although it is fair to say that the problem is still not solved.

How is colour constancy achieved? It is still something of a mystery as to how your visual system achieves colour constancy, but scientists do agree that there is more than one mechanism involved. All mechanisms in some way require comparisons of the reflected light from different locations across the scene; colour constancy is a fundamentally contextual phenomenon. The simplest mechanism is adaptation of the photoreceptors in the eye — these adjust their sensitivity according to how much they are stimulated. If the amount of long-wavelength light in the overall illumination increases, the 'red' receptors will reduce their sensitivity, and so maintain a stable output. This chromatic adaptation takes time — albeit seconds. Other mechanisms may be more nearly instantaneous, such as spatial contrast.

This is broadly the mechanism that Edwin Land proposed in his famous Retinex algorithm — so-named in order not to pin down the site to either retina or cortex. The Retinex compares the 'red' light reflected from a surface with the spatial average of 'red' light reflected from surrounding surfaces, and then does the same for the green and blue channels. These three 'lightnesses' yield colour. Although temporal adaptation and spatial contrast are both 'low-level' mechanisms which do not require any image segmentation or any interpretation of the scene, it is probable that long-range, near-instantaneous spatial comparisons do require cortical processing. In human colour constancy, 'higher-level' mechanisms may also contribute, involving object recognition and memory.

Colour memory must play a role, at least in that to know whether a surface colour has changed or not requires remembering its colour from previous viewings. Memory colour may also contribute. If you recognise a particular object as a banana, you may remember its typical yellow colour, and use any deviations from this memory colour to determine the illumination colour and then correct the colours of other objects. 'This banana is less yellow than it ought to be, so the illumination must be bluish.' It might even be that you use your own skin to calibrate the colours of other objects — that you literally carry a critical 'white balance' in your own hands.

What does the future hold for colour constancy research? Mondrians are unlike real coloured objects and colour adjustment tasks are unlike the tasks of everyday life. Mondrian stimuli do not possess specular highlights, surface irregularities, shading, shadows, mutual reflections or other reflection features due to three-dimensional shape, all of which, in theory, reveal information about the illumination colour. Neither can they reveal the role of memory colours of familiar objects. To determine whether colour constancy serves a vital purpose in improving object recognition, vision scientists need to measure the limits of colour constancy for real objects, using everyday tasks ('is this the same object you saw earlier?'). There is a healthy new trend for such research. This understanding may in turn enable robots to make better use of colour, and scientists to understand better the stages of colour processing in the brain.

Further reading

Arend, L., and Reeves, A. (1986). Simultaneous colour constancy. J. Opt. Soc. Am. A *3*, 1743–1751.

Bloj, M., Kersten, D., and Hurlbert, A.C. (1999). Perception of three-dimensional shape influences colour perception through mutual illumination. Nature *402*, 877–879.

Ebner, M. (2007). Colour Constancy. John Wiley & Sons Ltd, Chichester, England.

Hansen, T., Olkonnen, M., Walter, S., and Gegenfurtner, K. (2006). Memory modulates color appearance. Nat. Neurosci. *9*, 1367–1368.

Land, E.H. (1977). The retinex theory of color vision. Sci. Am. *237*, 108–128.

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Primer

Quorum sensing

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It is now appreciated that bacteria are highly interactive and exhibit a number of social behaviours, such as swarming motility, conjugal plasmid transfer, antibiotic resistance, biofilm maturation and virulence. Many of these behaviours are regulated by diverse quorum sensing systems which are found in both Gram-negative and Gram-positive bacteria. Quorum sensing refers to the phenomenon whereby the accumulation of 'signalling' molecules in the surrounding environment enable a single cell to sense the number of bacteria (cell density), so that the population as a whole can make a coordinated response. This often leads to autoinduction of the signal, and so a rapid increase of signal concentration in the surrounding environment is observed. At critical cell densities, the binding of a regulator protein to the signal leads to the switch on of genes controlled by quorum sensing and, therefore, a coordinated population response.

For a molecule to be classed as a quorum-sensing signal, there are a number of important criteria that need to be met: first, the production of the quorum-sensing signal should take place either during specific stages of growth or in response to particular environmental changes; second, the quorum-sensing signal should accumulate in the extra-cellular environment and be recognized by a specific bacterial receptor; third, the accumulation of a critical threshold concentration of the quorum-sensing signal should stimulate a concerted response; and fourth, the cellular response should extend beyond the physiological changes required to metabolize or detoxify the molecule.

Whilst the term 'quorum sensing' has been in general use since 1994, cell-to-cell communication

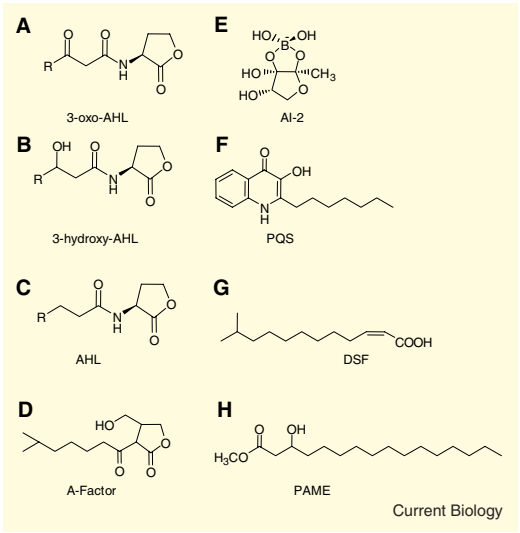

Figure 1. Structures of several quorum sensing signalling molecules.
(A) 3-oxo-AHL, *N*-(3-oxoacyl) homoserine lactone; (B) 3-hydroxy-AHL, *N*-(3-hydoxyacyl) homoserine lactone and (C) AHL, *N*-acylhomoserine lactone where R ranges from C1 to C15. (D) A-factor, 2-isocapryloyl-3-hydroxy-methyl-g-butyrolactone; (E) AI-2, autoinducer-2, furanosyl borate ester form; (F) PQS, Pseudomonas quinolone signal, 2-heptyl-3-hydroxy-4(1*H*)-quinolone; (G) DSF, 'diffusible factor', methyl dodecenoic acid; (H) PAME, hydroxyl-palmitic acid methyl ester.

in bacteria has a history that dates back to the early 1960s. Work on *Myxococcus xanthus* and *Streptomyces griseus* fruiting body formation and streptomycin production, respectively, empirically demonstrated that bacteria do not develop as individual cells in isolation. In 1970, Nealson and co-workers showed that by adding spent culture supernatants from the marine luminescent bacterium *Vibrio fischeri* to low cell density cultures, bioluminescence could be induced by an unknown compound they termed an autoinducer. The genetic mechanism required for autoinducer production and bioluminescene was identified in the early 1980s and the autoinducer identified as a member of the *N*-acyl homoserine lactone (AHL) family of molecules.

In the early 1990s it was shown that the production of the β-lactam antibiotic, 1-carbapen-2-em-3-carboxylic acid (carbapenem) by the terrestrial plant pathogen *Erwinia carotovora* was also regulated by AHLs. Since then, many Gram-negative species have been shown to have AHL quorum-sensing systems which regulate a wide variety of different phenotypes. Signalling is not restricted to Gram-negative bacteria, a number of Gram-positive organisms have been shown to employ small, modified oligopeptides as extracellular signalling molecules. These peptides activate gene expression by interacting with two-component histidine protein kinase signal transduction systems. For example, in *Staphylococcus aureus*, the expression of a number of cell-density-dependent virulence factors is regulated by the global regulatory locus *agr* (*accessory gene regulator*). To date, a number of diverse compounds have been identified as bacterial cell-to-cell quorum sensing signal molecules in both Gram-negative and Gram-positive bacteria (Figure 1). Importantly, virulence has been shown to be regulated by quorum sensing in a number of different organisms, including the opportunistic pathogen *Pseudomonas aeruginosa*. This has led to a widespread interest in being able to block cell-to-cell communication in bacteria, a process commonly termed 'quorum quenching'.

Since 1994, there has been an exponential increase in the number of publications on quorum sensing (to date >1500 articles) and the literature often assumes that just because a molecule produced by one cell influences the behaviour of another, then it should be classed as a 'signal'. We should be cautious when describing all quorum-sensing systems as signalling systems, as understanding the true nature of the interaction between cells is important if we are to successfully develop novel antimicrobials based on quorum sensing. For a molecule to be classed as a true signal, it should be beneficial for a receiver cell to respond and this response should benefit the producer cell. Alternatively, a molecule may be used as a cue for a receiver, which may guide a future action. In this case, the molecule may not benefit the producer and, therefore, cannot be strictly defined as a signal. A third possibility is that a molecule may 'coerce' a receiver cell into an action, which may be detrimental to its fitness, yet this action benefits the producer cell. Again, this cannot be classed as true signalling. One of the future challenges for researchers into quorum sensing is to determine which of these types of interaction they are observing when studying a particular bacterial system. This becomes especially important when an interaction between two distinct bacterial species is observed, a process known as bacterial cross-talk (see below).

What does quorum sensing regulate?

Quorum sensing has been shown to regulate a number of diverse phenotypes in both Gram-negative and Gram-positive bacteria, including antibiotic production, fruiting body development and sporulation (Figure 2). Importantly, quorum sensing has been demonstrated to be required for the full virulence of a number of important pathogenic organisms, including *P. aeruginosa, S. aureus, Burkholderia pseudomallei, Burkholderia cenocepacia* and *Vibrio cholerae*. The opportunistic pathogen *P. aeruginosa* uses both AHL and 2-alkyl-4-quinolones (AQs) as quorum-sensing signal molecules. In this organism, quorum sensing regulates the production of an arsenal of virulence determinants, including elastase, pyocyanin and lectins. Disruption of quorum sensing has been shown to reduce virulence in several animal hosts, including nematodes and mice. It is intriguing to note, however, that many clinical strains of *P. aeruginosa* isolated from the sputum of chronically infected cystic fibrosis patients, are defective in quorum sensing. This demonstrates that there is still much to learn regarding bacterial quorum sensing and host

interactions and this is likely to be a competitive future area of research.

Quorum sensing has also been demonstrated to be important in the development of biofilms. In the formation of biofilms, cells abandon the isolation of the planktonic mode of growth and group together to form organised 'slime-cities'. Biofilms are often highly resistant to ultra-violet irradiation, desiccation and treatment with antibacterial agents such as antibiotics. These structures often contain channels for the import of nutrients and the disposal of waste products and they may even contain specialist cells, which appear to have specific roles within the biofilm. Some people have described biofilms as akin to social insect societies such as those seen in ants. In several species of bacteria, disruption of the quorum sensing system has been shown to affect biofilm formation and differentiation. For example, in *P. aeruginosa,* inactivation of quorum sensing results in the formation of flatter, less structured biofilms than those seen in the corresponding wild type. Furthermore, when quorum sensing is disrupted, the biofilms formed are often more susceptible to treatment with biocides and antibiotics.

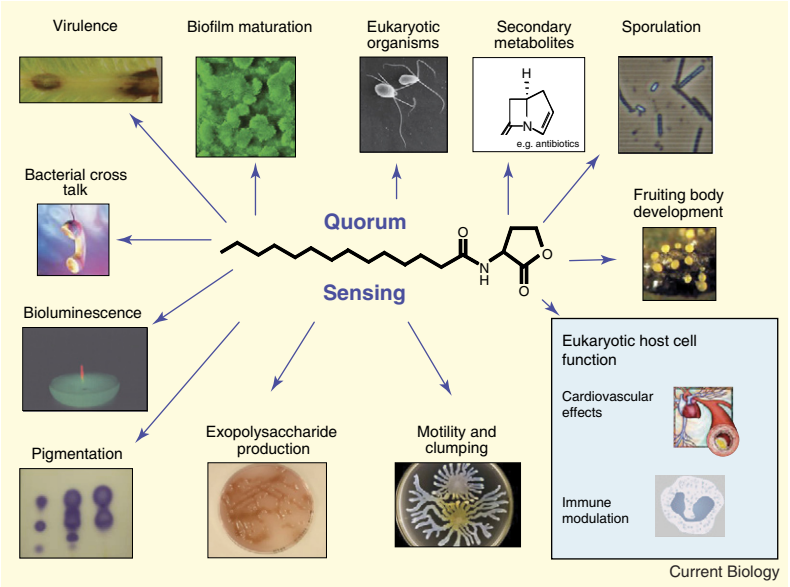

Figure 2. Phenotypes shown to regulated by quorum sensing in Gram negative and Gram positive bacteria. Sporulation picture (top right) courtesy John Heap; fruiting body picture (middle right) courtesy Michiel Voss and Greg Velicer.

Bacterial cross-talk and symbiosis

Initial research on quorum sensing focused on the roles signalling systems have in individual bacterial populations. However, the discovery that different Gram-negatives could make very similar if not identical signal molecules prompted the idea that these signals may be exploited as a cross-talk mechanism between very distinct organisms sharing the same environment. An example of this is *P. aeruginosa* and *Burkholderia cenocepacia* which co-exist in the lungs of cystic fibrosis patients. In this particular case, AHLs produced by *P. aeruginosa* can be perceived by *B. cenocepacia* but not vice versa, suggesting that in this particular example, the communication is unidirectional. Furthermore, cross-talk using quorum sensing signals can also take place between Gram-negative and Gram-positive bacteria. This is the case for *P. aeruginosa* and *S. aureus,* which also co-exist in the lungs of cystic fibrosis patients, where AHLs from the former can influence the expression of virulence determinants in the latter, and 2-alkyl-4-quinolones (AQs) can induce the formation of small colony variants and increase the resistance to antibiotics. If we examine more complex environments like the rhizosphere, where many different quorum-sensing signal producing bacteria co-exist, it would be easy to imagine the existence of a highly complex intercellular quorum sensing-driven signalling network which enables these poly-microbial communities to maintain an ecological balance.

In addition to controlling gene expression in bacterial populations, AHLs have also been found to be directly recognised by eukaryotic cells and even to influence the behaviour of eukaryotic organisms. AHLs have been shown in many different studies to have immuno-modulatory effects, influencing the production of cytokines that in turn will determine the type of immune response elicited upon infection. Furthermore, AHLs can also have cardiovascular effects by inducing relaxation of blood vessels. If we put these two effects into the context of infection it becomes apparent that bacteria have the power to influence immune responses, probably to their benefit, and stimulate the delivery of nutrients for their survival by increasing the blood supply.

Outside the clinical context, AHLs can also be perceived by eukaryotic organisms. This is the case with the green seaweed *Ulva*. This alga reproduces by zoospores which, once released from a fertile tip, need to search for adequate surfaces where they can settle and germinate. There is now substantial evidence showing that bacterial biofilms producing AHLs can influence the settlement of these spores and that this effect is a result of the sensing of these signal molecules by the zoospores. This is supported by the fact that the spores settle preferentially on microcolonies where the AHL concentration reaches its highest. Although the exact mechanism by which AHLs influence these responses is not known, these signal molecules can affect calcium influx in the spores which in turn controls their motility towards the surfaces where they eventually settle. From a biological perspective this appears to be an excellent symbiotic relationship, since the alga needs to be associated with bacteria

to differentiate and the bacteria probably associates with the alga for nutritional purposes, especially considering the poor availability of nutrients in sea water.

Plants have also been shown to respond to AHLs. There are two very interesting examples of this. Roots from the legume *Medicago truncatula* can respond to small concentrations of these signal molecules by changing the levels of more than 150 proteins. This indicates that this plant may have developed the ability to sense these signal molecules to induce global changes in its physiology upon the presence of AHL-producing bacteria. However, the real consequences of this interaction are not fully understood. Another interesting example is that of AHL-producing bacteria in the tomato rhizosphere. In this case, the tomato plant can obtain excellent benefits from the sensing of these signal molecules, as this results in increases in salicylic acid levels in plant leaves which enhances systemic resistance against fungal pathogens. This suggests that AHLs play a key role in the biocontrol activity of the rhizosphere bacteria.

Quorum-sensing signalling systems are quite vulnerable and can be quenched by other bacteria, eukaryotic cells or even eukaryotic organisms. A number of bacteria co-existing with AHL-producers have been found to produce enzymes which can degrade these signal molecules. This has been shown in soil, where *Bacillus* strains produce lactonase enzymes responsible for this activity. However, the biological significance of these is not fully understood, as it not known whether the main purpose of these enzymes is to quench quorum sensing-mediated responses. Interestingly, it should be noted that there are some organisms which have both the ability to synthesise as well as degrade quorum sensing molecules. An example of this is *P. aeruginosa* in which a number of enzymes have been shown to have the ability to inactivate AHLs although their main biological role may be other than the shutting down of quorum sensing-mediated responses.

The fact that quorum-sensing regulates the expression of key pathogenic traits makes it reasonable to assume that some eukaryotic organisms will develop mechanisms to protect themselves against bacterial damage. This is the case for the opportunistic fungal pathogen *Candida albicans* which, via the production of the cell–cell signalling molecule farnesol, can inhibit the production of AQs in *P. aeruginosa*. These two organisms live in close association in clinical environments where *P. aeruginosa* has been found to form biofilms on the surface of *C. albicans* hyphae, affect fungal morphology and even kill fungal cells with some of its exoproducts. Consequently, by inhibiting AQ production, *Candida* may be preventing the strong pressure on life-style and survival imposed by *P. aeruginosa.* The fact that farnesol is ubiquitous in nature suggests the possibility that many other organisms in nature may be capable of modulating the virulence of *P. aeruginosa*.

Another example of chemical inhibition of quorum sensing is provided by the macroalgae *Delisea pulchra.* This alga produces halogenated furanones which effectively antagonise AHL-mediated responses in many bacteria and could be one of the mechanisms used to prevent the colonisation of its surface by biofouling bacteria. Even human cells have now been found to synthesise enzymes which are able to degrade AHLs possibly as a strategy to prevent damage by bacterial pathogens. The approaches used by bacteria and eukaryotic organisms to inhibit quorum sensing-signalling has formed the basis for the development of novel antimicrobials to fight infectious diseases.

How can quorum sensing be maintained in natural populations?

One of the major challenges for evolutionary biologists is how to explain altruistic behaviours, where actions that increase another individual's fitness come at a cost to your own, as natural selection appears to favour selfish, uncooperative individuals. Little attention has been given to the evolutionary implications of quorum sensing, which in effect could be seen as altruistic behaviour by bacterial cells. In the quorum-sensing literature it is generally assumed that quorum sensing is selected for because it benefits the local group or population as a whole. However, evolutionary theory suggests that cooperative communication is only maintained by selection under fairly restrictive conditions. This raises the question whether quorum sensing in microbes is truly a cooperative behaviour. If it is then it should be subjected to invasion by social cheaters who gain the benefits of quorum sensing behaviour but do not pay any of the costs in performing the behaviours. How then could quorum sensing be maintained in natural populations without being overrun by cheats? One way is that quorum sensing signalling will be maintained by kin selection, that is, signalling will be maintained between close relatives. These questions have recently been explored from both theoretical and empirical perspectives, the results of which have been complementary. It does appear that quorum sensing communication in bacterial populations suffers from the same problem of exploitability by cheats that has been described many times in animals.

Further reading

Diggle, S.P., Gardner, A., West, S.A., and Griffin, A.S. (2007). Evolutionary theory of bacterial quorum sensing: when is a signal not a signal? Phil. Trans. R. Soc. Lond. B. *362,* 1241–1249.

Dudler, R., and Eberl, L. (2006). Interactions between bacteria and eukaryotes via small molecules. Curr. Opin. Biotechnol. *17,* 268–273.

Waters, C.M., and Bassler, B.L. (2005). Quorum sensing: cell-to-cell communication in bacteria. Annu. Rev. Cell. Dev. Biol. *21,* 319–346.

West, S.A., Griffin, A.S., Gardner, A., and Diggle, S.P. (2006). Social evolution theory for microorganisms. Nat. Rev. Microbiol. *4,* 597–607.

Williams, P., Winzer, K., Chan, W.C., and Camara, M. (2007). Look who's talking: communication and quorum sensing in the bacterial world. Phil. Trans. R. Soc. Lond. B. *362,* 1119–1134.

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