

1 Programmed cell death can increase the efficacy of microbial
2 bet-hedging

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4 **Abstract**

5 Programmed cell death (PCD) occurs in both unicellular and multicellular organisms.
6 While PCD plays a key role in the development and maintenance of multicellular organ-
7 isms, explaining why single-celled organisms would evolve to actively commit suicide has
8 been far more challenging. Here, we explore the potential for PCD to act as an accessory
9 to microbial bet-hedging strategies that utilize stochastic phenotype switching. We con-
10 sider organisms that face unpredictable and recurring disasters, in which fitness depends
11 on effective phenotypic diversification. We show that when reproductive opportunities are
12 limited by carrying capacity, PCD drives population turnover, providing increased oppor-
13 tunities for phenotypic diversification through stochastic phenotype switching. The main
14 cost of PCD, providing resources for growth to a PCD(-) competitor, is ameliorated by

15 genetic assortment driven by population spatial structure. Using three dimensional agent
16 based simulations, we explore how basic demographic factors, namely cell death and clonal
17 reproduction, can create populations with sufficient spatial structure to favor the evolution
18 of high PCD rates.

19 **Introduction**

20 Programmed cell death (PCD) describes a genetically encoded process of cellular suicide
21 that is often used as an umbrella term for more specific cell-death phenotypes (e.g., apop-
22 tosis, paraptosis, autophagy, chromatolysis, etc.) [1, 2, 3, 4, 5]. Anatomists first observed
23 PCD in the context of animal development during the 19th century [4]. Since then, a vast
24 body of literature has established the key role of PCD in both the generation [6, 7] and
25 maintenance of multicellular forms [1, 8]. Interestingly, PCD is also widespread among
26 distantly related unicellular organisms [9, 10, 11, 12, 13, 14, 15, 16]. The origin and main-
27 tenance of PCD within multicellular taxa has a straightforward evolutionary explanation
28 if the death of some cells provides a benefit to the organism as a whole. In contrast, the
29 evolution of PCD in unicellular organisms presents a conundrum: under what conditions
30 (and by what mechanisms) will natural selection favor organismal suicide?

31 Different mechanisms have been proposed to explain the existence of PCD among unicel-
32 lular taxa [15]. One category of hypotheses proposes that PCD is an altruistic trait favored
33 by kin or multilevel selection. These hypotheses propose that PCD may have evolved to
34 remove cells weakened by deleterious mutations, pathogens, or age-accumulated damage
35 [17, 18, 19, 20, 21, 22]. Removing such cells improves the health of other members of the
36 population either by preventing the spread of pathogens or making more resources available
37 to healthier cells. Another category of hypotheses considers PCD to be a pleiotropic side-
38 effect of genes under positive selection because of their pro-survival effects [15]. This would

39 imply that there is no direct adaptive benefit to PCD and its negative effects are simply
40 a tolerable side-effect of a beneficial pleiotropic trait. Finally, PCD may have evolved in
41 microbes because of its role in multicellular development. For example, PCD by a sub-
42 set of a bacterial population may be necessary to provide extracellular DNA that plays a
43 structural role in biofilm formation [23]. Unfortunately, few of these potential evolution-
44 ary explanations have been experimentally tested or mathematically modeled, and little is
45 known about the ecological conditions necessary for their evolution.

46 In this paper, we propose a novel evolutionary hypothesis for the origin and maintenance
47 of PCD in unicellular organisms: PCD serves as an accessory to microbial bet hedging. Bet-
48 hedging traits increase fitness in unpredictable environments in two possible ways. First,
49 they can spread risk among multiple phenotypes, each of which is well-suited to a possible
50 future environment (diversification bet-hedging) [24, 25]. Second, they can allow organisms
51 to pursue a generalist strategy that performs acceptably across a range of possible future
52 environments (conservative trait bet-hedging) [26]. Of the two types of bet-hedging, most
53 of the well-established traits act as diversification bet-hedging, but this may be because it
54 is more conspicuous than conservative trait bet-hedging [27]. Micro-organisms typically en-
55 act diversification bet hedging strategies through stochastic phenotype switching, in which
56 reproducing cells give rise to phenotypically distinct offspring with a low (typically 10^{-1}
57 to 10^{-5}) probability [28, 29]. Since the offspring can switch back to the original phenotype
58 at some low probability, stochastic phenotype switching typically generates bistable popu-
59 lations in which a single genotype exhibits two distinct phenotypes [30, 31]. Importantly,
60 stochastic phenotype switching requires generational turnover to create variation. Even at
61 relatively high rates of switching (10^{-3}), it still takes more than 1,000 generations for an
62 initially uniform population to reach maximum levels of phenotypic diversity [28].

63 Here we examine the conditions under which PCD increases the efficacy of microbial

64 bet-hedging by creating generational turnover, resulting in increased phenotypic diversity.
65 We analytically examine the co-evolution of PCD and stochastic phenotype switching in
66 an unpredictable environment in which more diversified populations have higher long-term
67 fitness. Although population size in our model is constrained by a carrying capacity,
68 PCD allows organisms to circumvent this limitation to reproduction. As organisms die,
69 they create opportunities for other organisms to reproduce and diversify via stochastic
70 phenotype switching. Thus PCD incurs both costs and benefits: some cells die, but if
71 surviving clonemates can use spared resources to divide, then the genotype as a whole will
72 become more diversified.

73 One possible downside of this strategy is that the resources made available by PCD
74 are susceptible to exploitation by low-PCD competitors. We show that across many cycles
75 of unpredictable environmental risk, exploitation by low-PCD competitors does not neces-
76 sarily overwhelm the long-term fitness advantage gained by the more diversified high-PCD
77 strain. Further, the cost of PCD is highly dependent on the degree of population struc-
78 ture and is reduced when individuals that die are more often replaced by nearby, related
79 clonemates. More importantly, we find that the conditions required for selection to favor
80 elevated PCD in our model are very permissive: elevated PCD can evolve in microbes with
81 a wide range of stochastic switching frequencies, in environments with a wide range of
82 disaster frequencies, and in populations with modest spatial structuring. We contextualize
83 these results using a spatially explicit biofilm simulation. We find that, even if the simu-
84 lation is initiated with a well-mixed population, the dynamics of occasional environmental
85 catastrophe and range expansion creates high levels of spatial structure, which rapidly fa-
86 vors the evolution of high rates of PCD. These results point to new adaptive explanations
87 for the evolution and maintenance of PCD in unicellular populations by focusing atten-
88 tion on the profoundly non-equilibrium nature of many microbial populations (particularly

89 those exploiting patchily-distributed, ephemeral resources).

90 **Results**

91 **Model**

92 We consider a competition between two microbial strains ($G1$ and $G2$) in an environment
93 that experiences frequent disasters (previously described in [28]). Each strain exhibits two
94 possible phenotypes: A and B . We denote the A and B phenotypes of the $G1$ genotype as
95 $A1$ and $B1$ and similarly $A2$ and $B2$ correspond to the phenotypes of $G2$. The only mean-
96 ingful difference between A and B phenotypes is their susceptibility to an environmental
97 disaster. Disasters occur randomly and target a single phenotype for annihilation— in this
98 way they are similar to other disasters that microbes might face such as antibiotic expo-
99 sure or immune system recognition. Following a disaster, the surviving types reproduce
100 until the total population is restored to some fixed amount, N . Phenotypic diversification
101 occurs via stochastic phenotype switching such that each time a strain reproduces there is
102 a probability (p_1 for $G1$ and p_2 for $G2$) that the alternate phenotype will be produced. In
103 between disaster events, there are rounds of PCD in which each strain loses cells according
104 to their own characteristic PCD rate, c_1 and c_2 . The population is restored to N in a
105 way that incorporates environmental structure or relatedness, characterized by a structure
106 parameter $r \in [0, 1]$ (shown in Figure 1). The r parameter determines the fraction of cells
107 lost to PCD that will be replaced by the same genotype. If $r = 0$, then no cells that died
108 via PCD will be replaced by the same genotype; if $r = 1$ then all cells will be replaced by
109 the same genotype. The equations in Eqn. 1 describe the dynamics of a single round of
110 PCD and repopulation. Discrete rounds of PCD and disasters continue until one or both
111 genotypes go extinct. A genotype is said to “win” if the opposing strain goes extinct first or

112 if at the end of a thousand rounds it makes up a higher percentage of the total population.
 113 Simulations were coded in the language python and are available in the Supplementary
 114 material.

$$\begin{aligned}
 A1_{t+1} &= A1_t - c_1 A1_t + c_1 r(1 - p_1) A1_t + p_1 r c_1 B1_t \\
 &\quad + c_2 (A2_t + B2_t)(1 - r) \left(\frac{A1_t}{(A1_t + B1_t)}(1 - p_1) + \frac{B1_t}{(A1_t + B1_t)} p_1 \right) \\
 B1_{t+1} &= B1_t - c_1 B1_t + c_1 r(1 - p_1) B1_t + p_1 r c_1 A1_t \\
 &\quad + c_2 (A2_t + B2_t)((1 - r)) \left(\frac{B1_t}{(A1_t + B1_t)}(1 - p_1) + \frac{A1_t}{(A1_t + B1_t)} p_1 \right) \\
 A2_{t+1} &= A2_t - c_2 A2_t + c_2 r(1 - p_2) A2_t + p_2 r c_2 B2_t \\
 &\quad + c_1 (A1_t + B1_t)((1 - r)) \left(\frac{A2_t}{(A2_t + B2_t)}(1 - p_2) + \frac{B2_t}{(A2_t + B2_t)} p_2 \right) \\
 B2_{t+1} &= B2_t - c_2 B2_t + c_2 r(1 - p_2) B2_t + p_2 r c_2 A2_t \\
 &\quad + c_1 (A1_t + B1_t)((1 - r)) \left(\frac{B2_t}{(A2_t + B2_t)}(1 - p_2) + \frac{A2_t}{(A2_t + B2_t)} p_2 \right)
 \end{aligned} \tag{1}$$

115 Cost of PCD

116 There is a direct cost to PCD because cells are killed. If all cells were replaced by the same
 117 strain then this would remove the cost. However, if replacement does not occur with perfect
 118 assortment (i.e. $r < 1$) then some of the cells that die will be replaced by members of the
 119 competing strain. We call the total number of a strain that are replaced by a competing
 120 strain the cost of PCD. Since our model has disasters interspersed with rounds of growth
 121 and PCD, we can calculate the expected cost of PCD during the period between disasters.
 122 If one strain uses PCD and the other does not then after t rounds of PCD/regrowth the

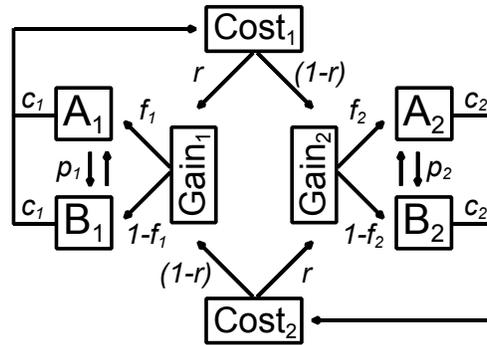


Figure 1: **Model schematic for the dynamics of the competition** Genotypes $G1$ and $G2$ switch between phenotypes A and B with a probabilities p_1 and p_2 , respectively. Organisms undergo PCD with probabilities c_1 and c_2 such that $c_1(A_1 + B_1)$ is the expected “cost” of PCD experienced by genotype $G1$. The population then regrows back to the carrying capacity N such that each genotype repopulates a fraction of its own cells that underwent PCD determined by the value of r . The repopulation is partitioned among phenotypes according to their relative frequency such that $f_1 = \frac{A_1}{A_1+B_1}$ and $f_2 = \frac{A_2}{A_2+B_2}$. The “gain” corresponds to the number of cell reproductive events reallocated to each genotype.

123 PCD strain will decrease by a factor shown in Eqn. 2.

$$(c(r - 1) + 1)^t \quad (2)$$

124 This factor has three terms which determine its magnitude: c , t , and r . As expected, the
125 cost of PCD is high for large values of c , i.e. the PCD rate. Similarly, if disasters are
126 infrequent, i.e. t is large, then the cost is high because it effectively increases the total
127 amount of PCD that occurs in between disasters. Since the r parameter determines how
128 many cells that undergo PCD are replaced by the same genotype, higher values of r reduce
129 the cost of PCD. When $r = 1$, Eqn. 2 is equal to 1 which means that there is no cost to
130 PCD. A parameter that is missing from the cost factor is the switching rate, p . This is
131 because the cost is absorbed by the strain as a whole without regard to how it is partitioned
132 into A and B types.

133 **Benefit of PCD**

134 While the cost of PCD is shared by all members of a genotype without regard to phenotype,
135 the benefit of PCD is determined by diversification among phenotypes. In our model, PCD
136 allows a strain more chances to diversify via stochastic phenotype switching by increasing
137 the number of reproductive events between disasters. Such diversification could manifest in
138 a more equal mix of the population among phenotypes, which can increase long-term fitness
139 by reducing variance in fitness across disasters. Alternatively, if there are few opportunities
140 for diversification between disasters (for example, strains with a very low rate of stochastic
141 switching), higher diversification rates can reduce a strain's possibility of extinction. We
142 will focus on this second manifestation because it is more crucial to the survival of the
143 PCD strain— failure to diversify can result in extinction.

144 We assume that one phenotype, say B , has just experienced a disaster and is no longer

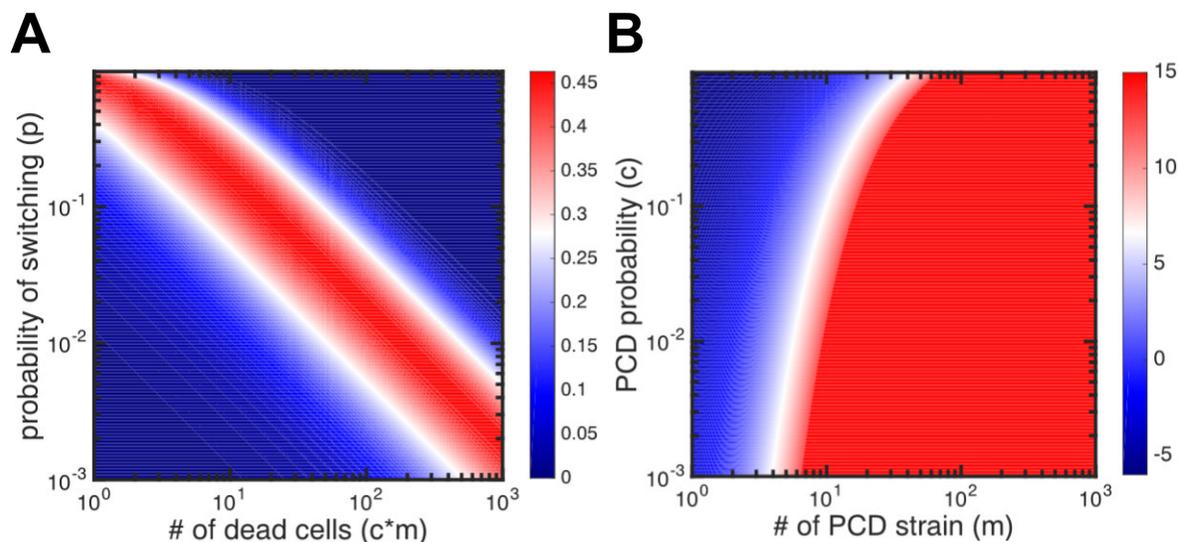


Figure 2: **Cost and benefit of PCD.** A) A contour plot of the benefit of PCD from Eq. 3 for a range of switching probabilities (p) and numbers of PCD cells ($c*m$) when $r = .75$. There is a narrow band where PCD is beneficial corresponding to $cmp = \text{const}$. Outside of this area, the switching rate is too high or too low to incur a benefit to PCD genotypes. B) A contour plot shows the \log_{10} relative benefit versus cost of PCD as expressed in Eq. 5 for a range of PCD probabilities (c) and number of cells (m) with $r = .5$ and $p = 10^{-6}$. The blue area corresponds to a greater cost while red areas correspond to a greater benefit. Since the plot is transformed by \log_{10} the benefit is many orders of magnitude (> 10) greater when the number of cells is larger than 10.

145 present in the population. Each strain only exists as the A phenotype with m members of
146 the PCD strain (A_1) and $N - m$ of the non-PCD strain (A_2). Prior to the next disaster,
147 each strain must produce B phenotypes or face extinction should the next disaster target A
148 phenotypes. Although there may be several rounds of PCD/regrowth in between disasters,
149 we investigate the consequences of just one round of PCD/regrowth. We calculate the
150 expected number of B types produced by each genotype assuming that the switching
151 probability is the same, i.e. $p_1 = p_2 = p$. The number of B types produced by the PCD
152 genotype ($B1$) is prc_1m and the non-PCD genotype ($B2$) is $p(1 - r)c_1m$. The difference
153 between $B1$ and $B2$ is determined solely by the r parameter. We note that the $B2$ types
154 produced by the non-PCD genotype only happen as a result of PCD undergone by $G1$
155 where replacement was not perfectly assortative ($r < 1$).

156 One advantageous situation for the PCD strain would be if it produced a B phenotype
157 but the non-PCD strain did not. The probability of this event is shown in Eqn. 3.

$$(1 - p)^{c_1m(1-r)} - (1 - p)^{c_1m} \quad (3)$$

158 The probability increases with the structure parameter r , reaching a maximum at $r = 1$.
159 It is highest at $p = 1 - (1 - r)^{c_1mr}$ which for $r \ll 1$ is approximately one expected
160 B phenotype produced by the PCD strain (see Figure 2A). At slower switching rates and
161 lower rates of PCD, the PCD strain is not likely to diversify. At higher switching rates and
162 higher rates of PCD the non-PCD strain is likely to diversify along with the PCD strain,
163 thereby reducing the relative advantage of PCD.

164 The probabilistic formulation of the benefit of PCD can also be compared with a prob-
165 abilistic formulation of the cost. We put the cost into a similar currency by considering
166 the probability that the PCD strain goes extinct because of PCD (shown in Eqn. 4). This

167 requires all m cells to undergo PCD and be replaced by the competing strain.

$$c_1^m(1-r)^m \quad (4)$$

168 In this formulation, the PCD strain faces the cost of going extinct from PCD but has
169 the benefit of diversifying when the competition does not. The relative probability of the
170 benefit to the cost is shown in Eqn. 5.

$$\frac{(1-p)^{c_1 m}((1-p)^{-r} - 1)}{(c_1(1-r))^m} \quad (5)$$

171 This relationship is low when p is very high ($p = 1$) or when p is very low $p = 10^{-6}$ and r
172 is small ($r = .5$). If we assume a low p , say $p = 10^{-6}$, and an unbiased r , i.e. $r = .5$, then
173 we can compare the ratio of probabilities for a range of values of c and m (see Figure 2B).
174 Effectively, if m is larger than 10 then *the benefit is more likely than the cost by over 10*
175 *orders of magnitude.*

176 **Interplay between cost and benefit**

177 While the benefit of PCD can outweigh the cost under some circumstances (Figure 2B), it is
178 not clear how often those circumstances arise. In a competition, it could be that it is rare for
179 both organisms to only exist in one phenotype after a cycle of a disaster and restoration to
180 carrying capacity. To evaluate how a genotype with PCD fares in competition, we compete
181 a PCD strain versus a non-PCD strain over a range of switching probabilities (p) and PCD
182 rates (c). The results shown in Figure 3A reveal that the PCD strain outcompetes the
183 non-PCD strain for intermediate switching probabilities when PCD is not too frequent,
184 i.e. $c < .1$. For PCD rates above .1, the cost is too high to compensate for any benefit.
185 For high switching probabilities, the benefit of PCD is diminished because it is likely that

186 the non-PCD strain will always diversify. Similarly, if the switching probability is too low
187 then the PCD strain cannot adequately diversify. For more structured environments, with
188 say $r = .9$ shown in Figure 3B, the PCD strain wins over a much larger area of parameter
189 space. This is because the higher value of r reduces the cost to PCD. If we fix the rate
190 of switching to $p = .1$ then we see that as the structure parameter increases, larger values
191 of c can be tolerated, i.e. higher PCD genotypes are successful (Figures 3C and 3D). In
192 addition, with increasing frequency of disasters, genotypes with higher values of PCD can
193 outcompete non-PCD genotypes—the benefit of diversification via PCD outweighs its cost.

194 **Cost when both strains have PCD**

195 Until now we have considered the case in which there is only one genotype that undergoes
196 PCD. Here, we investigate what happens when both strains exhibit PCD but at different
197 rates, i.e. $c_1 > c_2 > 0$. Similar to the derivation of Eqn. 2, we assume that both populations
198 undergo t rounds of PCD and regrowth. Genotype $G1$ starts with a population of $G1_0$ and
199 genotype $G2$ begins with $N - G1_0$. We compute the number of $G1$ genotypes at time t ,
200 $G1_t$, and find that this depends only on the PCD rates, the structure parameter r , and the
201 initial amounts of each genotype (see Eqn. 6).

$$G1_t = G1_0 \left(\frac{(c_1 + c_2 - \frac{c_2 N}{G1_0})((c_1 + c_2)(r - 1)/2 + 1)^t + \frac{c_2 N}{G1_0}}{c_1 + c_2} \right) \quad (6)$$

202 One interesting consequence of Eqn. 2 is that the higher PCD genotype, $G1$, can actually
203 increase in abundance over time. This occurs because the total lost by each genotype to
204 PCD depends on their abundance. With a fixed population size, as one genotype becomes
205 less abundant it pays less of a cost for PCD. In Eq. 6, PCD is a cost for $G1$ if the term
206 in parentheses is less than 1 which occurs if $G1_0 > \frac{c_2}{c_1 + c_2} N$. If the opposite is true then
207 there is actually a net flux to $G1$ and it can increase in number. Without the disruption

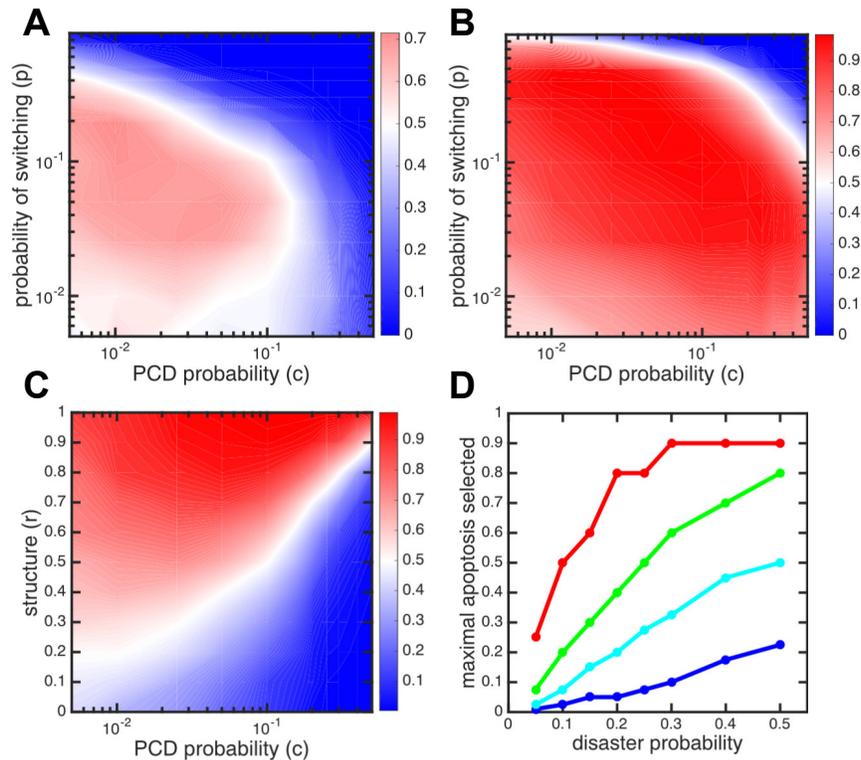


Figure 3: Competitions between PCD versus non-PCD genotypes. A) Contour plot shows the percentage of wins for the PCD strain versus the total number of competitions that ended in one strain winning, over a range of switching probabilities (p) and PCD probabilities (c) with $r = .5$ and disaster probability of $.1$. There is an intermediate range of switching probabilities and PCD probabilities where the PCD strain is more successful. The blue area corresponds to where PCD loses to non-PCD cells and the red area is where PCD wins. B) Same as A) but with $r = .9$. PCD is more successful (higher number of wins) over a larger area of parameter space. C) The success of PCD strains versus a non-PCD competitor are shown as a function of PCD rate c and the assortment parameter r . The disaster probability is $.1$ and the switch rate for both genotypes is $.1$. With increasing r PCD is more beneficial. D) The maximal amount of PCD selected is shown as a function of disaster probability for different values of r : $.3$ (blue), $.5$ (cyan), $.7$ (green), and $.9$ (red). Higher values of PCD are permitted for higher frequencies of disaster where there is greater benefit for diversification and in more structured environments where there is a lower cost to PCD.

208 of a disaster, the genotypes approach the equilibrium shown in Eqn. 7 (assuming $c_1 < 1$
209 and $r > -1$).

$$G1 = \frac{c_2}{c_1 + c_2} N \quad (7)$$

210 We note that the equilibrium does not depend on the value of the structure parameter r
211 as long as $r > 0$. Thus, as long as there is some chance that the higher PCD genotype
212 replaces some of the population it lost to cell death, then this equilibrium will be reached.
213 We note that in this calculation (in the absence of disasters) if $G2$ does not engage in PCD,
214 i.e. $c_2 = 0$, then $G1$ will eventually go extinct. If, however, both genotypes engage in PCD,
215 then they can coexist— at least until a disaster comes.

216 The above calculations show the possibility of coexistence in the absence of disasters
217 and stochasticity. We might expect that the combination of disasters and stochastic events
218 (phenotype switching) may interfere with long-term coexistence. Figure 4 shows an exam-
219 ple of coexistence between two PCD strains ($c_1 = .3$ and $c_2 = .1$) in a stochastic simulation
220 for 10^5 rounds. Disasters occur with a frequency of $.1$ which means on average we expect
221 10^4 disasters. Both genotypes switch with the same probability $p = .1$. Despite the fre-
222 quency of disasters and the different PCD rates, neither genotype goes extinct. Since the
223 simulations are stochastic, at some point one genotype will go extinct but the waiting time
224 until this event occurs can be long and require more than tens of thousands of disasters.

225 **Generation of spatial structure**

226 Our analyses thus far have relied on a notion of spatial structure r that determines how
227 much of the resources made available by PCD actually return to the genotype liberating
228 them through programmed cell death. Although our parameter r is a mathematically
229 convenient macroscopic description of population spatial structure, it is a complex variable
230 that is both an outcome and driver of individual-level (e.g., birth/death, motility) and

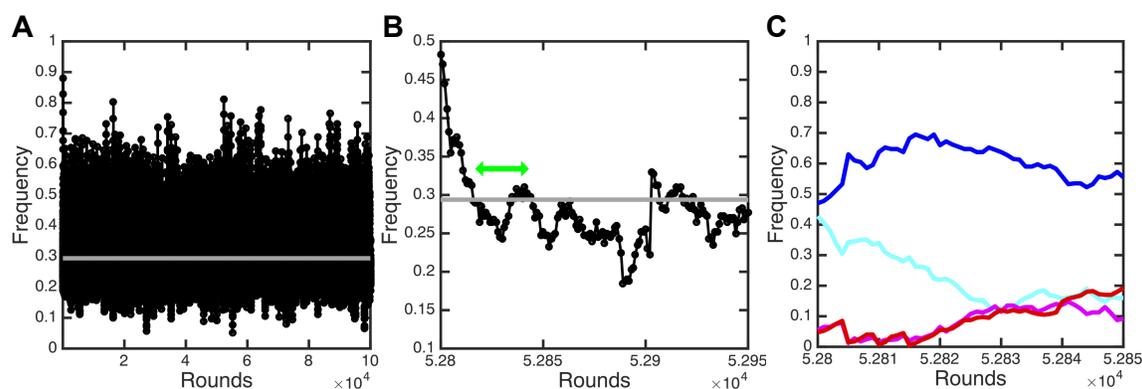


Figure 4: **Coexistence of PCD strains.** A) Two apoptotic strains with the same switching rate but different PCD rates ($c_1 = .3$, $c_2 = .1$) compete for 10^5 rounds without either going extinct. The switch probability is the same for both $p = .1$ and the disaster probability is $.1$. The black lines show the frequency of $G1$ as it fluctuates over time, rarely getting close to extinction. The gray line shows its average frequency over the simulation. B) A highlighted segment of the competition shows that as $G1$ gets low in frequency it is bolstered up by $G2$. An indication of this event can be seen in the area denoted by the green arrow. C) The dynamics of the genotypes separated into their A and B phenotypes are shown for the area denoted by the green arrow from B). The $G1$ phenotypes A (magenta) and B (cyan) and $G2$ phenotypes A (red) and B (blue) change in frequency as a result of PCD events. $G1$ and $G2$ experience the same increase in A phenotypes (red and magenta) due to differences in PCD rates and flux between genotypes. Even though the number of B for $G2$ (blue) is greater than the B for $G1$ (cyan), the A types recover by a similar amount— this corresponds to the populations approaching the equilibrium of coexistence.

231 population-level (e.g., selection, disturbance) processes. To examine whether sufficiently
232 high values of r that favor the evolution of PCD can emerge from demographic processes
233 of cell death and local reproduction, we construct a 3D agent-based model (ABM).

234 The agent-based model consists of cells distributed across patches that represent micro-
235 environments within 3D space (see Figure 5a). Each patch can hold 10 cells at which point
236 it reaches carrying capacity. Cells are mobile agents capable of reproduction and death. As
237 in our mathematical model, there are four types of cells corresponding to two genotypes,
238 each of which produce two phenotypes that are defined by their resistance/susceptibility to
239 disasters. The main difference between genotypes is that one undergoes PCD (the PCD+
240 strain), while one does not (the PCD- strain).

241 The ABM progresses via discrete time steps, during which cells and patches update the
242 state variables of itself and other agents in random order (see Supplementary material for
243 computer code). Every time step there is a fixed probability that a disaster will occur and
244 eliminate the susceptible phenotype. The choice of which phenotype is affected (what we
245 referred to as A and B in the analytical model described in previous sections) is determined
246 randomly and unbiasedly. Following the disaster, if there is space available in the patch,
247 cells reproduce and give rise to an alternative phenotype with a constant probability. After
248 reproduction, cells can migrate to new patches according to a fixed probability which is the
249 same for all cells. At the end of a time step, cells of the PCD genotype undergo cell death
250 probabilistically. Simulations end when a genotype goes extinct. We calculate r as the
251 average probability a PCD+ cell that dies in a patch will be replaced by another PCD+
252 cell.

253 Disaster influences assortment by determining the relative benefit of phenotypic di-
254 versification through PCD. This follows from the analytical work and can be seen in the
255 ABM simulations (Figures 5b and 5c). Overall, the relative performance of the PCD strain

256 increases with the frequency of disasters. Not only do disasters select for rapid phenotypic
257 diversification, but they also reduce the population to a relatively sparse state. Since the
258 effects of birth on assortment/structure outweigh those of migration at low population
259 densities, spatial structure (characterized by r) increases following a disaster. Thus, more
260 frequent disasters increase the relative benefit of phenotypic diversity as well as the mean
261 value of spatial structure (Figure 5d), both of which increase the benefit of PCD. The ben-
262 efits of PCD are reduced in environments with very high disaster probabilities, as carrying
263 capacity is rarely met so PCD offers fewer opportunities for increased reproduction, and
264 more frequent disaster increases the stochasticity of the simulation. In between disasters,
265 the PCD- strain increases in frequency and the value of r decreases. This happens because
266 cells migrate which dissipates community structure, and also because PCD- cells occasion-
267 ally replace dead PCD+ cells. This process would ultimately drive PCD+ strains extinct if
268 it were not for disasters which favor phenotypic diversification (and thus PCD) and create
269 spatial structure.

270 Discussion

271 While recent years have seen a growing awareness that programmed cell death (PCD) is
272 widespread among unicellular organisms [15, 16], few evolutionary hypotheses for its origin
273 have been theoretically or experimentally investigated. Here we propose a novel hypothesis
274 for the evolution of PCD in unicellular organisms: it increases the efficacy of microbial bet-
275 hedging by creating generational turnover that yields increased phenotypic diversity. Using
276 a simple bet-hedging model, we show analytically and computationally that PCD can be
277 adaptive. In our model, PCD is advantageous because it allows microbes to become more
278 phenotypically diversified, which is adaptive in the face of environmental uncertainty. It
279 can be costly, however, if cells that die provide reproductive opportunities for competitors,

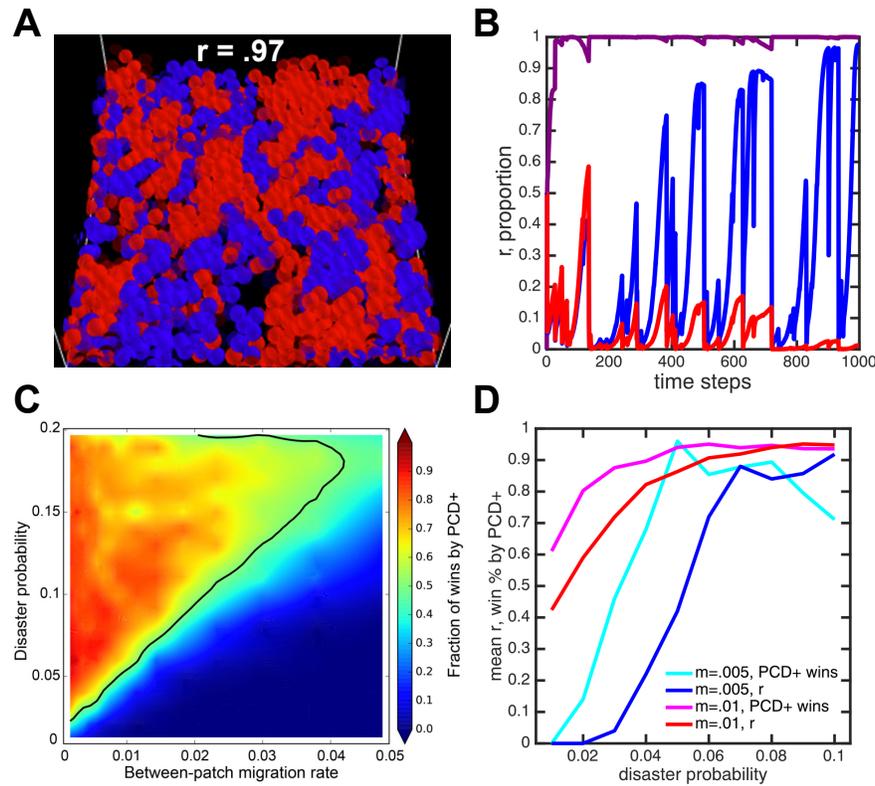


Figure 5: **Generation of structure (r) via population processes.** A) The 3D ABM shows that blue (PCD+) and red (PCD-) cells readily assort to create spatial structure. B) A characteristic time series of an ABM simulation showing the proportion of PCD+ (blue) and PCD- (red) as a function of the global carrying capacity as well as the value of r . Populations decrease following disasters. The ensuing recovery increases the value of r . Migration and PCD lead to a lowering of r and a decrease in the PCD+ strain. C) A contour plot shows the fraction of simulations won by PCD+ as a function of disaster probability and migration. More frequent disasters favor the PCD+ strain, but this is undermined by high rates of between-patch migration. The solid line represents equal competitiveness between PCD+ and - strains. 100 simulations were run for each of the 340 parameter combinations. D) The mean value of r and the fraction of wins by PCD+ are shown for two different migration probabilities¹⁸. In both cases, r and the fraction of wins increases with disasters.

280 not kin. In general, we find that fairly high rates of PCD can be favored by selection
281 assuming low rates of stochastic phenotype switching (making population turnover from
282 PCD more valuable) and high levels of genotypic assortment caused by spatial structure in
283 the population (allowing the genotype undergoing PCD to capture most of the resources
284 liberated by cellular suicide). Using an agent-based model we show that high rates of
285 genotypic assortment for PCD strains ($\approx .9$) can emerge through normal demographic
286 processes of reproduction and the bottlenecking effects of disasters.

287 Due to the seemingly selfless nature of PCD, potential explanations have typically
288 been sought at levels below (e.g. gene- [32] or phage-level; [33]) and above (e.g. colony-
289 , lineage- or population-level; [18, 22, 34]) that of the cell. It is also possible that the
290 appearance of evolved PCD may occasionally arise as a byproduct or “malfunction” of
291 traits that may confer cell-level advantages under certain environmental conditions [15].
292 Extant hypotheses for the adaptive significance of microbial PCD propose that it can
293 provide novel functionality, coordinating “prokaryotic developmental programs” [34, 35],
294 directing resources to mutant lineages that may be more capable of surviving a changing
295 environment [36], or limiting the spread of pathogens to relatives [37]. Our hypothesis,
296 that PCD can make bet-hedging strategies more effective, is both distinct from these
297 alternatives, and may be fairly general, as stochastic phenotypic diversification is used to
298 hedge against many different forms of risk [28, 38, 39, 40, 41, 42].

299 Why would organisms ever use PCD as a mechanism to diversify, when they could
300 simply avoid the costs of cell death and evolve faster switch rates? We see two scenarios
301 where PCD may be a useful tool. First, if evolving higher switch rates is either difficult or
302 costly, PCD may offer a workaround. A number of cellular mechanisms allow for stochastic
303 phenotype switching in microbes, including phase variation [43], contingency loci [44], and
304 positive feedback loops in gene expression [45]. One of the elegant aspects of PCD is that it

305 can increase the efficacy of any of these mechanisms without necessarily incurring complex
306 pleiotropic side-effects. Second, PCD may allow for tuning of switch rates to match different
307 environments that a microbe encounters. For example, if phenotypic diversification is
308 beneficial only in challenging environments, but is otherwise costly, then PCD linked to
309 an environmental cue correlated with the challenging environment may be a simple way to
310 plastically increase diversification rates.

311 Diversification through PCD may be an alternative to another common microbial bet-
312 hedging mechanism: variable dormancy, in which only some cells of a clonal genotype
313 become dormant [46, 47]. Individuals that become dormant, be it through persistence or
314 the formation of resting stages like spores or cysts, gain resistance to a broad spectrum of
315 environmental insults, but pay a considerable opportunity cost if the environment becomes
316 favorable to growth [48]. Our model suggests that another, subtler price of dormancy
317 is the missed opportunity to acquire resistance to future environmental stresses, through
318 increased phenotypic diversification. Intriguingly, there is evidence that PCD delays the
319 onset of dormancy (sporulation) during the transition to stationary phase in some bacteria
320 by supplying resources to starving survivors [48]. Although PCD by a subpopulation
321 may conceivably benefit surviving cells in the event of an abrupt shift back to favorable
322 conditions [49], our model suggests that increased diversification may be another benefit
323 of such population cycling.

324 Understanding the evolution of cellular suicide will require a plurality of approaches,
325 informed by real-world ecology, backed by rigorous mathematical modeling and direct
326 experimental investigation. We expect that there are many non-exclusive explanations for
327 why microbes evolve PCD, but unfortunately few of the numerous hypotheses put forward
328 in the literature have been mathematically investigated. Perhaps the main reason to model
329 various hypotheses for PCD is to determine whether the conditions required for its evolution

330 are permissive, or highly constrained. In this paper, for example, we find that high levels
331 of PCD are only supported when the population is structured, but we also find that simple
332 processes of death and clonal reproduction easily generate the necessary structure. While
333 much work remains before we have a complete understanding of altruistic suicide, it is well
334 worth the effort. Not only is it a topic of fundamental biological importance, it also has
335 the potential to help generate novel therapeutic interventions [23, 50].

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