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Evolutionary explanations for heterogeneous behavior in clonal bacterial populations

Rolf Kümmerli [1,*] and Steven A. Frank [2]

Cellular heterogeneity in clonal bacterial populations is widespread. Division of labor and bet hedging are common adaptive explanations for the function of such heterogeneity. We suggest group-level phenotypes via shareable molecules and variation in cellular vigor as two alternative evolutionary explanations for bacterial cellular heterogeneity.

Background

Clonal bacterial cells often vary in their phenotype. Why do individual cells differ even though they are genetically identical and share the same environmental conditions? This question has attracted a lot of interest among systems, molecular, and evolutionary biologists [1–3]. A certain level of heterogeneity seems inevitable given that cellular processes are intrinsically noisy [4]. But could there be more to it? Could natural selection favor heterogeneity among clonal individuals because it provides fitness advantages? Here, we first introduce the two most popular adaptive explanations – division of labor and bet hedging [2]. Then, we offer two alternative, underappreciated evolutionary explanations – group-level phenotypes and variation in cellular vigor – which might be common causes for cellular heterogeneity.

Division of labor and bet hedging

Studies of phenotypic heterogeneity in bacteria typically explain cellular diversity by division of labor or bet hedging. Table 1 highlights four representative examples. Example A – division of labor: *Salmonella enterica* Typhimurium segregates in two phenotypes when invading the intestinal mucosa of mice [5]. Some cells express the type III secretion system (T3SS) to cause a host inflammatory response that clears the native microbiome. The cells that do not express T3SS gain an enhanced opportunity to establish an infection. Example B – division of labor: *Bacillus subtilis* cells segregate into surfactin- and matrix-producing phenotypes during sliding motility on surfaces [6]. Surfactin-producing cells reduce the friction between cells and the surface, which allows the matrix-producing cells to form bundles and trigger colony expansion. Example C – bet hedging: in the presence of both glucose and cellobiose, *Lactococcus lactis* cells first consume glucose. After glucose exhaustion, the population segregates into cells that start to express cellobiose degrading enzymes and cells that stop growing while continuing to express glucose-related metabolic enzymes [7]. The latter group remains ready to consume glucose should this sugar become available again. In effect, the heterogeneous cellular population has hedged its bets against unpredictable environmental changes. Example D – bet hedging: *Pseudomonas aeruginosa* temporally segregates into two subpopulations when engaging in quorum sensing (QS) communication [8]. One fraction of cells immediately commits to the expression of quorum-sensing regulated traits, while the other fraction delays quorum-sensing commitment until a higher cell density is reached. Heterogeneity at intermediate cell density could allow populations to quickly revert to a QS-off state should environmental fluctuations lead to an unforeseen drop in population density.

Are there alternative evolutionary explanations for cellular heterogeneity?

Division of labor and bet hedging are intuitive explanations for the scenarios described above, but are they the only ones? Considerable levels of heterogeneity in cellular behavior are observed in many studies. Intuitive adaptive explanations are often lacking. For example, *P. aeruginosa* produces two iron-scavenging siderophores (pyoverdine and pyochelin) under iron limitation. A single-cell study revealed high cellular heterogeneity (Figure 1A) in the expression of genes involved in siderophore synthesis [9]. Division of labor does not seem to explain the observed patterns because heterogeneity also occurred under iron-rich conditions, in which the siderophores are not needed [9]. Additionally, cells that expressed high levels of pyochelin also expressed high levels of pyoverdine with no specialization on one of the types. No connection to bet hedging is apparent. We use this example to introduce our alternative evolutionary explanations for heterogeneity.

Group-level phenotypes

Bacteria often secrete beneficial compounds into the environment that are subsequently shared among neighboring cells. Examples include iron-scavenging siderophores and nutrient-degrading enzymes [10]. With shareable secreted molecules, one cell may secrete a lot, reproducing less because of the cost of producing the secreted molecules. Another cell may secrete little and reproduce more. The secretion level per cell may not matter very much because the overall fitness of the clone depends only on the group's total secretion level and not on the per-cell secretion level. Put another way, group-level phenotype is important, whereas individual cellular phenotype is not. With weak selection acting on fluctuations in the expression of each cell around some average value, the regulatory mechanisms controlling secretion output may lack precision [11]. Consequently, cellular heterogeneity follows without any benefit from division of labor or bet hedging. Group-level phenotype could explain the heterogeneity in siderophore investment observed in *P. aeruginosa*

Table 1. Example studies advocating division of labor or bet hedging as adaptive explanations for phenotypic heterogeneity in clonal bacterial populations

Species	System	Phenotype 1	Phenotype 2	Adaptive explanation	Refs
Salmonella enterica	Gut infection	Type III secretion system activated	Gut colonizer	Division of labor	[5]
Bacillus subtilis	Colony migration	Biosurfactant production	Matrix production	Division of labor	[6]
Lactococcus lactis	Diauxic shift	Glucose enzyme synthesis	Cellobiose enzyme synthesis	Bet hedging	[7]
Pseudomonas aeruginosa	Quorum sensing	Instant communicators	Delayed communicators	Bet hedging	[8]

(Figure 1A) [9]. This hypothesis predicts that the amount of heterogeneity between cells should increase the more a secreted compound is shared within the group. For example, different siderophores vary in their diffusibility and in their potential for sharing [12]. Thus, highly diffusible and more shareable siderophores may associate with relatively high cellular heterogeneity in siderophore production (Figure 1B), whereas membrane-associated or slowly diffusing siderophores may associate with low cellular heterogeneity in siderophore production (Figure 1C).

Heterogeneity in cellular vigor

Vigor describes the capacity of a cell to complete fitness-enhancing tasks. Clonal

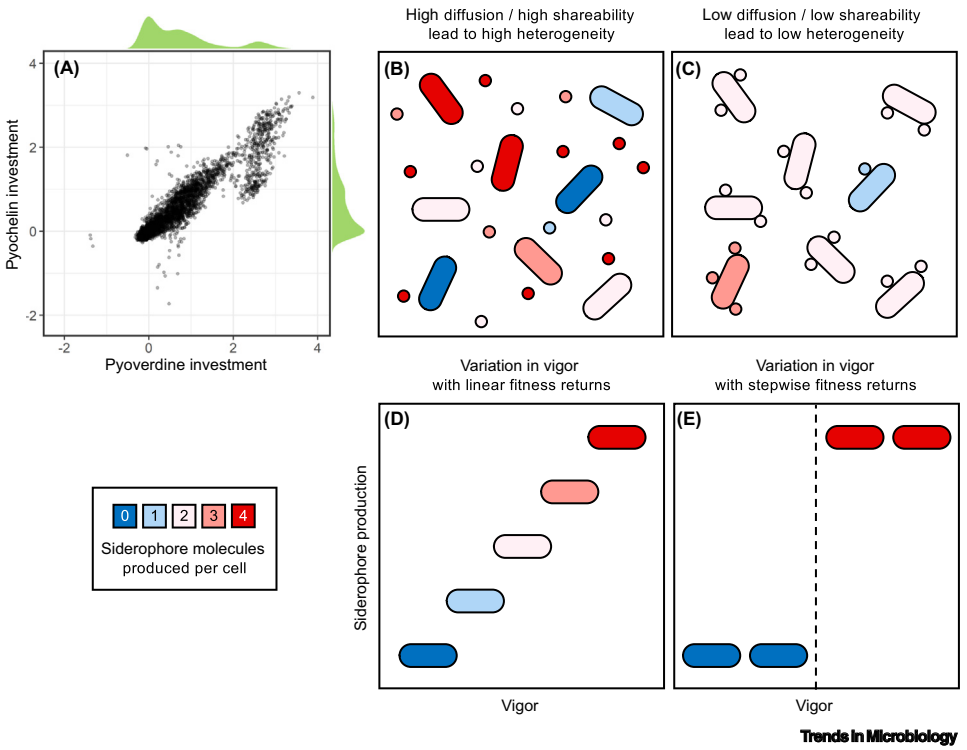

Figure 1. Phenotypic heterogeneity induced by group-level phenotypes and variation in cellular vigor. (A) Phenotypic heterogeneity in the expression of siderophore genes (pyoverdine and pyochelin) among cells in a clonal population of the bacterium *Pseudomonas aeruginosa*. The data are from Figure S8 in Mridha and Kümmerli [9] using gene expression reporters to measure siderophore investment (log-transformed fluorescence units) of single cells (semi-translucent dots). The observed cellular heterogeneity is high, with no apparent connection to division of labor or bet hedging. The green histograms show cellular gene expression distributions across 3477 cells. (B) Siderophores have group-level phenotypes when secreted molecules can be shared within the group and deliver iron to all cells regardless of a cell's own siderophore production level. In such group-level phenotypes, selection is weak on fluctuations in individual trait expression. Thus, we predict heterogeneity to be particularly high for highly diffusible and shareable molecules. (C) By contrast, heterogeneity should be relatively low for less diffusible and less shareable molecules because each cell depends on its own secretion level. (D) Variation in the capacity of cells to complete fitness-enhancing tasks, which we call vigor, can spur heterogeneity in trait expression. For example, a trait such as siderophore production may increase with cellular vigor. We expect a steady rise in trait expression if fitness benefits increase steadily with vigor. (E) By contrast, a step increase in fitness with respect to trait expression predicts bimodal trait expression. The dashed line indicates the threshold value above which cells start to invest in nonvital fitness-enhancing tasks. In all schemes (B–E), oval shapes represent bacteria that vary in their siderophore production level from zero (dark blue) to four (dark red) molecules. The different siderophore production levels reflect arbitrary values and are used for illustration purposes. Siderophores are depicted as circles matching the color of their producer.

cells within a bacterial population likely vary in their vigor, leading to nongenetic differences between individuals [13]. A cell's age, nutrient stocks, stress, or level of internal damage can influence its vigor. At any moment in time, there will be significant variation in cellular vigor and the expression of phenotypes [14]. Individuals with low vigor might primarily invest in their core metabolism to ensure survival and basic growth. Individuals with high vigor might, in addition to core metabolism, also invest in auxiliary traits that are non-essential for survival but provide additional fitness benefits. If those benefits increase steadily with trait expression, then auxiliary trait expression may increase steadily with vigor (Figure 1D). By contrast, if there is a stepwise increase in fitness benefits then bimodal auxiliary trait expression may arise, in which expression switches around a threshold value of vigor (Figure 1E). Variability in vigor is likely to be common [13,14] and may be the most frequent cause of cellular heterogeneity in clonal populations. For example, the heterogeneity in siderophore production observed in *P. aeruginosa* (Figure 1A) may arise from heterogeneity in vigor. Experimentally, one could manipulate cellular vigor to test predictions about how particular traits vary in expression in response to changing vigor.

Concluding remarks

Heterogeneity in trait expression commonly occurs in bacteria. Division of labor and bet hedging are two intuitive explanations for why heterogeneity may be beneficial for individuals and groups (Table 1). We emphasized two additional explanations for heterogeneity. First, when phenotypes occur at the group level, as often happens for extracellular molecular secretions, heterogeneity can arise because selection acts strongly on group-level expression but only weakly on individual-level expression. In this case, heterogeneity is not directly beneficial but instead arises as a consequence of imprecise regulatory mechanisms, a secondary outcome of extracellular sharing of resources. Second, variable cellular vigor favors relatively weak cells to reduce expression of nonvital traits and favors relatively strong cells to increase allocation to those nonvital traits that provide additional benefits. With this type of cellular heterogeneity, each cell adjusts its expression in relation to its vigor in order to optimize fitness at the level of the clonal group. These ideas extend the conceptual framework for cellular heterogeneity in microbial populations and enhance our general understanding of microbial design [15].

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Declaration of interests

No interests are declared.

[1]Department of Quantitative Biomedicine, University of Zürich, Zürich, Switzerland
[2]Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA

*Correspondence:
rolf.kuemmerli@uzh.ch (R. Kümmerli).

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