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Current Opinion in Genetics & Development

Macromotives and microbehaviors: the social dimension of bacterial phenotypic variability

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Bacterial phenotypic variability — the display of multiple distinct phenotypes in a genetically homogenous population of bacteria — emerges as an adaptive response to conflicting challenges. This creates an opportunity for social interactions which are able to dynamically redistribute cell fates within a community and to directly share the benefits of the different fates. While social interactions between cell fates can optimize community behavior, they also make the community vulnerable to exploitation. The aim of this review is to emphasize the social roles of phenotypic variability and introduce it as a communal rather than a single-cell property. Specifically, we present two prevalent perspectives on the forces shaping social interactions between cell fates — engineering optimality and social stability — and review recent works combining engineering, developmental and social evolution analyses in light of this distinction.

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Current Opinion in Genetics & Development 2011, **21**:759–767

This review comes from a themed issue on
Genetics of system biology
Edited by Norbert Perrimon and Naama Barkai

Available online 26th October 2011

0959-437X/$ – see front matter

DOI 10.1016/j.gde.2011.09.011

Introduction

Bacteria are almost never alone, due to the celerity with which they divide to populate ever-changing niches with communities comprised of millions of individuals. Bacterial phenotypic variability — the display of multiple distinct phenotypes in a genetically homogenous population of bacteria — could emerge as an adaptive response in an environment with conflicting challenges [1–6]. The consensual view on how a genetically homogenous bacterial population can give rise to multiple phenotypes is that phenotypic variability is almost unavoidable for gene networks designed to induce a switch-like change in behavior as a function of an environmental cue (Figure 1a). Predominantly, this includes gene networks with a positive feedback [7], but other thresholding mechanisms have also been shown to result in phenotypic variability [8]. Multiple cell fates can easily arise given an appropriate network by intrinsic cellular noise or by micro-environmental changes (Figure 1a). The mechanistic basis for variability as a probabilistic, single-cell property is more thoroughly discussed in an accompanying paper by Balaban [56] (in this issue).

Social interactions between bacteria offer a way to — often drastically — affect reproductive success of the community. The advantage of this additional layer of complexity is that it is able relatively fast to redistribute fitness across different cell fates, and thereby synergize the separate advantages of each cell fate. However, it also makes the population susceptible for exploitation by cheaters.

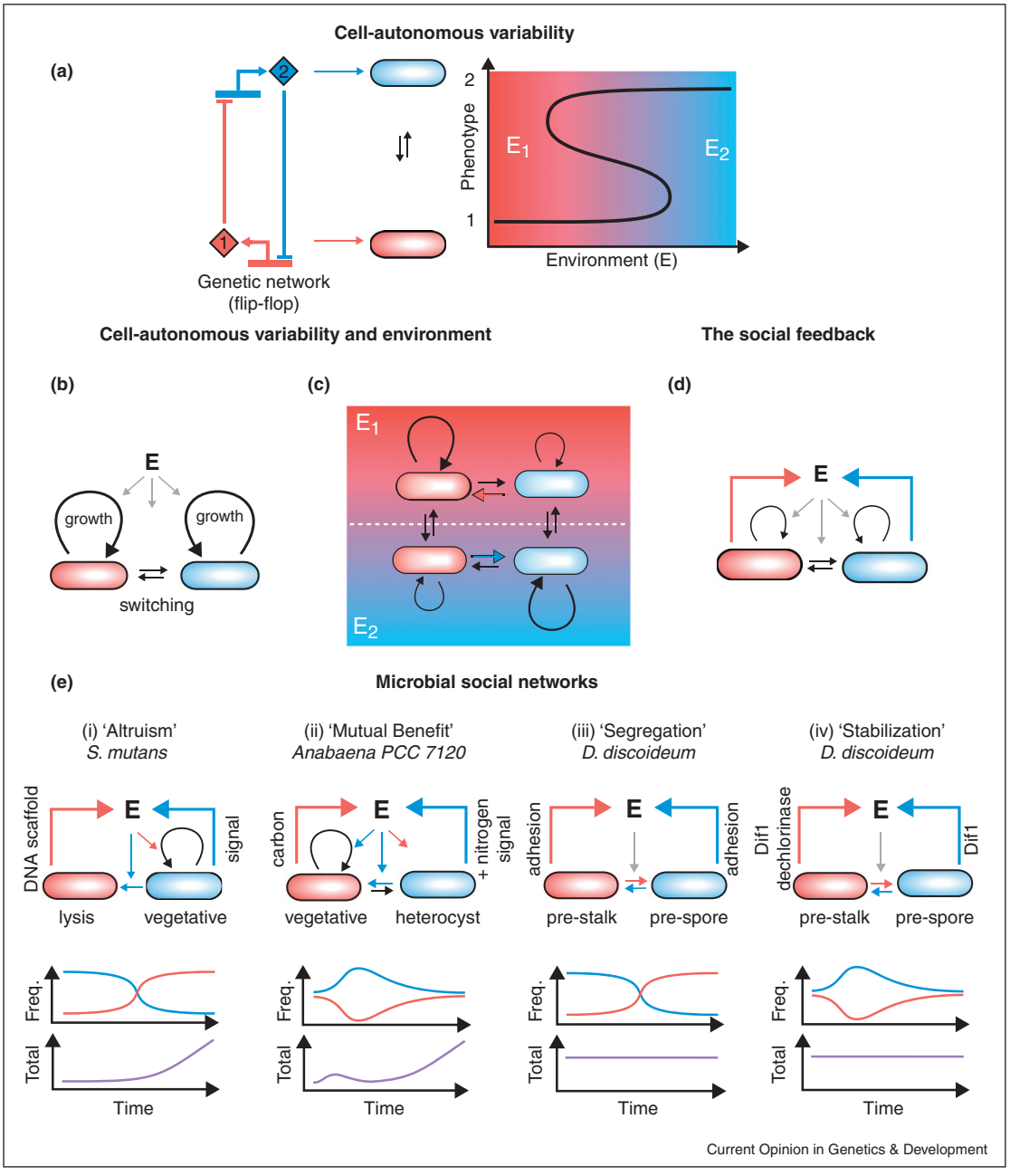
The aim of this review is to highlight the importance of social interactions between differentiated fates and to argue that phenotypic variability is often a communal property. We discuss how the propensity of bacteria to social interactions impacts the distribution of cell fates in the community and how can this allocation be utilized to optimize community behavior. We then proceed to the difficulties posed on social interactions by the threat of exploitation and the way this influences optimality of community behavior, social evolution of phenotypic variability and the evolution of genetic variability.

Phenotypic variability as a communal property

While the structure of genetic networks determines the possible phenotypic fates, the environment plays a critical role in determining the frequency of the different phenotypes. It does so by both 'regulating' the switching probabilities between the various fates and by 'selecting' the growth rate of specific phenotypes (Figure 1b). All the known mechanisms that lead to phenotypic variability in bacteria appear to be cell-autonomous — each cell 'decides' for itself whether to switch to one phenotype or another. As such, phenotypic variability can be demonstrated and studied as a probabilistic single-bacterium property especially, if the environment fluctuates with time between conditions that favor different phenotypes (Figure 1c) [9–12]. For example, probabilistic differentiation between a growing and non-growing cell fate can benefit bacteria if the environment fluctuates in the level of antibiotics [13,14].

Although attractive in its simplicity, a single-cell view of phenotypic variability is often insufficient for understanding its impact. This is because bacteria normally reside in large communities and the behavior of one bacterium

Figure 1



The social context of microbial phenotypic variability. **(a)** Phenotypic variability is driven by cell-autonomous gene networks, for example, the flip–flop architecture (left) will lead to a bistable solution under some conditions (right). **(b)** Simple phenotypically variable population in some environment (E) can be characterized by its switching rates between phenotypes and the growth rates of the two phenotypes. **(c)** If the two phenotypes show opposite differential growth in two environments (E_1,E_2) then phenotypic variability (either with or without switching rate regulation) will be selected. **(d)** Social interactions between the cells are generally mediated by the feedback cells have on their environment — for example, by secretion of enzymes, antibiotics, or signals. **(e)** Four examples for social interactions between cell-fates in microorganisms. For each example a network diagram shows the relation between fate and the type of social interactions. Cartoon graphs show the change in time in the frequency and total population size after a perturbation to the system. (i) *Streptococcus mutans* programmed cell death is a form of altruism. A quorum sensing signal is used to control the differentiation of a subpopulation of *S. mutans* into a programmed cell death pathway [40•]. The lysing subpopulation supplies the DNA scaffold needed for the biofilm formation [41]. (ii) Heterocyst development in *Anabaena* leads to 'mutual benefit'. The heterocyst fix nitrogen and the vegetative photosynthetic cells fix carbon, required element for both cell types. Heterocyst 'switching' frequency is also determined by a signal emanating from the heterocyst [18]. We note that the environment in the case of *Anabaena* is intracellular. (iii) Differential sorting in *Dictyostelium discoideum* [17] leads to segregation. The pre-stalk cells aggregate by differential attachment to their own cell-type. This does not conform to fate switching, but is similar to it. (iv) Switching regulation of pre-spore and pre-stalk cells in *Dictyostelium discoideum* [17] stabilizes fate frequency. Pre-spore fate is suppressed and pre-stalk fate enhanced by the secretion of the signaling molecule Dif-1 by the pre-stalk cells. This is countered by Dif-1 dechlorinase secreted by the pre-spore cells.

may crucially affect the behavior of others (Figure 1d). The influence of such interactions between different cell fates cannot be reduced to or observed at the single-cell level but can only be studied at the community level.

Most of social interactions between bacteria are indirectly mediated by the effects they exert on their environment (Figure 1d). These include the secretion of various materials; enzymes that modify the nutrients in the environment, molecules that enhance or impede motility, antibiotics that kill other bacteria and signals (generally known as 'quorum sensing' signals in microbiology [15]) that directly elicit a response from other bacteria.

Social interactions between cell fates mediated by phenotypic variability

Classical model systems where social interactions between cell fates have been examined include the formation of fruiting bodies in *Myxococcus xanthus* [16] and the amoeba *Dictyostelium discoideum* [17], the formation of heterocyst in cyanobacteria [18] and of lysis-mediated production of bacteriocins [19] (Figure 1e). A useful model for studying social interactions between cell fates is the soil bacteria *Bacillus subtilis*, a classical system for differentiation due to its sporulation and genetic competence cell fates [20]. A series of studies in the last decade demonstrated the prevalence of phenotypic variability in numerous pathways including sporulation [21] and competence [22,23], production of exo-degradative enzymes [24], motility [25], matrix production [26,27•], surfactant secretion [27•,28] and release of antibiotics [29,30•]. All the above processes come into effect within a developing biofilm — a surface-adhered community of cells — and result in the formation of multiple cell fates [29]. Many of these differentiated fates are social, affecting the fate of other cells and specifically, the fate of other differentiated states.

From a dynamical perspective, social interaction between cell fates can modify their frequencies in the population in a way that will reflect the 'needs' of the community for their respective function. It is therefore tempting to define fitness at the level of the community and analyze the way allocation of cell fates maximize it.

The ability of social interactions to dynamically change the allocation of cell fates in a community has been demonstrated in several works which studied the transition to sporulation in *B. subtilis* biofilms (Figure 2a). One remarkable (and in fact macabre) example concerns the role of cannibalism in this transition [31•]. Spo0A is a major master regulator governing inter-cellular matrix production at low activity levels and the initiation of sporulation at high levels. It has been previously shown that matrix producers secrete an antibiotic (known as Skf) to which they are immune but Spo0A-inactive cells are not. Therefore matrix producers kill and cannibalize the Spo0A-inactive population [32]. Lopez *et al.* examined this process in the context of a bacterial biofilm and found that the wild-type cells display a much longer and gradual transition to the spore cell fate compared to an Skf null mutant [31•]. They hypothesize that the Skf-dependent procrastination of the switch to sporulation may lengthen the period of bet-hedging between spores and actively growing cells. This delay may be beneficial under conditions of fluctuating nutrients.

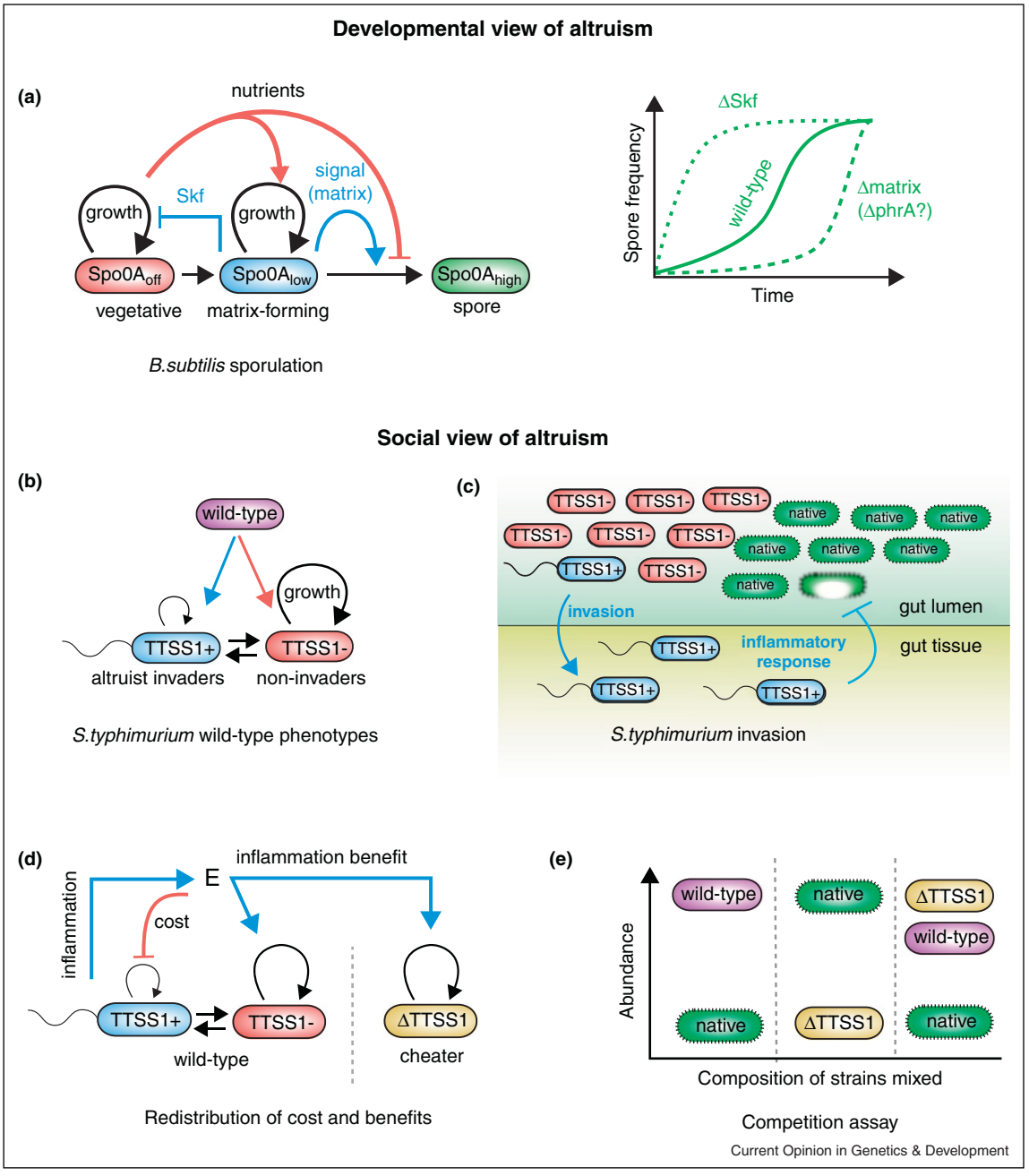
Another recent theoretical work showed how *B. subtilis* cells can utilize quorum sensing to better predict the costs and benefits of turning into spores in a given environment [33•]. The transition to sporulation is negatively regulated by nutrient levels. It is also positively regulated by a quorum-sensing system, whose signal (phrA) is produced only by cells with low Spo0A activity which are still growing and not by the sporulating subpopulation. The signal strength therefore reflects the density of growing cells in the population. By properly integrating the information from nutrient levels and quorum sensing, it was shown that the cells can compute the level of nutrients available per cell which is a better predictor of the benefits of growth versus sporulation than the absolute level of nutrients. Additional work has demonstrated how the matrix itself is governing the transition to sporulation by specifically activating a kinase of Spo0A — this, again, makes the transition into sporulation dependent on the state of the community [34].

Social evolution of phenotypic variability

Most experimental data on the behavior of communities are taken from experiments where the community comprised a single genotype. The reasoning used to analyze and interpret the data is then formulated in the language of optimality — some function of the community (usually related to long-term growth) is optimized by the specific network of interactions. Social evolution, however, shows that it is indispensable to also consider the consequences of social conflict between genotypes within the community even if it contradicts clonal optimality [35,36] (see Box 1). Analysis of social conflict requires a very different type of analysis of the social network — one needs to define the fitness of social interactions, divide it into cost and benefit terms and examine who is benefiting and who is paying the cost of an action and if there lies a potential for a cheater genotype which enjoys the social benefits without paying its costs.

Conflict and exploitation are easy to understand if one of the phenotypes is lethal — the death of cells exhibiting it benefits other cells with different phenotype. In such a case, a non-lethal mutant strain will act as a 'cheater', exploiting the benefits arising from the death of the wild-type subpopulation without paying the ultimate price of this death. For example, in the case of the Skf system described in the previous section, a mutant that

Figure 2



The developmental (a) and social (b–e) views of altruism. **(a)** Three cell fates with different level of active Spo0A exist during biofilm formation with increasing levels corresponding to vegetative, matrix former and spore fates (left). The matrix-former is secreting killing factors (Skf and others) which kill the Spo0A-inactive cells and probably utilizes lysed cells as nutrients. Transition to sporulation is in turn regulated by nutrient levels, by the matrix itself and by a quorum sensing signal. The transition to sporulation (right) in the wild-type is slower than in an skf mutant but faster than in a matrix mutant (and maybe also a signaling mutant). The delay may be adaptive under fluctuating nutrient levels by extending the period of bet-hedging. (b–e) *Salmonella* virulence as an altruistic cell fate. **(b)** Wild type *Salmonella* displays phenotypic variability of a virulent cell fate, expressing flagella and secretion system and an avirulent cell fate not expressing these systems. **(c)** Virulent bacteria which invade the gut lamina cause an immune inflammatory response. This inhibits the natural gut flora growth more than the gut residing *Salmonella*. The chances of virulent bacteria to be propagated to the next host are low [44•]. **(d)** The virulent phenotype is therefore an altruist that indirectly benefits the avirulent phenotype. It will therefore benefit also a constitutively avirulent mutant which does not pay the cost of altruism. **(e)** Infection by wild-type *Salmonella* results in its high abundance and lowers the abundance of resident gut flora. An avirulent 'cheater' mutant will not be able to compete with the residing gut bacteria. When an avirulent mutant is mixed with the wild-type it invades the gut alongside the wild-type.

Box 1 The differences between engineering and social evolution approaches to optimality

Systems-level analysis often tries to prove that some network design is optimizing a cellular function. For example, switching rate between phenotypes in a bet-hedging strategy has been analyzed as an optimality problem under conditions of fluctuating environments [13]. The integration of quorum-sensing signals has been analyzed and found to optimize the information content of the different signals [54].

When considering social interactions between phenotypes (as any other type of social engagement) an optimal strategy may be susceptible to exploitation by suboptimal strategies. In this case, evolution will converge on an evolutionary stable strategy which cannot be invaded by other strategies. While the formal theory of this difference is well rooted in both evolutionary biology [35] and game theory [55], an illustrative example which follows the model of Ref. [44•] demonstrates the difference between optimal altruist allocation frequency from an engineering perspective and social perspective. Generalizing on the model presented in this work, we assume that a population can split into two phenotypes — an altruist (e.g. virulent *Salmonella* phenotype) that makes a public good (inflammatory response) and a beneficiary (avirulent phenotype) which benefits from the altruist act. We assume that the growth rate of the beneficiary, Φ, is a monotonously increasing function of the frequency of both altruist and beneficiary. Therefore, increasing the frequency of altruist results in a growth cost (by reducing the frequency of beneficiary phenotype) but leads to an altruism benefit. If the frequency of the altruist is q, then the fitness of the strain can be written as:

$$\Phi(q) = \Phi_{Private}(1-q) \times \Phi_{Public}(q)$$

If the population is clonal, Φ can be optimized to yield a maximal fitness at the frequency, q_{opt}^{eng}, which follows:

$$\left.\frac{d\Phi(q)}{dq}\right|_{q_{opt}^{eng}} = 0 \Rightarrow \left.\frac{d\log\Phi_{Private}(1-q)}{dq}\right|_{q=q_{opt}^{eng}} = \left.\frac{d\log\Phi_{Pubic}(q)}{dq}\right|_{q=q_{opt}^{eng}}$$

To study the social consequences of competition between strains, we consider the stability of a strain with frequency value q_1 to invasion by a strain with a different value q_2. We assume that the direct growth benefit $\Phi_{Private}$ is private, affecting only the specific strain, while the indirect benefit of the altruist Φ_{Public}, is public and benefits both strains equivalently. We also need to define the population structure — how well related are bacteria in a community. The simple population structure used in Ref. [44•] implies that the invading strain's frequency in a mixed community cannot exceed a fraction ($1/M$) of the population (e.g. if a local population is routinely propagated through a bottleneck of M bacteria). Therefore the fitness of the two strains will be:

$$\Phi(q_i) = \Phi_{Private}(1-q_i) \times \left(\frac{M-1}{M}\Phi_{Public}(q_1) + \frac{1}{M}\Phi_{Public}(q_2)\right), \quad i = 1,2$$

From this expression one can show that an evolutionary stable frequency, q_{opt}^{soc} which cannot be invaded by mutants with close values of q or by the cheater strain, follows the equation:

$$\left.\frac{d\log\Phi_{Private}(1-q)}{dq}\right|_{q=q_{opt}^{soc}} = -\frac{1}{M}\left.\frac{d\log\Phi_{Public}(q)}{dq}\right|_{q=q_{opt}^{soc}}$$

This is equivalent to optimizing a new fitness function:

$$\Phi^{soc}(q) = \Phi_{Private}(1-q) \times (\Phi_{Public}(q))^{1/M}$$

This optimization function will yield suboptimal values of altruist allocation frequency compared to the engineering case, unless $M = 1$ which is exactly the case where strains are never mixed and social optimality converges to engineering optimality.

constitutively expresses the Skf-immunity gene may act as a cheater. The phenomenon of self-sacrificing altruistic subpopulation (Figure 1e) is prevalent in many bacterial systems. Classical examples include the release of bacteriocins by autolysing bacteria [19,37] and the autolysis of most bacteria in a developing *Myxococcus* fruiting body [16,38]. More recently, many additional species have been shown to utilize DNA from lysed cells as part of their biofilm matrix [39], a trait which is sometimes regulated by quorum sensing [40•,41,42].

Recently, a different form of self-sacrificing altruism was experimentally demonstrated to be crucial for the propagation and virulence of enteropathogenic bacteria [43••,44•]. These pathogens, for example *Salmonella typhimurium*, have to invade the existing gut microbiota. It has been previously shown that only a subpopulation of *Salmonella* expresses virulence factors (Figure 2b) which enable gut tissue invasion. It has been demonstrated that while the immune response kills invading *Salmonella* cells the immune inflammatory response in the gut is beneficial to the gut residing, non-invasive, subpopulation. This is because the non-invading *Salmonella* is more resistant to the inflammatory response than native gut microbiota (Figure 2c) [43••,44•]. In this scenario, it is expected for an avirulent mutant to act as a 'cheater' (Figure 2d). Stecher *et al.* indeed demonstrated that an avirulent mutant cannot establish itself in the mice gut,

but is able to establish itself when mixed with the wild-type (Figure 2e) [43••].

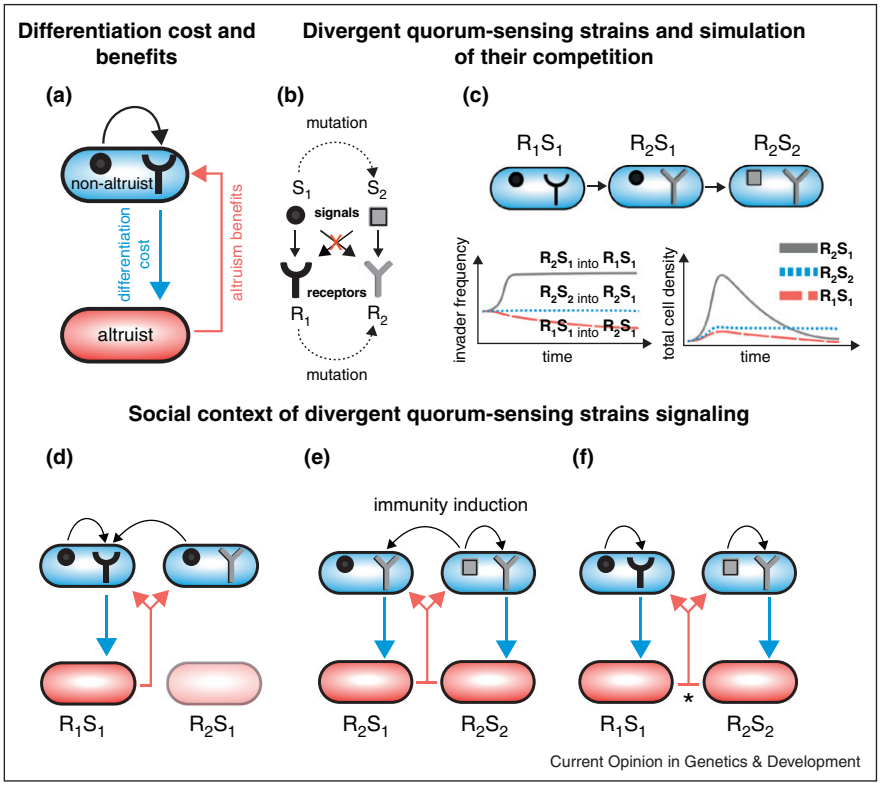
How is cooperation sustained in the face of 'cheaters'? The general answer is that structured population may group together cells with higher relatedness [36]. In the case of *Salmonella*, relatedness may increase by the extreme population bottlenecks that *Salmonella* undergoes before invasion. While bottlenecks maintain cooperation, the level of investment in the virulent phenotype may be lower than the optimal level for a clonal population [44•] (Box 1).

Social conflict poses a new 'design criterion' on the topology of the gene-regulatory networks underlying social interactions — how to minimize exploitation by other genotypes, specifically (but not only), by mutants [45••,46,47]. Understanding the interplay between optimality and social resilience requires an analysis of the social stability of a given regulatory network.

Phenotypic variability can drive and sustain genetic variability

While a 'cheater' genotype may impose a burden on the bacterial community that needs to be minimized, its long-term survival is limited by its reduced fitness in isolation. However, more conditional social strategies where a strain behaves differently in the presence of another strain may be successful also in isolation and can drive genetic diversification. In this context, Eldar developed a model that suggests how diversification of bacterial intercellular signaling can result from social interactions [48••]. Intriguingly, many species of bacteria show rapid diversification of their quorum-sensing communication systems — a

Figure 3



Social engagement can lead to diversification of bacterial signaling. **(a)** We assume that a secreted signaling molecule (S) can activate a corresponding receptor (R) (black arrow between signal and receptor). Active receptor then regulates cost (blue arrow) and social benefit (red arrow), such as the differentiation of an altruist lethal phenotype (red cell) which benefits the non-altruist phenotype (blue cell). **(b)** We assume that original signal (S_1) and receptor (R_1) can mutate into different alleles (S_2,R_2). Each allele of signal activates only its corresponding receptor allele. **(c)** A new signaling pathway can evolve sequentially through an intermediate with altered receptor (top). Simulation of competitions between a mutant strain and its ancestor in simple environments (bottom). A receptor mutant (R_2S_1) will invade into its wild-type ancestor (R_1S_1) but cannot be invaded by it. A receptor/signal double mutant (R_2S_2) maintains its frequency in the presence of its ancestor (and will increase in frequency in structured environments). Shown are the frequency of invader and its total level as a function of time. (d–e) Social interaction explains the invasion patterns. **(d)** The receptor mutant (R_2S_1) invades its ancestor by cheating — it does not produce the altruist cell fate but enjoys the benefits of its altruist phenotype. **(e)** A double mutant (R_2S_2) is immune to its ancestor's (R_2S_1) cheating — the new signal (S_2) induces altruist differentiation in both strains as they carry the same receptor (R_2). **(f)** The two signaling pathways co-exist. If one of the strains is in minority, its contribution to the altruist fate (marked by asterisk) will be lower and it will therefore exploit altruists of the other strain. This negative frequency selection leads to co-existence of the two strains.

signal from one strain will not activate the receptor of another strain of the same species (e.g. [49–51], Figure 3a,b). It is difficult to explain such diversification, because a change in signaling specificity requires changes to both receptor and signaling genes, but a change in only one of them will lead to a non-functional signaling system.

Eldar has shown that these changes can occur sequentially with the receptor mutating first and the signal following, if quorum-sensing regulates a cooperative act [48••] (e.g. the probabilistic differentiation into an altruist phenotype, Figure 3a). In this case, diversification into a new signaling system will occur by rounds of 'cheating' receptor mutation (which does not invest in cooperation, Figure 3c,d [52]) followed by 'cheating immunity' signaling compensatory mutation. 'Cheating immunity' arises as the double mutant with new receptor and new signal induces its ancestor, receptor-only mutant, to cooperate by activating its receptor (Figure 3e). Finally, the two alternative signaling systems will stably co-exist (Figure 3f) as the minority strain always invests less in the cooperative act when the two strains are mixed, because its signal is weaker. This 'facultative cheating' mechanism is able to maintain co-existence between genetically diverged strains within the population.

Outlook

Many facets of phenotypic variability can only be understood if one considers social interactions between cell fates in a community. Social interactions between fates enable the dynamical allocation of fates in the community according to its 'optimal' needs. This allows the benefit of the different fates to be shared by the community. Analysis of social interactions in our opinion would benefit from merging two different perspectives — engineering and social evolution. The reason being that their concepts of optimality may result in very different levels of predicted social interactions (Box 1). The engineering (or developmental) perspective assumes the prevalence of clonal population and treats it as a multicellular organism whose function is evolutionarily optimized. In contrast, the social perspective assumes that the colony is always threatened by the invasion of suboptimal exploiting genotypes and therefore social optimality is dictated by the stability against exploitation.

The relative importance of engineering optimality and social evolution stability for microbial populations not only depends on the ecology of the bacteria but can also be shaped by the structure of their social interaction networks. An important and exciting future challenge is therefore to devise tools that will relate a network structure to the notions of optimality and compare the results to the predictions of the two views. From an experimental perspective, this will require to relate traits like gene expression or community morphology to fitness in an ecologically meaningful manner [45••]. From the perspective of social evolution, the requirement will be to measure the costs and benefits of the various cell fates, as well as conducting appropriate competition between genotypes in mixed populations. To this end, synthesis of the engineering and social evolution approaches might be ineluctable and will greatly benefit both fields which are currently studied by different communities and only rarely interact [53].

Acknowledgements

Research in AE laboratory is supported by the International Human Frontier Science Program Organization Career Development award, by a Marie Curie reintegration grant and by the Israeli Academy of Science. PR is a VATAT post-doctoral fellow. We wish to thank D. Sprinzak, O. Shaya and S. Pollak for valuable suggestions and critical reading of the manuscript. We apologize to those of our colleagues whose works while highly regarded could not be cited due to the space limitations.

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