Cooperation in the dark: signalling and collective action in quorum-sensing bacteria

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The study of quorum-sensing bacteria has revealed a widespread mechanism of coordinating bacterial gene expression with cell density. By monitoring a constitutively produced signal molecule, individual bacteria can limit their expression of group-beneficial phenotypes to cell densities that guarantee an effective group outcome. In this paper, we attempt to move away from a commonly expressed view that these impressive feats of coordination are examples of multicellularity in prokaryotic populations. Here, we look more closely at the individual conflict underlying this cooperation, illustrating that, even under significant levels of genetic conflict, signalling and resultant cooperative behaviour can stably exist. A predictive two-trait model of signal strength and of the extent of cooperation is developed as a function of relatedness (reflecting multiplicity of infection) and basic population demographic parameters. The model predicts that the strength of quorum signalling will increase as conflict (multiplicity of infecting strains) increases, as individuals attempt to coax more cooperative contributions from their competitors, leading to a devaluation of the signal as an indicator of density. Conversely, as genetic conflict increases, the model predicts that the threshold density for cooperation will increase and the subsequent strength of group cooperation will be depressed.

Keywords: communication; sociality; altruism; homoserine lactone; bacterial ecology

1. INTRODUCTION

Cooperative behaviour in bacterial populations is commonplace. Colonies cooperatively modify their environment in many ways, for instance by the release of enzymes, fibrous building materials or antibacterial agents (Fuqua *et al.* 1996; Bassler 1999). *Erwinia carotovora*, an important plant pathogen, provides a well-studied example. Established colonies of this species produce a range of digestive enzymes and antibacterial agents that ensure efficient digestion of the host in the near absence of bacterial competitors. Intriguingly, individual bacteria only begin to secrete these 'cooperative' enzymes once a distinct density threshold has been passed (Bainton *et al.* 1992; Jones *et al.* 1993).

In order to tailor phenotypic expression to changing cell densities, bacteria face the problem of assessing group density. Through the release of low-molecular-weight signalling molecules, quorum-sensing bacteria (QSB) are able to monitor and respond to changes in bacterial density (Fuqua *et al.* 1996; Robson *et al.* 1997). Once a particular density threshold is crossed, cooperative behaviour (e.g. the production of tissue-degrading enzymes in *E. carotovora*) is switched on.

The ubiquity of quorum-sensing in bacterial ecology is only beginning to emerge, with examples ranging from the control of swarming behaviour to bioluminescence (reviewed by Fuqua *et al.* 1996; Robson *et al.* 1997; Bassler 1999). The best-documented system is that of luminescence in the marine bacterium *Vibrio fischeri*. When *V. fischeri* cells are diluted into fresh medium, bioluminescence is not observed until the mid-logarithmic phase of

growth. The activation of the *lux* genes required for light emission is dependent on the accumulation of a small diffusible auto-inducer molecule or bacterial pheromone, N-(3-oxohexanoyl) homoserine lactone (HSL) (Eberhard *et al.* 1981). Accumulation of HSL above a threshold level occurs only at high cell densities, as found symbiotically within the light organs of fishes or in stationary-phase *in vitro* cultures. The production of HSL is directed by the *luxI* gene product. HSL diffuses freely throughout the bacterial colony and is detected by its receptor, the *luxR* gene product. This receptor-HSL complex then, in turn, activates the transcription of *luxI* and the structural *lux* genes, resulting in light emission (Engebrecht *et al.* 1983). In broad outline, this mechanism appears to be ubiquitous across quorum-sensing bacteria (see Bassler (1999) for a discussion of non-HSL mechanisms of quorum-sensing).

The generic problem of group cooperation to achieve a common goal has recently been addressed analytically by Brown (1999) in a model of parasite-induced host manipulation. Under this model, group size is a key parameter determining the extent of cooperation (e.g. the contributions to host manipulation). The most striking prediction is that of the existence of a group-size threshold to cooperative behaviour strongly reminiscent of the behaviour of QSB. Indeed, the most compelling qualitative support for the threshold phenomenon can be found in the pattern of density-dependent cooperation within expanding populations of bacteria.

The additional strategic complexity of producing and responding to a signalling molecule adds an extra dimension to the system captured in the earlier model, which describes an evolutionarily stable strategy (ESS) of cooperative effort for QSB under the assumption of honest cost-free group-size information. More realistically, such information can only be acquired at some cost. As in any signalling system, conflicts of interest between signallers and receivers can potentially favour 'dishonest' and

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manipulative mutants that elicit responses beneficial to themselves, to the detriment of others (Dawkins & Krebs 1978; Krebs & Dawkins 1984). Bacteria that produce exceptional quantities of a signalling molecule, for example, may benefit because they are more likely to elicit cooperative efforts from others. Reliable communication may thus prove stable only if signalling entails sufficient cost to render dishonesty unprofitable (Zahavi 1975, 1977, 1987; Grafen 1990; Johnstone 1997, 1998a; though see Maynard Smith (1994) and Bergstrom & Lachmann (1998)).

Brown's (1999) model is expanded here to a two-trait ESS model describing the optimal level of cooperation (i.e. production of group-beneficial traits), and of signalling, in QSB as a function of relatedness, costs to the individual, benefits to the group and demographic parameters.

2. A TWO-TRAIT ESS MODEL

We are interested in the possibility of a reliable quorum-signalling equilibrium at which the concentration of a signalling molecule or pheromone accurately reflects cell density, permitting bacteria to adjust their investment in cooperation in relation to colony size. Let s denote an individual's investment in the production of the signalling molecule, and t denote the total concentration of the molecule that it experiences (which will depend on the colony size, n). Individual investment in cooperation, which may vary in relation to signal concentration, will be denoted by $m(t)$.

We assume that investment in signalling and investment in cooperation both have a negative impact on individual fitness; investment in cooperation, however, has a positive impact on group fitness. The fitness of an individual bacterium thus depends on its individual investments in signalling and cooperation, and on the average level of investment in cooperation across all colony members. Formally, writing $w(n, s, m, \bar{m})$ for the fitness of an individual that invests s in signal production and m in cooperation, in a colony of size n in which the average level of investment in cooperation is $\bar{m}$, we assume that

$$w(n, s, m, \bar{m}) = (I(m) \times G(n\bar{m})) - s. \quad (1)$$

$I(m)$ is the individual fitness function, a declining function of m , and $G(n\bar{m})$ is the group fitness function, a rising function of summed cooperation.

At an evolutionarily stable quorum-signalling equilibrium, the level of investment in cooperation at any given pheromone concentration, denoted $m^*(t)$, and the level of investment in signalling, denoted s^* , must be such that no mutant type investing a different amount in either cooperation or signalling can enjoy a fitness advantage over the rest of the population. Assuming that group members are related by a coefficient r , this implies that

$$\frac{\partial w(n, s, m, \bar{m})}{\partial m} + \left(\frac{1 + r(n-1)}{n} \right) \frac{\partial w(n, s, m, \bar{m})}{\partial \bar{m}} = 0, \text{ for all } n, \quad (2a)$$

and

$$\int f(n) \left[\frac{\partial w(n, s, m, \bar{m})}{\partial s} + (1 + (n-1)r)m^*(ns^*) \times \left(\frac{\partial w(s, m, \bar{m})}{\partial m} + \frac{\partial w(s, m, \bar{m})}{\partial \bar{m}} \right) \right] dn = 0, \quad (2b)$$

where all derivatives are evaluated at $s = s^*$, $m = \bar{m} = m^*(ns^*)$, and $f(n)$ denotes the probability density distribution of colony sizes experienced by an individual (we treat colony size/density as a continuous variable). Equation (2a) implies that there is no marginal gain from a deviation in cooperative effort at equilibrium, and equation (2b) implies that (averaging over the range of colony sizes that an individual may experience) there is no marginal gain from a deviation in signalling effort at equilibrium (we assume that a change in signal production will lead to a change in an individual's own level of cooperative effort as well as in the cooperative efforts of others in the colony).

From equation (1) it is clear that the marginal costs and benefits of investment in cooperation are independent of signalling effort. Consequently, one can first solve equation (2a) to determine the stable level of cooperative effort at any given group size and pheromone concentration, and then go on to solve equation (2b) for s^* , the stable level of signalling effort.

(a) Stable cooperative effort

Brown (1999) derives stable cooperative efforts for several different forms of the function w (his model assumes cost-free information regarding colony size, without the need for signalling, but is otherwise identical to the present analysis). Here we focus on the simple case of linear I and G functions, for which

$$w(n, s, m, \bar{m}) = (1 - cm)(p + n\bar{m}) - s, \quad (3)$$

where c represents the cost of cooperation and p represents passive fitness (i.e. the fitness of a non-cooperating bacterium in a group of non-cooperators). Substituting equation (3) into equation (2a), we can solve to obtain the stable level of cooperative effort:

$$m^*(ns^*) = \begin{cases} \frac{1 + r(n-1) - cp}{c(1 + r(n-1) + n)} & \text{for } n \geq \frac{cp - (1-r)}{r} \\ 0 & \text{for } n < \frac{cp - (1-r)}{r}. \end{cases} \quad (4)$$

Note that (assuming $r > 0$) equation (2a) yields a negative value of m^* for colony sizes less than a critical group size, in which circumstances the stable level of cooperative effort will be zero (as negative effort is not possible).

(b) Stable signalling effort

Having determined the stable level of cooperative effort, we can substitute the expression for $m^*(ns^*)$ given in equation (4), together with equation (1), into equation (2b), to obtain (after some rearrangement) an expression for s^* :

Figure 1. Equilibrium cooperative effort, m^* , as a function of the pairwise relatedness among colony members (r) and $\log_{10}$ colony size (n), for parameter values $c = 1$ and $p = 100$.

$$\int_{n \geq \frac{cp - (1-r)}{r}} f(n) \left[-1 + (1 + r(n-1)) \left(\frac{r(cp+1) + cp - 1}{s^*c(r(n-1) + n + 1)^2} \right) \times \left(n - cp - 2cn \left(\frac{1 + r(n-1) - cp}{c(r(n-1) + n + 1)} \right) \right) \right] dn + \int_{n < \frac{cp - (1-r)}{r}} f(n) [-1] dn = 0 \quad (5a)$$

$$\Rightarrow s^* = \int_{n \geq \frac{cp - (1-r)}{r}} f(n) (1 + r(n-1)) \left(\frac{r(cp+1) + cp - 1}{c(r(n-1) + n + 1)^2} \right) \times \left(n - cp - 2cn \left(\frac{1 + r(n-1) - cp}{c(r(n-1) + n + 1)} \right) \right) dn. \quad (5b)$$

To proceed further, we must specify the distribution of group sizes experienced by individual bacteria, $f(n)$. In § 3, stable levels of cooperative effort and signalling effort are illustrated graphically for the case where colony size, n , is distributed normally.

3. RESULTS

Figure 1 shows stable individual cooperative effort (m^*) as a function of pairwise relatedness among colony members, r , and $\log_{10}$ colony size (n), for parameter values $c = 1$ and $p = 100$. The assumption of a non-zero passive fitness introduces a fixed cost of cooperation, independent of group density. This fixed cost explains the absence of cooperation ($m^* = 0$) at low densities and low levels of relatedness. As density increases, the potential for cooperatively achieving a significant group benefit increases, until a threshold is reached (whose position is sensitive to p , c and r). Above the threshold, cooperation becomes an ESS, as group density is sufficiently large to generate enough group fitness to offset a reduction in the passive-fitness component. Increasing r also leads to greater levels of cooperation. It is important to note, however, that cooperation remains stable under a range of parameter values even in largely unrelated groups and for a range of group-fitness functions.

Figure 2a shows stable signalling effort (s^*) as a function of relatedness and $\log_{10}$ mean colony size ($\bar{n}$) for the

Figure 2. Equilibrium signalling effort, s^* , as a function of the pairwise relatedness among colony members (r) and $\log_{10}$ mean colony size ($\bar{n}$), for parameter values $c = 1$ and $p = 100$. Colony sizes are assumed to be drawn from a normal distribution, with coefficients of variation equal to (a) 0.2 and (b) 0.4.

same parameter values used in figure 1, assuming that the colony size that an individual experiences is drawn from a normal distribution with a coefficient of variation equal to 0.2 (i.e. with a standard deviation equal to 20% of the mean colony size). Figure 2b shows equivalent results assuming that the coefficient of variation is equal to 0.4. The graphs reveal that both mean colony size and relatedness have a significant impact on signalling effort (while the degree of variation in colony size is of less importance). The former effect arises simply because investment in cooperation is restricted to large colonies (as illustrated in figure 1), so that there is little to be gained by signalling unless individuals have a reasonable chance of experiencing high cell densities. The impact of kinship, however, is more complex.

The level of relatedness is important in regulating the degree of conflict experienced within a colony (Brookfield 1998). As relatedness approaches 1, the equilibrium signal cost, q^* , tends towards zero, in accordance with the predictions of other models of biological signalling (Godfray 1991; Maynard Smith 1991; Johnstone & Grafen 1992; Reeve 1997; Noldeke & Samuelson 1999). This does not imply that perfectly related colonies should not signal, rather that as genetic conflict decreases so the strength of signalling should decrease to the lowest intensity capable of functioning effectively as an indicator of

bacterial density. Were we to incorporate noise and the possibility of error into the present model, some level of signal cost would still be required to ensure effective communication even at very high levels of relatedness; as in the models cited above, however, we have assumed that cost is required only for the maintenance of honesty (see Johnstone (1998b) for a discussion of this issue).

As relatedness is lowered, and conflict increases, the model predicts an increase in signalling intensity driven by a competitive devaluation of signal strength. This elevated signalling reaches a peak in poorly related colonies (for the parameters used in figure 2, the peak is around $r=0.35$ when $\bar{n}$ is large), where individuals signal intensely in order to manipulate their competitors into greater and/or earlier acts of cooperation. At even lower levels of relatedness, signal cost starts to decline once again (also in accordance with the predictions of previous signalling models; see, for example, Johnstone & Grafen (1992)). This trend reflects the fact that when relatedness is low, selection favours reduced investment in group cooperation, even at high densities (figure 1); consequently, the cost of competitive signalling outweighs the diminished benefit of the competitive reward.

4. DISCUSSION

The growing field of research into QSB suggests that the use of small diffusible signal molecules to coordinate group-level cooperation is widespread in bacteria, possibly ubiquitous across prokaryotes (Bassler 1999). In this paper, we have drawn attention to the strategic conflicts that may play a part in explaining the diversity of signal expression and cooperative responses in QSB. Like other forms of communication, quorum sensing and signalling are potentially subject to disruption by 'cheats'. As we have shown, however, reliable assessment of group density is still possible, provided that signal production is sufficiently costly. The need for a costly signal is in accordance with the 'handicap principle' of Zahavi (1975, 1977, 1987); in contrast to other handicap-signalling models, however, cost does not serve to differentiate between signallers that differ in quality or state.

Most previous discussions and models of signalling have focused on individual display, and even those studies that have dealt with groups of signallers typically emphasize the competitive rather than the cooperative aspects of their interaction (e.g. Godfray 1995; Yachi 1995; Rodriguez-Girones *et al.* 1996; Johnstone 1999). One notable exception is the study by McComb *et al.* (1994) of roaring and numerical assessment in contests between groups of female lions. These authors employed playback experiments to simulate territorial intrusion and found that defending females were less likely to approach playbacks of a group of three intruders than those of a single intruder; moreover, on occasions when they did approach three intruders, they did so more cautiously (the size and composition of the defenders' own group also had an effect). Lions thus seem to be capable of numerical assessment based on communal display, and the authors suggested that equivalent skills may be widespread in social species. This form of group-size assessment is not directly equivalent to quorum sensing, since it involves interaction between hostile groups, each of which stands

to gain by exaggerating its apparent size, rather than the assessment of the size of a single group by its members. It does suggest, however, that communal or group signalling (in which many signallers contribute to a joint signal) is not restricted to QSB.

The models presented here represent significant simplifications of the biological reality, as currently understood. One important simplification concerns the auto-inducing nature of HSL. The exact mechanism and timing of HSL auto-induction is subject to considerable variation between bacterial species (Salmond *et al.* 1995), yet it appears to be a widespread violation of the rule $t=ns^*$ in HSL-based quorum-signalling systems. Acceleration of signal production is in keeping, however, with the expectation of competitively driven signal inflation near the threshold value for n (figure 2). Alternatively, the signal auto-induction may only be of significance in the post-threshold upregulation of the quorum-regulated genes, leading to a rapid implementation of the cooperative phenotype (Salmond *et al.* 1995).

The model also assumes continuous availability of information on n , whereas it seems that QSB are able to recognize only discrete pre- and post-threshold states. This may be less of a limitation, as it appears that certain QSB are able to recognize and respond to multiple threshold points, through the existence of multiple quorum-sensing systems, each tied to a distinct biological response (Bassler 1999). Thus, for example, a family of hierarchical *luxR* homologues may be coordinating a diverse array of physiological processes in a density-dependent manner. It may also be the case that a simple decision rule based on one or only a few threshold inputs gives a good approximation to the game-theoretic optima presented in the models.

In an earlier theoretical study, Brookfield (1998) simulated the evolution of quorum sensing, given discrete presence-absence genotypes for both the cooperative and the signalling traits, and found that polymorphism is the expected outcome. In contrast, our model assumes that both traits are continuous, allowing the establishment of a single monomorphic ESS, m^*q^* . Considering the nature of the traits under investigation (e.g. the quantity of exoenzyme secretion and the quantity of HSL secretion), a continuous representation appears reasonable. Nonetheless, should a cooperative trait prove to be intrinsically discrete, our qualitative findings concerning signal escalation in imperfectly related colonies would still apply. Further clarification would follow from empirical investigations of trait variability both within and between closely related bacterial strains.

The interchangeability of signal molecules between diverse bacterial species raises the complex issues of inter-specific communication, interference and eavesdropping. Recent studies have highlighted the capacity for inter-species interactions in natural environments. The capacity to respond to both intra- and interspecies signals could allow a bacterium to modify its phenotype in response to both same-species cell density and the species proportion in a mixed bacterial community (Bassler 1999). For instance, the work of Pierson *et al.* (1998) suggests that the plant pathogen *Pseudomonas aureofaciens* can eavesdrop on the signal production of its bacterial rivals, and respond if necessary by the production of phenazine to eliminate

its competitors. Interactions between bacterial pathogens and their hosts also offer a potential role for quorum signalling. Quorum signalling offers an important advantage of surprise to a bacterial invader, aiding the coordination of a rapid and overwhelming attack. On the other hand, reliance on quorum signalling allows for the possibility of signal interference as a strategy for host defence. Manefield *et al.* (1999) illustrated that the seaweed *Delisea pulchra* produces a number of molecules that specifically target and inhibit quorum-sensing-controlled functions in bacteria. From a theoretical perspective, these and other complications present an impressive array of problems for future research.

In conclusion, we present a simplified model of the evolutionary ecology of cooperation and communication in QSB. The experimentally malleable nature of the key parameters driving this model ($r, \bar{n}$), together with short bacterial-generation times, offer an exciting opportunity for experimental investigation of the evolution of cooperation and communication in bacteria.

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