metabolite, only



**Figure 1.** Overview of the model: the pathway is initiated by a carrier protein and comprised of  $n_1$  upstream enzymes defining the sub-pathway  $P_1$  and  $n_2$  downstream enzymes defining the sub-pathway  $P_2$ . Allocation of the proteome is driven by two parameters: (i)  $C_{opt}$  coincides with the adaptive proteome fraction dedicated to one of the reaction of the full pathway  $P_1 + P_2$  when all enzyme concentrations are identical (the case of no differential allocation – see section Optimal metabolic allocation); (ii) the parameter  $\delta$  (resp.  $1 - \delta$ ) corresponds to the fraction dedicated to the first sub-pathway  $P_1$  (resp.  $P_2$ ). The extracellular dynamic of the nutrient (metabolite 0) is based on a constant replenishment-degradation process – see Materials and Methods section. Within the pathway, each reaction follows Briggs Haldane kinetics (with or without reversibility) where enzyme efficiency is constant and studied as a parameter. These reactions each provide a fitness yield, shown above as lightning, proportional to the amount of product produced and to the specific yield of energy gain set for reactions (1 energy unit per reaction per nutrient for upstream enzymes and 5 for downstream enzymes in the above example) – see section on overexpression. Fitness is simultaneously impeded by the cost of expression – production and crowding – and, depending on the subsection again, by the toxicity resulting from the total concentration of metabolites. The metabolite produced at the end of the upstream pathway  $P_1$  is susceptible to leak through the membrane according to a permeability  $\eta_p$  – studied as a parameter – and the gradient of concentration between the extra and intracellular environments. Once in the extracellular environment, it is available to other cells but may also be degraded (like metabolite 0)

94 compatible with diffusion rates reported for acetate or glycerol. We also find that MCF requires  
 95 moderate to high levels regarding the intracellular selective constraints acting on metabolites along  
 96 the pathway.

## 97 Results

### 98 Optimal metabolic allocation and cell constraints

#### 99 Evolution of the overall expression of metabolic enzymes

100 We first assume that all enzymes have an equal concentration and consider its evolution. Increas-  
 101 ing concentration enhances the efficiency of catalysis and thus the production of energy, but with  
 102 diminishing returns [34, 46]. It also incurs costs, firstly due to the actual energy cost of making  
 103 proteins, and secondly because high protein concentrations in the cell decrease the efficiency of  
 104 reactions due to cell packing. The former is captured in our model by a linear cost inflicted to extra  
 105 production, and the latter through a penalty on  $k_f$ , whose effect has been estimated [39, 47] and  
 106 modelled in previous studies [38] – [see Model and 34].

107 To approach the case of reactions involved in the carbon cycle [48], we consider a pathway  
 108 comprised of 40 reactions and initiated by facilitated diffusion through a transporter [49], where  
 109 energy is produced at each step in the process – see Figure 1 – and assessed the effect of reaction  
 110 yield – see SM Figure S3. Reactions follow irreversible Briggs-Haldane kinetics [50], with kinetic  
 111 constants set by default half an order of magnitude higher than the median observed for enzymes  
 112 involved in the central metabolism [51] ( $k_f = 10^{6.25} M^{-1} s^{-1}$ ,  $k_{cat} = 10^{2.25} s^{-1}$ ) – see SM Table S2 for the

113 full set of parameters we used; besides, we also consider the case where reactions are reversible  
114 – see SM Text S1.2. Setting these efficiencies above the average found for enzymes involved in  
115 central carbon metabolism is conservative as it diminishes the selective pressure that promotes  
116 higher enzyme expression, and thus ensures that we are not examining an implausible range of  
117 values (*eg.* because the whole set would be biased by some enzymes that need not be efficient  
118 due to specific metabolic features). The local context of reactions – including reaction reversibility  
119 and metabolite toxicity – may also affect metabolic efficiency, as captured by a linear degradation  
120 rate of each metabolite  $\eta$  in this instance of the model [34] – see SM Text S1.2 for more complex  
121 selective pressures. Nutrients are added to the environment at a constant rate  $\alpha$  and degraded at  
122 a linear rate  $\beta$  – the latter also applying to metabolites released by cells in the medium (see below)  
123 – whose levels are such that without cells, substrate concentration equilibrates at  $1M$ .

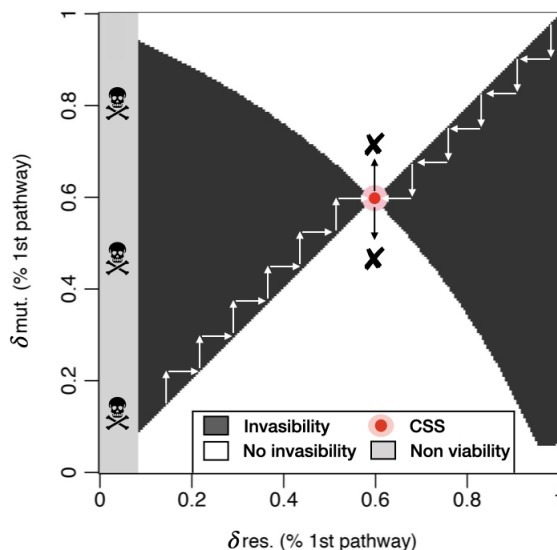
124 For all combinations of the parameters above considered, the evolutionarily expected concen-  
125 trations of the 40 enzymes in the pathway sum up to 15 – 20 % of the whole proteome (see SM  
126 – section Text S1). As we have shown previously, adaptive outcomes depend on a complex inter-  
127 action between cell constraints, enzymes concentrations and kinetic efficiencies that cannot be  
128 captured through the influence of classical experimenters' parameters (such as  $K_M$  or the activity  
129 constant  $k_{cat}[E_{tot}]/K_M$ , for instance) alone [34]. The highest fraction is self-evidently obtained in  
130 conditions where selection for the rates of reactions is acute, such that increasing concentration  
131 becomes beneficial, to a certain extent, despite amplifying intracellular crowding and production  
132 costs. These predictions are consistent with estimates among unicellular species [48]: in most  
133 cases, enzymes involved in the carbon cycle constitute approximately 20 % of the proteome. In  
134 the remaining of this study, the overall concentration of enzymes in the pathway is considered  
135 fixed at its evolutionary expectation by default, *i.e.* that obtained for the specific combination of  
136 parameters studied when assuming an identical allocation all along the pathway.

### 137 **Overexpression in upstream reactions**

138 We then studied how cells should distribute enzyme expression along a pathway split into two  
139 parts of equal lengths. This is a proteome allocation problem, since the overall concentration is  
140 fixed to an (adaptive) optimum as just described; we study the evolution of the part of this over-  
141 all concentration allocated to the first half  $P_1$  of the metabolic pathway,  $\delta$ , which we assume can  
142 change by mutation in the range  $[0, 1]$  –  $\delta = 0$  coinciding with no investment in  $P_1$  such that all of  
143 the resources are allocated to  $P_2$ , while  $\delta = 1$  corresponds to all resources being allocated to  $P_1$   
144 (and none to  $P_2$ ).

145 The evolution of  $\delta$  is modelled using adaptive dynamics [32, 43, 44], as is appropriate when the  
146 fate of a mutant can depend on the environment shaped by one (or several) resident genotype(s).  
147 Here the resident strategy may impact the equilibrium concentrations of the nutrient and of the  
148 metabolites produced along the pathway. Nonetheless at this stage, each metabolite is considered  
149 to be unable to diffuse across the membrane, such that their concentration in the environment re-  
150 mains zero. Therefore in this case, the evolutionary outcome is always a single allocation strategy  
151  $\delta$ , as exemplified in Fig. 2. In this figure, whatever the initial resident strategy in place in the pop-  
152 ulation, evolutionary trajectories will converge to  $\delta \approx 0.6$ , and, once in place in the population (as  
153 resident) this latter strategy will be stable against the invasion by mutants with any other  $\delta$ . These  
154 features make an enhanced expression of upstream reactions – with  $\delta \approx 0.6$  – the evolutionarily  
155 expected outcome, also described as a convergent stable strategy – CSS, hereafter.

156 In the presence of degradation, the adaptive investment in the first part of the pathway is gen-  
157 erally above 0.5 : remarkably,  $\delta$  evolves to more than 0.6 even at very low degradation rates where  
158 the resulting loss in metabolites is less than 1% along the pathway (see Table S3 of SM for an  
159 analysis of the influence of degradation on the loss of metabolites and Figure S5 of SM for more  
160 details). One factor that pushes upstream expression towards higher adaptive values is the selec-  
161 tive pressure imposed by transporters – see Figure S5-B of SM for the influence of transporters.  
162 Yet, the process also holds when this influence is set apart. A plausible explanation is that within



**Figure 2.** Example of the outcome of Adaptive Dynamics shown through a pairwise invasibility plot – PIP, hereafter – where black (resp. white) areas stand for positive (resp. negative) invasive fitness. The grey area represents an area where no strategy is viable because it cannot produce enough energy to reach the level set as corresponding to the demographic steady-state even when only one individual is present. When a mutant arrives in the population of resident strategies, it takes the place of the resident strategy if its invasion fitness is positive, a process represented by the white arrows. Here, below the CSS, mutants with higher values (black area above the left lower corner to right upper corner bisector) than the resident invades and evolution pushes resident to converge towards the CSS, while above the CSS, it is the other way around. This PIP shows how cells should spread their content, according to the respective fractions  $\delta$  and  $1 - \delta$ , between upstream and downstream enzymes and exemplifies that under the specific set of parameters chosen, they should prioritise upstream reactions since  $\delta > 0.5$ , as was generally found – see text for more details.

163 an irreversible pathway as the one modelled, upstream enzymes not only concur to fitness di-  
164 rectly through the energy generated by their respective reaction, but also through their indirect  
165 contributions to downstream reactions – see SM Text S2 and Figure S6 for the analysis of this phe-  
166 nomenon through a more tractable model showing that the optimal strategy should always be to  
167 prioritise upstream reactions. This is also consistent with the fact that this unequal allocation holds  
168 when downstream reactions produce far more energy than their upstream counterparts so that  
169 upstream reactions contribute mostly indirectly to fitness (and is significantly heightened in the  
170 opposite case): even when increasing the yield of the reactions in the second half of the pathway  
171 tenfold as described for the carbon cycle [52],  $\delta$  remains close to 0.6. Besides, the irreversible loss  
172 of metabolites caused by an increase in the degradation rate increases the asymmetry in fitness  
173 contributions further and thereby tends to increase the adaptive ratio of upstream to downstream  
174 enzyme expression – see Figures S5 and S15 of SM.

175 Such asymmetries in fitness contributions might help explain why enzymes catalysing reactions  
176 more upstream tend to face stronger selection [53–55]. Overexpression of upstream enzymes can  
177 nonetheless be – at least, partly – counteracted by selection for homogeneity in metabolite con-  
178 centrations, as is the case when toxicity is high (Figure S7-B of SM) and equally spread, and it also  
179 depends on reactions reversibility, including that of the transporter, inasmuch as reversibility also  
180 shifts the balance between direct and indirect contributions of each subpathway: if downstream  
181 reactions are more reversible than upstream ones, cells should prioritise them, and vice versa –  
182 see Figure S7-A of SM. Be that as it may, considering realistic combinations of these pressures –  
183 moderate toxicity and degradation rates as well as the average reversibility found in central carbon  
184 metabolism [56] tends to corroborate the need for upstream overexpression, as shown in Section



185 Text S2.2 of SM. One constraint that can reshuffle the deck of cards is the permeability of the cell  
186 membrane, which was hitherto not considered.

## 187 **Membrane permeability and cross-feeding**

### 188 **Membrane permeability impacts proteome allocation**

189 Membranes are only permeable to a few metabolites, owing to their unique chemical features  
190 [40]. In our model, we allowed the metabolite produced by the last reaction of the first half of  
191 the metabolic pathway to diffuse – see the model overview in Figure 1 and the Model section for  
192 details – with permeability rates ranging from  $P = 10^{-12} \text{ dm}\cdot\text{s}^{-1}$  to  $10^{-4} \text{ dm}\cdot\text{s}^{-1}$ , in line with empirical  
193 estimates [40]. Metabolites making their way across the membrane have two consequences. First,  
194 they are lost for the cell that has produced them, which may act as a selective pressure for limiting  
195 diffusion. Second, they may accumulate in the external environment and be available to other  
196 individuals as a resource.

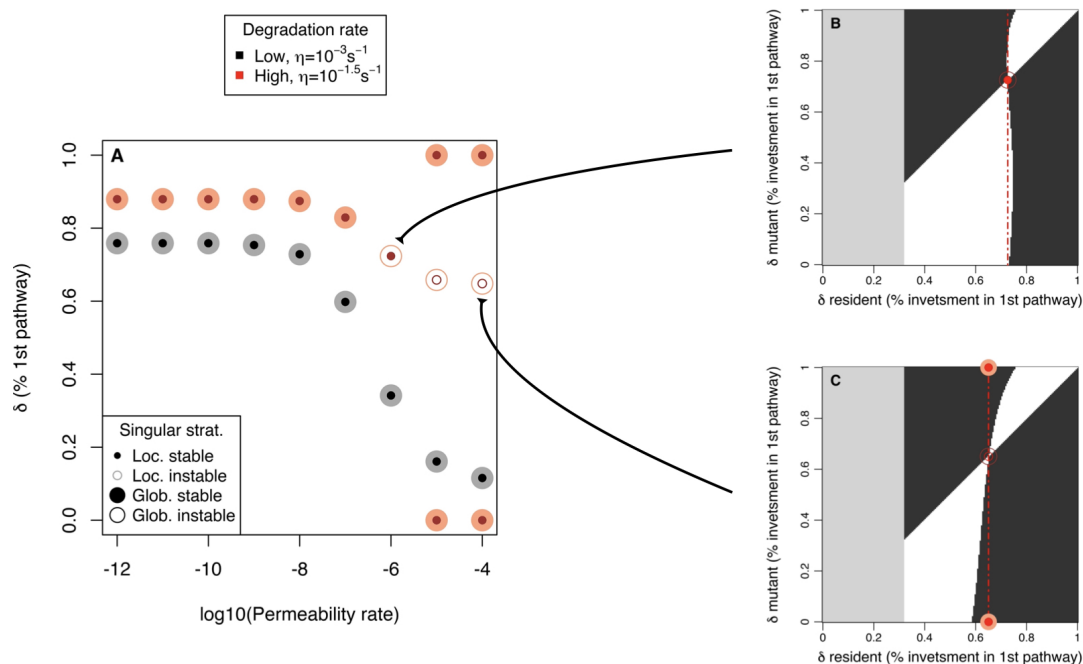
197 A cell has little leverage to limit outward diffusion; the most obvious solution is to use the  
198 metabolite before it is lost, which in our model is possible through an increase in the concentration  
199 of enzymes acting on the second part of the pathway. This is indeed what happens: the optimal  
200 allocation shifts from a higher concentration in the first part of the pathway – owing to the afore-  
201 mentioned factors – to a higher concentration in the second part as permeability increases, as  
202 shown for instance in Fig. 3 in the case of a low degradation rate (grey dots). The results presented  
203 in Fig. 3 are for proteins with kinetic parameters being slightly higher than the median reported for  
204 enzymes in the carbohydrate and energy pathway [51]. Higher efficiencies consistently produce a  
205 qualitatively similar result of a downward shift in allocation to the first part of the metabolic path-  
206 way,  $P_1$ , as permeability increases (see Figure S12 in SM). This outcome is also robust to the possible  
207 existence of different energetic yields along a pathway – see Figure S8 for the influence of yield on  
208 the optimal allocation – and most often holds when introducing other mechanistic constraints –  
209 see Figures S13 and S14.

### 210 **High permeability coefficients can promote cross-feeding**

211 The overinvestment in the second part of pathway,  $P_2$ , limits the loss of the leaky metabolite. As  
212 permeability increases, the investment in  $P_2$  must increase further, but this strategy becomes less  
213 efficient such that the external concentration of the metabolite rises. This, in turn, may give a se-  
214 lective advantage to genotypes that give up preventing the metabolite's leakage by increasing their  
215 contribution to the first or second part of the pathway. From that point on, a new ecological niche  
216 may emerge, that can ultimately result in an evolutionary diversification between two genotypes.  
217 These situations can be identified by a specific type of pairwise invasibility plots where the singular  
218 strategy is convergent but evolutionarily unstable – as shown in Fig. 3-C for the case of a high degra-  
219 dation rate, the singular strategy at  $\delta \approx 0.65$  can be invaded by nearby mutants – called a branching  
220 point. An adaptive diversification may occur at a branching point, requiring that we study the fate  
221 of mutants invading a pair of coexisting strategies, instead of a single one, through trait evolution  
222 plots (SM Figure S9).

223 Branching points indeed lead to an adaptive diversification as the diffusion of the intermediate  
224 metabolite increases above  $10^{-6} \text{ dm}\cdot\text{s}^{-1}$ , with the two most extreme strategies,  $\delta = 0$  and  $\delta = 1$ ,  
225 evolving and forming a stable coalition (see SM - Text S3.1.2). This is characteristic of a complete  
226 metabolic cross-feeding, where a genotype transforms a nutrient into a metabolite released in  
227 the environment, and a second genotype feeds exclusively on that metabolite. For moderately  
228 high permeability rates – around  $P = 10^{-6} \text{ dm}\cdot\text{s}^{-1}$  – the evolutionary outcome is more dubious as  
229 invasion is only possible by distant mutants; the result, in this case, will depend on the distribution  
230 of mutational effects and cannot be studied using classic adaptive dynamics methods.

231 It should be noted that the results in Figure 3 correspond to a metabolic pathway with unequal  
232 contributions of reactions to the energy needs of the cell (downstream reactions produce more



**Figure 3.** Permeability influences the evolution of strategies of enzyme allocations along a metabolic pathway, with the occurrence of cross-feeding at high permeability coefficients depending on the degradation rate. Two degradation rates are considered here (two other ones in Fig. S8 of SM) : low  $\eta = 10^{-3} \text{ s}^{-1}$  in grey and moderate  $\eta = 10^{-1.5} \text{ s}^{-1}$  in red. The enzymes catalysing reactions in either of two parts of the metabolic pathway, with their relative concentrations represented by  $\delta$  (% 1st pathway), have kinetic parameters typical of those intervening in the carbon cycle ( $k_f = 10^{6.25} \text{ M}^{-1} \cdot \text{s}^{-1}$  and  $k_{\text{cat}} = 10^{2.25} \text{ s}^{-1}$ ); we also assume here that reactions in the second part  $P_2$  of the pathway produce 10 times more energy than reactions in the first part  $P_1$ . For low permeability rates (below  $10^{-6} \text{ dm} \cdot \text{s}^{-1}$ ), the evolutionarily expected strategy is always globally and locally stable – as in Figure 2 – and consists in investing more in the first part of the pathway as discussed in the text – and described in further details through Fig. S5 and S15 (see the latter for the same enzyme efficiencies). Increasing permeability coefficients above  $10^{-7} \text{ dm} \cdot \text{s}^{-1}$  results in a decrease in the investment in the first part of the pathway,  $\delta$ , with different consequences for low and high degradation rates. At low degradation rates (in grey on panel A), lowering  $\delta$ , *i.e.* increasing the investment in the second part, remains the most efficient strategy. At high degradation rates (in red on panel A), however, high permeability coefficients result in singular strategies that are both convergent (they evolve from any starting  $\delta$ ) and evolutionarily unstable (they can be invaded by mutants with close (locally unstable) or distant (globally unstable) allocation strategy  $\delta$  – see panel C). This can lead to adaptive diversification, resulting in a stable coalition of strategies, that is, a population made of coexisting genotypes with different values of  $\delta$ . The coalition can be determined (see SM - Figure S9) ; it is comprised of genotypes with  $\delta = 1$  (expressing only the first part of the pathway  $P_1$ ) and of genotypes with  $\delta = 0$  (expressing only the second part  $P_2$ ), corresponding to metabolic cross-feeding. Panel B represents a permeability  $P = 10^{-6} \text{ dm} \cdot \text{s}^{-1}$  for which the singular strategy is globally instable but locally stable, so that the evolutionary outcome should be contingent to the mutational landscape.

233 than upstream ones, as observed on average in the carbon cycle). Equalling the energy contribu-  
 234 tions of all reactions often prevents the occurrence of cross-feeding. Yet, cross-feeding emergence  
 235 was less sensitive to sub-pathway theoretical yields when we introduced the altogether realistic  
 236 possibility of transporters and upstream enzymes co-expression, as exemplified on Figure S11.  
 237 Reversibility and toxicity may also be involved in defining the critical point where cross-feeding  
 238 coalitions can appear – see Section Text S3.3. Overall, the tipping point of  $P = 10^{-6} \text{ dm} \cdot \text{s}^{-1}$  seems  
 239 robust across all internal selective environments, albeit not being a sufficient condition as it also  
 240 depends on the specific combination of constraints associated with these environments.

241 More generally, the evolution of cross-feeding appears contingent on many factors. For in-  
 242 stance, selection on the overall concentration of enzymes contributing to the pathway is impor-



243 tant (eg. because it can reduce diffusive constraints), such that allowing higher concentrations  
244 precludes the occurrence of cross feeding. This is because increasing downstream concentrations,  
245 which may efficiently deal with metabolite diffusion, comes at a lower cost under these circum-  
246 stances. This illustrates that the occurrence of cross-feeding may critically depend on the other  
247 tasks – and their contributions to fitness, which was not directly considered here, though the degra-  
248 dation rate may partly capture the behaviour – performed by cells and thereby on selection acting  
249 on their dedicated proteome, as the global proteome concentration may only vary to a small ex-  
250 tent [40]. No less important should be the size of cells since smaller ones mechanically come with  
251 higher relative leakiness due to larger surface-to-volume ratios, which could in turn favor the occur-  
252 rence of cross-feeding. Cells can nonetheless adapt their size to their content, at least to a certain  
253 extent [37], which may limit the costs incurred by an increase in concentration and prevent the  
254 occurrence of cross-feeding.

255 Finally, while in our model the efficiency of a reaction may only be changed through enzyme con-  
256 centrations, kinetic parameters may also evolve and thus represent a relevant alternative in some  
257 specific parts of the metabolic network. The evolution of cross-feeding could thus also be con-  
258 tingent on the relative availability of mutations changing concentration *versus* kinetic parameters.  
259 Therefore, while this model includes much of the available information about enzyme kinetics and  
260 the selective constraints acting on the proteome, actually predicting how and when cross-feeding  
261 should evolve will require more efforts to better understand the building of global epistasis along  
262 metabolic networks and how critically this depends on the environment.

## 263 Discussion

264 The fact that few metabolites – acetate and glycerol, noticeably – are more likely involved in the  
265 evolution of cross-feeding has been a conundrum for as long as experiments have revealed this  
266 phenomenon [25]. Here, we have put forward an explanation based on the necessity for a cell to  
267 optimise its proteome allocation, accounting for incurred costs and physical constraints like the dif-  
268 ferential permeability of a cell's membrane to metabolites. Indeed, contrary to other metabolites,  
269 acetate is in constant chemical equilibrium with the highly diffusive acetic acid [42], and glycerol  
270 readily leaks towards the environment [40], which may predispose them to be involved in cross-  
271 feeding.

272 That metabolites rather upstream in a metabolic pathway – and therefore of potential use to  
273 generate more energy – will create a novel ecological niche when released is straightforward. But  
274 whether evolution will take this path when some genotypes have the potential to reduce the leak  
275 is worthy of a careful examination. In a number of cases that we have considered and that fall into  
276 the realistic range of parameters, proteome allocation will evolve in such a way that it prevents, or  
277 at least limits, the diffusion of the molecule. Only under some restricted conditions – that we have  
278 shown to coincide with the features of the two aforementioned metabolites – will cross-feeding  
279 evolve, characterised by a functional specialisation between a part of the population that trans-  
280 forms the nutrient into the diffusive metabolite, and another part that uses the metabolite as a  
281 carbon source, echoing work on digital evolution that similarly pointed to the possible contingency  
282 of cross-feeding [57, 58].

283  
284 Beyond the existence of intrinsic cellular leakiness, one process that may facilitate the emergence  
285 of cross-feeding, or that may be co-opted to foster its efficacy when it is in place, is the cheap  
286 uptake and secretion of metabolites (eg. through facilitated transport) [42, 59, 60]. Even a slight  
287 leakage may for instance be enough to sustain a cross-feeder with high affinity transporters such  
288 as the one reported in *E.coli* for acetate [61]. This metabolic strategy, where cells actively give up  
289 a metabolite even though it still has the potential to bring fitness contributions, is often known  
290 as overflow metabolism [62, 63] and also frequently involves acetate. We did not consider the  
291 existence of a specific cost to the second part of the pathway, as has been documented in the past

292 to explain overflow [64, 65]; nor did we account for other possible costs such as the existence of  
293 a localised toxicity [66] or the extra membrane occupancy involved in cellular respiration [67, 68].  
294 These processes may promote the advent of cross-feeding, for they could bring extra fitness to an  
295 organism expressing one or the other part of the pathway.

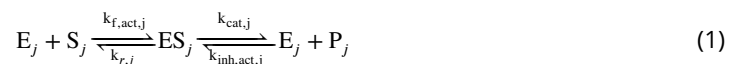
296 From a broader ecological perspective, nutrients are hardly ever constantly renewed in nature;  
297 they are subject at least to some stochasticity, and most often than not occur through unpredictable  
298 periods of feast and famine. Insight into how proteome allocation may evolve in this context, and  
299 whether it should likely involve cross-feeding, can be gained from the existence of so-called diauxic  
300 shifts, where the phenotype can switch from the production of acetate to its consumption when the  
301 medium is enriched in this nutrient [59, 69]. In this case, it appears that the environment could be  
302 used to store intermediate metabolites, both increasing a genotype's ability to uptake and use  
303 glucose when it is present, and its ability to await its renewal otherwise. Such plasticity in  
304 expressing different parts of a metabolic pathway should prevent, to some extent, the evolution of  
305 fixed specialists (as considered here) and further hinder the evolution of cross-feeding. We thus  
306 postulate that cross-feeding might only evolve in environments stable enough to prevent the evolution  
307 of plasticity.

308 Biology is actually teeming with interactions and emergent properties that complicate the big  
309 picture [70]. And, if functional simplicity may exist at the ecological scale [71], the ultimate  
310 underpinnings behind community assemblies are still blurred by the combination between a long and  
311 tumultuous evolutionary history and the wide prevalence of high order interactions, both within and  
312 between organisms, and in relation to the environment [57, 72, 73]. Addressing these community-level  
313 questions cannot bypass the existence of lower level features such as the epistatic relationships  
314 stemming from the joint effect of enzyme kinetic parameters [74] and their expression along  
315 pathways [75]; nor can we study metabolic evolution without a careful examination of their impact  
316 on the cellular micro-environment. We believe that the present study, by accounting for these  
317 interactions at a primordial ecological stage, will help explain how (some of) these communities  
318 appeared in the first place and how this may have then fuelled the scaffolding process underlying  
319 the building of microbial societies.

## 320 Models

### 321 Metabolic Model of fitness

322 Cell fitness results from the biomass and energy produced along a metabolic pathway (eg. ATP).  
323 The pathway is initiated by carrier proteins with  $V_{T_m} = 1mM/s$  and  $K_T = 10mM$  (see SM Text S1 for  
324 details and justification), passively transporting nutrients inside cells and whose features are based  
325 on those for glucose in yeasts, as detailed elsewhere [34]. Nutrients are added (resp. eliminated) at  
326 a constant rate  $\alpha$  (resp.  $\beta$ ) in the external environment. The metabolic pathway is linear, comprising  
327  $N_r$  (40 in the paper, but see SM for other values) reactions catalysed by enzymes whose levels of  
328 expression may evolve. The product of the  $j^{ieth}$  reaction is used as the substrate of the next one.  
329 These metabolites are constantly degraded at a rate  $\eta$  or fuelling toxicity  $T$  according to processes  
330 justified elsewhere [34] and also detailed at the beginning of the SM (introduction of section Text S1  
331 and subsection Text S1.2). Each reaction (j) follows either reversible or irreversible Briggs Haldane  
332 kinetics [76]:



Irreversible reactions are modeled by setting  $k_{inh} = 0$  - in this simple setting, the system can be solved for each pair of successive reactions since they do not feedback on upstream reactions, so that one has to solve  $N$  quadratic equations of the form:

$$V m_j \frac{[S_j]}{[S_j] + K_{M,j}} = V m_{j+1} \frac{[S_{j+1}]}{[S_{j+1}] + K_{M,j+1}}, \quad (2)$$

333 where  $V_m$  and  $K_M$  depends on microscopic parameters  $k_f$  and  $k_{cat}$ , as well as enzyme concentra-  
334 tions while  $\eta$  is the degradation rate (leakiness is modelled in a similar manner than the degrada-  
335 tion rate but depends on the gradient between internal and external concentrations so that it also  
336 impacts the environment through the latter). Otherwise, reversibility is spread equally between  
337 backward parameters  $k_r$  and  $k_{inh,act}$  (eg. if  $K_{rev} = 1/9$ ,  $k_r = k_{cat}/3$  and  $k_{inh,act} = k_{f,act}/3$ ) and the system  
338 of  $N$  equations is solved using Broyden's method [77]. The actual values for  $k_{inh,act}$  and  $k_{f,act}$  depend  
339 on the cellular constraints – see next subsection of Model.

340 Each reaction produces energy. This is a simplification as some reactions in the carbon cycle do  
341 not, unimportant as we consider the global expression of large portions of a pathway. We consider  
342 the case where contributions are equal along the pathway as well as other more realistic setups.

### 343 Cellular constraints

344 Cell proteomes face two intrinsic constraints: (i) the cost of protein expression and (ii) the burden  
345 entailed by molecular crowding. We model (i) through a cost linear with protein expression, pro-  
346 portional to constant  $c$  [36]. We considered values of  $c$  such that the whole cytoplasmic proteome  
347 – the enzymes in the pathway and other free enzymes – costs 5 to 50% of the whole cell budget. We  
348 model molecular crowding (ii) through a non-linear decrease of diffusion [38, 39] that changes the  
349 affinity constant  $k_f$  to  $k_{f,act}$  in the model with irreversible reactions, according to equation:

$$k_{f,act} = k_f \cdot 10^{-([E_{other}] + \sum_{i=1}^{40} [E_{tot,i}]) / [M_b]}, \quad (3)$$

350 where  $[E_{tot,i}] = [E_i] + [ES_i]$ ,  $[M_b] = 3 \cdot 10^{-3} M$  represents the scaling factor for the effect of diffusion,  
351 while  $[E_{other}]$  denotes the sum of the concentrations of other cytoplasmic proteins than the 40  
352 under consideration. In the model with reversible reactions,  $k_{inh}$  is also affected by crowding, which  
353 is a conservative assumption as reverse reactions might be less sensitive to the diffusive process  
354 owing to the preexisting co-localisation between substrate molecules and enzymes.

355 Our model includes three processes involving the metabolites produced that select for the en-  
356 hancement of enzyme activity, drawing a complex trade-off on the coexpression of enzymes: (1)  
357 metabolites can be lost, either because they are involved in parasitic reactions or because they are  
358 subject to targeted degradation [78], modelled through a linear degradation rate  $\eta$ ; (2) metabolites  
359 can be toxic for the cell, for they engage in parasitic reactions, for instance through promiscuous in-  
360 teractions [79]; (3) highly reversible reactions within a pathway may also require efficient enzymes  
361 to maintain a high net flux [80]. We considered these three processes in various instances of our  
362 model, as described in SM Text S1; the results presented in the paper mainly comprise the action  
363 of a linear degradation rate, which provides a good qualitative understanding of how processes  
364 impacting the metabolites also impact selection on enzymes. Finally, the permeability of cell mem-  
365 brane to a given metabolite also acts as a constraint, which is introduced here by considering that  
366 one metabolite in the pathway diffuses passively at a rate  $\eta_d$  – on Figure 1, we show where this  
367 process occurs.

### 368 Ecological equilibria

369 As to perform the adaptive dynamics analysis, we need to determine the ecological equilibrium set  
370 by a given genotype or coalition. To this aim, we first compute the value of the net flux (number  
371 of energy units per individual per unit of time)  $\Phi_{net,N}$  for a given population size  $N$ . As long as this  
372 flux is lower (or higher) than an arbitrary value  $\Phi_{net} = 10^{-4} M \cdot s^{-1}$ ,  $N$  is increased (or decreased)  
373 at the next iteration (by one unit when the flux gets close enough from this value). The algorithm  
374 is stopped, either when the difference between these fluxes is lower than  $10^{-6} \Phi_{net}$ , or when it os-  
375 cillates between two neighbouring values ( $N_{eq}$  is then set to the average between these values).  
376 The ecological equilibrium, is defined by the nutrient and metabolite concentrations in the envi-  
377 ronment at this demographic equilibrium. Fitness values of strategies are then calculated in these  
378 conditions of equilibrium.

## 379 **Adaptive Dynamics of enzyme expression**

380 We use Adaptive Dynamics [32, 43, 44] to model the evolution of enzyme expression along the  
381 metabolic pathway. This framework consider rare mutations, such that a resident “strategy” – cor-  
382 responding to a given expression pattern – is assumed to have reached its demographic equilib-  
383 rium before a mutant strategy appears in the population. At this demographic equilibrium, births  
384 compensate for deaths in the population, resulting in a concentration of nutrients specific of the  
385 resident (see previous section). The fitness of any mutant strategy is then determined for each  
386 resident equilibrium, which enables the drawing of Pairwise Invasibility Plots (PIPs) representing  
387 for each pair the ability of a rare mutant to invade the resident strategy, based on a comparison  
388 between the growth rate of the mutant and that of the resident. These plots are used to identify  
389 singular strategies and their properties, as defined in [43]. A particular type of singular strategies,  
390 branching points, may be indicative of a diversification in the population, which we further study by  
391 drawing areas of mutual invasibility (where both strategies invade when rare), before computing  
392 the ecological equilibrium for each coalition – composed of two resident strategies instead of one  
393 – in that space. We then calculate the growth rate of mutants nearby each strategy in the coalition  
394 to identify coalitions that are stable (they cannot be invaded by any of the nearby mutants) and  
395 convergent (there exist evolutionary trajectories towards them), hence identifying evolutionarily ex-  
396 pected communities after diversification has occurred [81, 82]. This latter process is summarised  
397 on trait evolution plots – TEPs – that we have shown in SM.

398 PIPs were generally drawn for 250 strategies, unless lower resolution was sufficient to capture  
399 the trend. In order to determine the optimal allocation, we set the total proteome content to its  
400 optimal value as determined without the influence of permeability. An individual whose strategy  
401 is to invest as much in the first part of the pathway than in the second part corresponds precisely  
402 to this case. Notice that, besides using a two step process, solving the systems to find nutrient and  
403 metabolite concentrations required to use R package ‘nleqslv’ with Broyden’s method [77].

## 404 **Settings for the models**

405 A list of the basic settings can be found at the end of section Text S1.1 - SM. We varied them within  
406 their biological realistic ranges. This allowed us to identify key drivers of the diversification process  
407 that eventually result in cross-feeding, as discussed in the results section. The extensive analysis  
408 of parameters can be found in SM, especially in Texts S1 and S3.

## 409 **Acknowledgment**

410 We thank T.Lenormand for helpful discussions that led us to consider the evolution of cross-feeding.  
411 This preprint was created using the LaPreprint template (<https://github.com/roaldarbol/lapreprint>) by  
412 Mikkel Roald-Arbøl.

## 413 **Author contributions**

414 FL, ER, FM and VD designed the research. FL and ER developed the models and analyzed the results.  
415 FL and ER wrote the first draft while FM and VD contributed further improvements.

## 416 **References**

- 417 [1] Sewall Wright. “The genetical structure of populations”. In: *Annals of Eugenics* 15.1 (1949),  
418 pp. 323–354. DOI: <https://doi.org/10.1111/j.1469-1809.1949.tb02451.x>. URL: <https://doi.org/10.1111/j.1469-1809.1949.tb02451.x>.
- 420 [2] Francisco J Ayala et al. “Genetic differentiaiton during the speciation process in Drosophila”.  
421 eng. In: *Evolution* 28.4 (1974), pp. 576–592. ISSN: 1558-5646 (Electronic); 0014-3820 (Linking).  
422 DOI: [10.1111/j.1558-5646.1974.tb00791.x](https://doi.org/10.1111/j.1558-5646.1974.tb00791.x).

- 423 [3] G. F. Gause. "Experimental Studies on the Struggle for Existence : I. Mixed Population of Two  
424 Species of Yeast". In: *Journal of Experimental Biology* 9.4 (1932), pp. 389–402. DOI: [10.1242/jeb.  
425 9.4.389](https://doi.org/10.1242/jeb.9.4.389). URL: <https://doi.org/10.1242/jeb.9.4.389>.
- 426 [4] G. Hardin. "The competitive exclusion principle." In: *Science* 131 3409 (1960), pp. 1292–7.
- 427 [5] Bruce R. Levin. "Coexistence of Two Asexual Strains on a Single Resource". In: *Science* 175.4027  
428 (1972), pp. 1272–1274. URL: <http://www.jstor.org/stable/1733726>.
- 429 [6] Rutger Hermsen et al. "A growth-rate composition formula for the growth of E. coli on co-  
430 utilized carbon substrates". In: *Molecular Systems Biology* 11.4 (2015), p. 801. DOI: [https://doi.  
431 org/10.15252/msb.20145537](https://doi.org/10.15252/msb.20145537). URL: <https://doi.org/10.15252/msb.20145537>.
- 432 [7] Xin Wang et al. "Growth strategy of microbes on mixed carbon sources". In: *Nature Commu-  
433 nications* 10.1 (2019), p. 1279. DOI: [10.1038/s41467-019-09261-3](https://doi.org/10.1038/s41467-019-09261-3). URL: [https://doi.org/10.1038/  
s41467-019-09261-3](https://doi.org/10.1038/<br/>434 s41467-019-09261-3).
- 435 [8] G. E. Hutchinson. "The Paradox of the Plankton". In: *The American Naturalist* 95.882 (1961),  
436 pp. 137–145. DOI: [10.1086/282171](https://doi.org/10.1086/282171). URL: <https://doi.org/10.1086/282171>.
- 437 [9] S. C. Stearns. "Trade-Offs in Life-History Evolution". In: 3.3 (1989), pp. 259–268. DOI: [10.2307/  
438 2389364](https://doi.org/10.2307/2389364). URL: <http://www.jstor.org.inee.bib.cnrs.fr/stable/2389364>.
- 439 [10] Richard E. Michod. "On the transfer of fitness from the cell to the multicellular organism".  
440 In: *Biology and Philosophy* 20.5 (2005), pp. 967–987. DOI: [10.1007/s10539-005-9018-2](https://doi.org/10.1007/s10539-005-9018-2). URL:  
441 <https://doi.org/10.1007/s10539-005-9018-2>.
- 442 [11] Hila Sheftel et al. "The geometry of the Pareto front in biological phenotype space". In: *Ecology  
443 and Evolution* 3.6 (2013), pp. 1471–1483. DOI: <https://doi.org/10.1002/ece3.528>. URL: <https://doi.org/10.1002/ece3.528>.
- 444
- 445 [12] Aleksej Zelezniak et al. "Metabolic dependencies drive species co-occurrence in diverse mi-  
446 crobial communities". In: *Proceedings of the National Academy of Sciences* 112.20 (2015), pp. 6449–  
447 6454. DOI: [10.1073/pnas.1421834112](https://doi.org/10.1073/pnas.1421834112). URL: <https://doi.org/10.1073/pnas.1421834112>.
- 448 [13] Fernando Baquero et al. "The Origin of Niches and Species in the Bacterial World". In: *Frontiers  
449 in microbiology* 12 (2021), pp. 657986–657986. DOI: [10.3389/fmicb.2021.657986](https://doi.org/10.3389/fmicb.2021.657986). URL: <https://pubmed.ncbi.nlm.nih.gov/33815348>.
- 450
- 451 [14] Robert B. Helling, Christopher N. Vargas, and Julian Adams. "Evolution of <em>Escherichia  
452 coli</em> During Growth in a Constant Environment". In: *Genetics* 116.3 (1987), p. 349. URL:  
453 <http://www.genetics.org/content/116/3/349.abstract>.
- 454 [15] Richard E. Lenski et al. "Long-Term Experimental Evolution in Escherichia coli. I. Adaptation  
455 and Divergence During 2,000 Generations". In: 138.6 (1991), pp. 1315–1341. URL: [http://www.  
456 jstor.org/stable/2462549](http://www.jstor.org/stable/2462549).
- 457 [16] Daniel E. Rozen and Richard E. Lenski. "Long-Term Experimental Evolution in Escherichia coli.  
458 VIII. Dynamics of a Balanced Polymorphism." In: *The American Naturalist* 155.1 (2000), pp. 24–  
459 35. DOI: [10.1086/303299](https://doi.org/10.1086/303299). URL: <https://doi.org/10.1086/303299>.
- 460 [17] Benjamin H. Good et al. "The dynamics of molecular evolution over 60,000 generations". In:  
461 *Nature* 551.7678 (2017), pp. 45–50. DOI: [10.1038/nature24287](https://doi.org/10.1038/nature24287). URL: [https://doi.org/10.1038/  
nature24287](https://doi.org/10.1038/<br/>462 nature24287).
- 463 [18] D S Treves, S Manning, and J Adams. "Repeated evolution of an acetate-crossfeeding poly-  
464 morphism in long-term populations of Escherichia coli." In: *Molecular Biology and Evolution*  
465 15.7 (1998), pp. 789–797. DOI: [10.1093/oxfordjournals.molbev.a025984](https://doi.org/10.1093/oxfordjournals.molbev.a025984). URL: [https://doi.org/10.  
1093/oxfordjournals.molbev.a025984](https://doi.org/10.<br/>466 1093/oxfordjournals.molbev.a025984).
- 467 [19] Richard E Lenski. "Experimental evolution and the dynamics of adaptation and genome evo-  
468 lution in microbial populations". In: *The ISME Journal* 11.10 (2017), pp. 2181–2194. DOI: [10.  
469 1038/ismej.2017.69](https://doi.org/10.1038/ismej.2017.69). URL: <https://doi.org/10.1038/ismej.2017.69>.



- 470 [20] Glen D'Souza et al. "Ecology and evolution of metabolic cross-feeding interactions in bacteria".  
471 In: *Natural Product Reports* 35.5 (2018), pp. 455–488. DOI: [10.1039/C8NP00009C](https://doi.org/10.1039/C8NP00009C). URL: <http://dx.doi.org/10.1039/C8NP00009C>.  
472
- 473 [21] Nick W. Smith et al. "The Classification and Evolution of Bacterial Cross-Feeding". In: *Frontiers*  
474 *in Ecology and Evolution* 7 (2019), p. 153. ISSN: 2296-701X. DOI: [10.3389/fevo.2019.00153](https://doi.org/10.3389/fevo.2019.00153). URL:  
475 <https://www.frontiersin.org/article/10.3389/fevo.2019.00153>.
- 476 [22] Magdalena San Roman and Andreas Wagner. "An enormous potential for niche construction  
477 through bacterial cross-feeding in a homogeneous environment". In: *PLOS Computational*  
478 *Biology* 14.7 (2018), e1006340-. URL: <https://doi.org/10.1371/journal.pcbi.1006340>.
- 479 [23] Margie Kinnersley et al. "Ex Uno Plures: Clonal Reinforcement Drives Evolution of a Simple  
480 Microbial Community". In: *PLOS Genetics* 10.6 (2014), e1004430-. URL: [https://doi.org/10.](https://doi.org/10.1371/journal.pgen.1004430)  
481 [1371/journal.pgen.1004430](https://doi.org/10.1371/journal.pgen.1004430).
- 482 [24] Tobias Großkopf et al. "Metabolic modelling in a dynamic evolutionary framework predicts  
483 adaptive diversification of bacteria in a long-term evolution experiment". In: *BMC Evolutionary*  
484 *Biology* 16.1 (2016), p. 163. DOI: [10.1186/s12862-016-0733-x](https://doi.org/10.1186/s12862-016-0733-x). URL: <https://doi.org/10.1186/s12862-016-0733-x>.  
485
- 486 [25] Magdalena San Roman and Andreas Wagner. "Acetate and glycerol are not uniquely suited  
487 for the evolution of cross-feeding in *E. coli*". In: *PLOS Computational Biology* 16.11 (2020),  
488 e1008433-. URL: <https://doi.org/10.1371/journal.pcbi.1008433>.
- 489 [26] Douwe Molenaar et al. "Shifts in growth strategies reflect tradeoffs in cellular economics."  
490 eng. In: *Mol Syst Biol* 5 (2009), p. 323. ISSN: 1744-4292 (Electronic); 1744-4292 (Linking). DOI:  
491 [10.1038/msb.2009.82](https://doi.org/10.1038/msb.2009.82).
- 492 [27] Robert E. Beardmore et al. "Metabolic trade-offs and the maintenance of the fittest and the  
493 flattest". In: *Nature* 472.7343 (2011), pp. 342–346. DOI: [10.1038/nature09905](https://doi.org/10.1038/nature09905). URL: [https://doi.](https://doi.org/10.1038/nature09905)  
494 [org/10.1038/nature09905](https://doi.org/10.1038/nature09905).
- 495 [28] Romain Gallet et al. "The evolution of bacterial cell size: the internal diffusion-constraint hy-  
496 pothesis". In: *The Isme Journal* 11 (2017), 1559 EP -. URL: [http://dx.doi.org/10.1038/ismej.2017.](http://dx.doi.org/10.1038/ismej.2017.35)  
497 [35](http://dx.doi.org/10.1038/ismej.2017.35).
- 498 [29] Torsten Held, Daniel Klemmer, and Michael Lässig. "Survival of the simplest in microbial evo-  
499 lution". In: *Nature Communications* 10.1 (2019), p. 2472. DOI: [10.1038/s41467-019-10413-8](https://doi.org/10.1038/s41467-019-10413-8).  
500 URL: <https://doi.org/10.1038/s41467-019-10413-8>.
- 501 [30] Meike T. Wortel et al. "Evolutionary pressures on microbial metabolic strategies in the chemo-  
502 stat". In: *Scientific Reports* 6.1 (2016), p. 29503. DOI: [10.1038/srep29503](https://doi.org/10.1038/srep29503). URL: [https://doi.org/10.](https://doi.org/10.1038/srep29503)  
503 [1038/srep29503](https://doi.org/10.1038/srep29503).
- 504 [31] Meike T. Wortel et al. "Metabolic enzyme cost explains variable trade-offs between microbial  
505 growth rate and yield". In: *PLOS Computational Biology* 14.2 (2018), e1006010-. URL: [https:](https://doi.org/10.1371/journal.pcbi.1006010)  
506 [//doi.org/10.1371/journal.pcbi.1006010](https://doi.org/10.1371/journal.pcbi.1006010).
- 507 [32] M. Doebeli. "A model for the evolutionary dynamics of cross-feeding polymorphisms in mi-  
508 croorganisms". In: *Population Ecology* 44.2 (2002), pp. 59–70. DOI: [10.1007/s101440200008](https://doi.org/10.1007/s101440200008).  
509 URL: <https://doi.org/10.1007/s101440200008>.
- 510 [33] Thomas Pfeiffer, Sebastian Bonhoeffer, and Associate Editor: Peter J. Morin. "Evolution of  
511 Cross-Feeding in Microbial Populations". In: 163.6 (2004), E126–E135. DOI: [10.1086/383593](https://doi.org/10.1086/383593).  
512 URL: <http://www.jstor.org/stable/10.1086/383593>.
- 513 [34] Florian Labourel and Etienne Rajon. "Resource uptake and the evolution of moderately effi-  
514 cient enzymes". In: *Molecular Biology and Evolution* (2021). DOI: [10.1093/molbev/msab132](https://doi.org/10.1093/molbev/msab132). URL:  
515 <https://doi.org/10.1093/molbev/msab132>.



- 516 [35] Andreas Wagner. "Energy Constraints on the Evolution of Gene Expression". In: *Molecular*  
517 *Biology and Evolution* 22.6 (2005), pp. 1365–1374. ISSN: 0737-4038. DOI: [10.1093/molbev/](https://doi.org/10.1093/molbev/msi126)  
518 [msi126](https://doi.org/10.1093/molbev/msi126). eprint: <https://academic.oup.com/mbe/article-pdf/22/6/1365/3879110/msi126.pdf>. URL:  
519 <https://doi.org/10.1093/molbev/msi126>.
- 520 [36] Michael Lynch and Georgi K Marinov. "The bioenergetic costs of a gene". In: *Proceedings of the*  
521 *National Academy of Sciences of the United States of America* 112.51 (2015), pp. 15690–15695.  
522 DOI: [10.1073/pnas.1514974112](https://doi.org/10.1073/pnas.1514974112). URL: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4697398/>.
- 523 [37] Moshe Kafri et al. "The Cost of Protein Production". In: *Cell Reports* 14.1 (2016), pp. 22–31.  
524 DOI: <https://doi.org/10.1016/j.celrep.2015.12.015>. URL: [https://www.sciencedirect.com/science/](https://www.sciencedirect.com/science/article/pii/S221112471501428X)  
525 [article/pii/S221112471501428X](https://www.sciencedirect.com/science/article/pii/S221112471501428X).
- 526 [38] Ken A. Dill, Kingshuk Ghosh, and Jeremy D. Schmit. "Physical limits of cells and proteomes".  
527 In: *Proceedings of the National Academy of Sciences* 108.44 (2011), p. 17876. DOI: [10.1073/pnas.](https://doi.org/10.1073/pnas.1114477108)  
528 [1114477108](https://doi.org/10.1073/pnas.1114477108). URL: <http://www.pnas.org/content/108/44/17876.abstract>.
- 529 [39] Pablo M. Blanco et al. "Macromolecular diffusion in crowded media beyond the hard-sphere  
530 model". In: *Soft Matter* 14 (16 2018), pp. 3105–3114. DOI: [10.1039/C8SM00201K](https://doi.org/10.1039/C8SM00201K). URL: [http://](http://dx.doi.org/10.1039/C8SM00201K)  
531 [dx.doi.org/10.1039/C8SM00201K](http://dx.doi.org/10.1039/C8SM00201K).
- 532 [40] R. Milo, R. Phillips, and N. Orme. "Cell Biology by the Numbers". In: (2016). Ed. by New York:  
533 Garland Science.
- 534 [41] Mario Orsi, Wendy E. Sanderson, and Jonathan W. Essex. "Permeability of Small Molecules  
535 through a Lipid Bilayer: A Multiscale Simulation Study". In: *The Journal of Physical Chemistry*  
536 *B* 113.35 (2009), pp. 12019–12029. DOI: [10.1021/jp903248s](https://doi.org/10.1021/jp903248s). URL: [https://doi.org/10.1021/](https://doi.org/10.1021/jp903248s)  
537 [jp903248s](https://doi.org/10.1021/jp903248s).
- 538 [42] Stéphane Pinhal et al. "Acetate Metabolism and the Inhibition of Bacterial Growth by Acetate".  
539 In: *Journal of bacteriology* 201.13 (2019), e00147–19. DOI: [10.1128/JB.00147-19](https://doi.org/10.1128/JB.00147-19). URL: [https://](https://pubmed.ncbi.nlm.nih.gov/30988035)  
540 [pubmed.ncbi.nlm.nih.gov/30988035](https://pubmed.ncbi.nlm.nih.gov/30988035).
- 541 [43] S. A. H. Geritz et al. "Evolutionarily singular strategies and the adaptive growth and branching  
542 of the evolutionary tree". In: *Evolutionary Ecology* 12.1 (1998), pp. 35–57. DOI: [10.1023/A:](https://doi.org/10.1023/A:1006554906681)  
543 [1006554906681](https://doi.org/10.1023/A:1006554906681). URL: <https://doi.org/10.1023/A:1006554906681>.
- 544 [44] J. Hofbauer and K. Sigmund. "Adaptive dynamics and evolutionary stability". In: *Applied Math-*  
545 *ematics Letters* 3.4 (1990), pp. 75–79. DOI: [https://doi.org/10.1016/0893-9659\(90\)90051-C](https://doi.org/10.1016/0893-9659(90)90051-C). URL:  
546 <https://www.sciencedirect.com/science/article/pii/089396599090051C>.
- 547 [45] Thomas Pfeiffer and Stefan Schuster. "Game-theoretical approaches to studying the evolu-  
548 tion of biochemical systems". In: *Trends in Biochemical Sciences* 30.1 (2005), pp. 20–25. DOI:  
549 <https://doi.org/10.1016/j.tibs.2004.11.006>. URL: [https://www.sciencedirect.com/science/article/](https://www.sciencedirect.com/science/article/pii/S0968000404002968)  
550 [pii/S0968000404002968](https://www.sciencedirect.com/science/article/pii/S0968000404002968).
- 551 [46] D L Hartl, D E Dykhuizen, and A M Dean. "Limits of adaptation: the evolution of selective  
552 neutrality". In: *Genetics* 111.3 (1985), pp. 655–674. URL: [https://pubmed.ncbi.nlm.nih.gov/](https://pubmed.ncbi.nlm.nih.gov/3932127)  
553 [3932127](https://pubmed.ncbi.nlm.nih.gov/3932127).
- 554 [47] Steven S Andrews. "Effects of surfaces and macromolecular crowding on bimolecular reac-  
555 tion rates." eng. In: *Phys Biol* 17.4 (2020), p. 045001. ISSN: 1478-3975 (Electronic); 1478-3967  
556 (Linking). DOI: [10.1088/1478-3975/ab7f51](https://doi.org/10.1088/1478-3975/ab7f51).
- 557 [48] Wolfram Liebermeister et al. "Visual account of protein investment in cellular functions". In:  
558 *Proceedings of the National Academy of Sciences* 111.23 (2014), p. 8488. DOI: [10.1073/pnas.](https://doi.org/10.1073/pnas.1314810111)  
559 [1314810111](https://doi.org/10.1073/pnas.1314810111). URL: <http://www.pnas.org/content/111/23/8488.abstract>.

- 560 [49] Arnošt Kotyk. "Mobility of the free and of the loaded monosaccharide carrier in *Saccharomyces cerevisiae*". In: *Biochimica et Biophysica Acta (BBA) - Biomembranes* 135.1 (1967), pp. 112–  
561 119. DOI: [https://doi.org/10.1016/0005-2736\(67\)90013-2](https://doi.org/10.1016/0005-2736(67)90013-2). URL: <http://www.sciencedirect.com/science/article/pii/0005273667900132>.  
562  
563
- 564 [50] G E Briggs and J B Haldane. "A Note on the Kinetics of Enzyme Action". In: *The Biochemical journal* 19.2 (1925), pp. 338–339. DOI: [10.1042/bj0190338](https://doi.org/10.1042/bj0190338). URL: <https://pubmed.ncbi.nlm.nih.gov/16743508>.  
565  
566
- 567 [51] Arren Bar-Even et al. "The Moderately Efficient Enzyme: Evolutionary and Physicochemical Trends Shaping Enzyme Parameters". In: *Biochemistry* 50.21 (2011), pp. 4402–4410. DOI: [10.1021/bi2002289](https://doi.org/10.1021/bi2002289). URL: <https://doi.org/10.1021/bi2002289>.  
568  
569
- 570 [52] Michael Cox and David Nelson. *Lehninger Principles of Biochemistry*. Vol. 5. 2000. DOI: [10.1007/978-3-662-08289-8](https://doi.org/10.1007/978-3-662-08289-8).  
571
- 572 [53] Anthony J. Greenberg, Sarah R. Stockwell, and Andrew G. Clark. "Evolutionary Constraint and Adaptation in the Metabolic Network of *Drosophila*". In: *Molecular Biology and Evolution* 25.12 (2008), pp. 2537–2546. DOI: [10.1093/molbev/msn205](https://doi.org/10.1093/molbev/msn205). URL: <https://doi.org/10.1093/molbev/msn205>.  
573  
574  
575
- 576 [54] Kevin M. Wright and Mark D. Rausher. "The Evolution of Control and Distribution of Adaptive Mutations in a Metabolic Pathway". In: *Genetics* 184.2 (2010), p. 483. DOI: [10.1534/genetics.109.110411](https://doi.org/10.1534/genetics.109.110411). URL: <http://www.genetics.org/content/184/2/483.abstract>.  
577  
578
- 579 [55] Carrie F. Olson-Manning et al. "Evolution of Flux Control in the Glucosinolate Pathway in *Arabidopsis thaliana*". In: *Molecular Biology and Evolution* 30.1 (2012), pp. 14–23. DOI: [10.1093/molbev/mss204](https://doi.org/10.1093/molbev/mss204). URL: <https://doi.org/10.1093/molbev/mss204>.  
580  
581
- 582 [56] Xin Li et al. "A database of thermodynamic properties of the reactions of glycolysis, the tri-carboxylic acid cycle, and the pentose phosphate pathway". In: *Database* 2011 (2011). DOI: [10.1093/database/bar005](https://doi.org/10.1093/database/bar005). URL: <https://doi.org/10.1093/database/bar005>.  
583  
584
- 585 [57] Nuno M. Oliveira, Rene Niehus, and Kevin R. Foster. "Evolutionary limits to cooperation in microbial communities". In: *Proceedings of the National Academy of Sciences* 111.50 (2014), p. 17941. DOI: [10.1073/pnas.1412673111](https://doi.org/10.1073/pnas.1412673111). URL: <http://www.pnas.org/content/111/50/17941.abstract>.  
586  
587  
588
- 589 [58] Jeroen Meijer, Bram van Dijk, and Paulien Hogeweg. "Contingent evolution of alternative metabolic network topologies determines whether cross-feeding evolves". In: *Communications Biology* 3.1 (2020), p. 401. DOI: [10.1038/s42003-020-1107-x](https://doi.org/10.1038/s42003-020-1107-x). URL: <https://doi.org/10.1038/s42003-020-1107-x>.  
590  
591  
592
- 593 [59] Brice Enjalbert et al. "Acetate fluxes in *Escherichia coli* are determined by the thermodynamic control of the Pta-AckA pathway". In: *Scientific Reports* 7.1 (2017), p. 42135. DOI: [10.1038/srep42135](https://doi.org/10.1038/srep42135). URL: <https://doi.org/10.1038/srep42135>.  
594  
595
- 596 [60] Alan R. Pacheco, Mauricio Moel, and Daniel Segrè. "Costless metabolic secretions as drivers of interspecies interactions in microbial ecosystems". In: *Nature Communications* 10.1 (2019), p. 103. DOI: [10.1038/s41467-018-07946-9](https://doi.org/10.1038/s41467-018-07946-9). URL: <https://doi.org/10.1038/s41467-018-07946-9>.  
597  
598
- 599 [61] Joana Sá-Pessoa et al. "SATP (YaaH), a succinate–acetate transporter protein in *Escherichia coli*". In: *Biochemical Journal* 454.3 (2013), pp. 585–595. DOI: [10.1042/BJ20130412](https://doi.org/10.1042/BJ20130412). URL: <https://doi.org/10.1042/BJ20130412>.  
600  
601
- 602 [62] G N Vemuri et al. "Overflow metabolism in *Escherichia coli* during steady-state growth: transcriptional regulation and effect of the redox ratio." eng. In: *Appl Environ Microbiol* 72.5 (2006), pp. 3653–3661. ISSN: 0099-2240 (Print); 1098-5336 (Electronic); 0099-2240 (Linking). DOI: [10.1128/AEM.72.5.3653-3661.2006](https://doi.org/10.1128/AEM.72.5.3653-3661.2006).  
603  
604  
605

- 606 [63] Olga Ponomarova et al. "Yeast Creates a Niche for Symbiotic Lactic Acid Bacteria through  
607 Nitrogen Overflow". In: *Cell Systems* 5.4 (2017), 345–357.e6. DOI: [https://doi.org/10.1016/j.cels.  
608 2017.09.002](https://doi.org/10.1016/j.cels.2017.09.002). URL: <https://www.sciencedirect.com/science/article/pii/S2405471217303903>.
- 609 [64] Karl Peebo et al. "Proteome reallocation in Escherichia coli with increasing specific growth  
610 rate." eng. In: *Mol Biosyst* 11.4 (2015), pp. 1184–1193. ISSN: 1742-2051 (Electronic); 1742-2051  
611 (Linking). DOI: [10.1039/c4mb00721b](https://doi.org/10.1039/c4mb00721b).
- 612 [65] Markus Basan et al. "Overflow metabolism in Escherichia coli results from efficient proteome  
613 allocation". In: *Nature* 528.7580 (2015), pp. 99–104. DOI: [10.1038/nature15765](https://doi.org/10.1038/nature15765). URL: <https://pubmed.ncbi.nlm.nih.gov/26632588>.  
614
- 615 [66] Harry Kemple et al. "Flux, toxicity, and expression costs generate complex genetic interac-  
616 tions in a metabolic pathway". In: *Science Advances* 6.23 (), eabb2236. DOI: [10.1126/sciadv.  
617 abb2236](https://doi.org/10.1126/sciadv.abb2236). URL: <https://doi.org/10.1126/sciadv.abb2236>.
- 618 [67] Kai Zhuang, Goutham N Vemuri, and Radhakrishnan Mahadevan. "Economics of membrane  
619 occupancy and respiro-fermentation". In: *Molecular Systems Biology* 7.1 (2011), p. 500. DOI:  
620 <https://doi.org/10.1038/msb.2011.34>. URL: <https://doi.org/10.1038/msb.2011.34>.
- 621 [68] Mariola Szenk, Ken A. Dill, and Adam M. R. de Graff. "Why Do Fast-Growing Bacteria Enter  
622 Overflow Metabolism? Testing the Membrane Real Estate Hypothesis". In: *Cell Systems* 5.2  
623 (2017), pp. 95–104. DOI: <https://doi.org/10.1016/j.cels.2017.06.005>. URL: [https://www.  
624 sciencedirect.com/science/article/pii/S2405471217302338](https://www.sciencedirect.com/science/article/pii/S2405471217302338).
- 625 [69] Pierre Millard et al. "Control and regulation of acetate overflow in Escherichia coli". In: *eLife*  
626 10 (2021), e63661. DOI: [10.7554/eLife.63661](https://doi.org/10.7554/eLife.63661). URL: <https://doi.org/10.7554/eLife.63661>.
- 627 [70] Joy Bergelson et al. "Functional biology in its natural context: A search for emergent simplic-  
628 ity". In: *eLife* 10 (2021), e67646. DOI: [10.7554/eLife.67646](https://doi.org/10.7554/eLife.67646). URL: [https://doi.org/10.7554/eLife.  
629 67646](https://doi.org/10.7554/eLife.67646).
- 630 [71] Joshua E. Goldford et al. "Emergent simplicity in microbial community assembly". In: *Science*  
631 361.6401 (2018), p. 469. DOI: [10.1126/science.aat1168](https://doi.org/10.1126/science.aat1168). URL: [http://science.sciencemag.org/  
632 content/361/6401/469.abstract](http://science.sciencemag.org/content/361/6401/469.abstract).
- 633 [72] Djordje Bajic and Alvaro Sanchez. "The ecology and evolution of microbial metabolic strate-  
634 gies". In: *Current Opinion in Biotechnology* 62 (2020), pp. 123–128. DOI: [https://doi.org/10.1016/  
635 j.copbio.2019.09.003](https://doi.org/10.1016/j.copbio.2019.09.003). URL: <https://www.sciencedirect.com/science/article/pii/S0958166919300722>.
- 636 [73] Sylvie Estrela et al. "Metabolic rules of microbial community assembly". In: *bioRxiv* (2020),  
637 p. 2020.03.09.984278. DOI: [10.1101/2020.03.09.984278](https://doi.org/10.1101/2020.03.09.984278). URL: [http://biorxiv.org/content/early/  
638 2020/03/11/2020.03.09.984278.abstract](http://biorxiv.org/content/early/2020/03/11/2020.03.09.984278.abstract).
- 639 [74] David Heckmann, Daniel C. Zielinski, and Bernhard O. Palsson. "Modeling genome-wide en-  
640 zyme evolution predicts strong epistasis underlying catalytic turnover rates". In: *Nature Com-  
641 munications* 9.1 (2018), p. 5270. DOI: [10.1038/s41467-018-07649-1](https://doi.org/10.1038/s41467-018-07649-1). URL: [https://doi.org/10.  
642 1038/s41467-018-07649-1](https://doi.org/10.1038/s41467-018-07649-1).
- 643 [75] Sergey Kryazhimskiy. "Emergence and propagation of epistasis in metabolic networks". In:  
644 *eLife* 10 (2021), e60200. DOI: [10.7554/eLife.60200](https://doi.org/10.7554/eLife.60200). URL: <https://doi.org/10.7554/eLife.60200>.
- 645 [76] J. B. S. Haldane. *Enzymes*. London, New York: Longmans, Green, 1930, vii, 235 p. URL: [//catalog.  
646 hathitrust.org/Record/001580201](https://catalog.hathitrust.org/Record/001580201).
- 647 [77] Berend Hasselman. *Solve Systems of Nonlinear Equations - package 'nleqslv'*. Tech. rep. Version  
648 3.3.2. 2018.
- 649 [78] Alessio Peracchi. "The Limits of Enzyme Specificity and the Evolution of Metabolism". In:  
650 *Trends in Biochemical Sciences* 43.12 (2018), pp. 984–996. DOI: [https://doi.org/10.1016/j.  
651 tibs.2018.09.015](https://doi.org/10.1016/j.tibs.2018.09.015). URL: <https://www.sciencedirect.com/science/article/pii/S0968800418302007>.

- 652 [79] Thomas D. Niehaus and Katie B. Hillmann. "Enzyme promiscuity, metabolite damage, and  
653 metabolite damage control systems of the tricarboxylic acid cycle". In: *The FEBS Journal* 287.7  
654 (2020), pp. 1343–1358. DOI: <https://doi.org/10.1111/febs.15284>. URL: <https://doi.org/10.1111/febs.15284>.  
655
- 656 [80] R Heinrich, S Schuster, and H G Holzhütter. "Mathematical analysis of enzymic reaction systems using optimization principles." eng. In: *Eur J Biochem* 201.1 (1991), pp. 1–21. ISSN: 0014-2956 (Print); 0014-2956 (Linking). DOI: [10.1111/j.1432-1033.1991.tb16251.x](https://doi.org/10.1111/j.1432-1033.1991.tb16251.x).  
657  
658
- 659 [81] Ulf Dieckmann and Richard Law. "The dynamical theory of coevolution: a derivation from stochastic ecological processes". In: *Journal of Mathematical Biology* 34.5 (1996), pp. 579–612. DOI: [10.1007/BF02409751](https://doi.org/10.1007/BF02409751). URL: <https://doi.org/10.1007/BF02409751>.  
660  
661
- 662 [82] Ulf Dieckmann and Michael Doebeli. "On the origin of species by sympatric speciation". In: *Nature* 400.6742 (1999), pp. 354–357. DOI: [10.1038/22521](https://doi.org/10.1038/22521). URL: <https://doi.org/10.1038/22521>.  
663