

# Cooperation and conflict in quorum-sensing bacterial populations

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**It has been suggested that bacterial cells communicate by releasing and sensing small diffusible signal molecules in a process commonly known as quorum sensing (QS)<sup>1–4</sup>. It is generally assumed that QS is used to coordinate cooperative behaviours at the population level<sup>3,5</sup>. However, evolutionary theory predicts that individuals who communicate and cooperate can be exploited<sup>6–10</sup>. Here we examine the social evolution of QS experimentally in the opportunistic pathogen *Pseudomonas aeruginosa*, and show that although QS can provide a benefit at the group level, exploitative individuals can avoid the cost of producing the QS signal or of performing the cooperative behaviour that is coordinated by QS, and can therefore spread. We also show that a solution to the problem of exploitation is kin selection, if interacting bacterial cells tend to be close relatives. These results show that the problem of exploitation, which has been the focus of considerable attention in animal communication, also arises in bacteria.**

An exponentially increasing body of research suggests that communication between bacterial cells can occur by QS. The uptake of QS signalling molecules has two consequences. First, they regulate a variety of behaviours such as the production and secretion of diverse exoproducts, which have various uses: virulence factors that damage the host, nutrient scavenging molecules (for example siderophores), compounds for providing structure for growth in biofilms, and surfactants for facilitating movement. Most of these exofactors seem to provide a benefit to surrounding cells in the local population and represent ‘public goods’ or cooperative behaviours<sup>11,12</sup>. Second, the uptake of signal molecules also leads to an increase in production of the signal molecules (termed autoinduction). This often leads to positive feedback at high cell densities and to a marked increase in the cooperative production of exoproducts<sup>4</sup>. The assumption here is that many cooperative behaviours, such as the production of exoproducts, are not worth performing until a sufficient number of cells (a quorum) are present, and that QS provides a mechanism for stimulating such behaviours only when high enough cell population densities are reached.

It is frequently assumed that QS has been selected for because it optimizes population growth or survival<sup>3,5</sup>. However, evolutionary theory shows that a behaviour can be favoured by selection only if it increases the inclusive fitness of the individual actor, which is often not the same as maximizing population fitness<sup>7–10,12</sup>. In particular, a large body of evolutionary theory, and empirical studies on a range of other organisms, have shown that cooperation and communication can generate conflicts of interest between individuals<sup>6–9</sup>. QS seems to involve using cooperative, honest communication to coordinate other cooperative behaviours, and so a double evolutionary problem arises. Specifically, populations are at risk from the invasion of selfish cheats, who exploit either the signalling or the exoproduct production of others<sup>1,2,13,14</sup>. A cheat that does not produce QS molecules can

benefit from monitoring the local cell density without investing effort into the dissemination of this information (‘signal negative’). Alternatively, a cheat could produce the signal but not increase the production of exoproducts in response (‘signal blind’), exploiting (and encouraging) the cooperative production of exoproducts by other cells.

Here we examine one of the best-understood QS systems, that of *Pseudomonas aeruginosa*, a Gram-negative bacterium, capable of causing disease in plants and animals, including humans<sup>15</sup>. QS is crucial to the success and virulence of *P. aeruginosa* because it controls behaviours such as biofilm development, swarming motility and the production of an arsenal of extracellular virulence factors that are capable of causing extensive tissue damage, bloodstream invasion and consequently promoting systemic dissemination<sup>4,15</sup>. In this bacterium, QS is controlled by two pathways that regulate the production of *N*-acyl homoserine lactone (AHL) signalling molecules (see Supplementary Fig. 1). These two systems are termed *las* and *rhl*, and use different AHL signal molecules, synthesized by means of the signal generators LasI (*N*-(3-oxododecanoyl)-L-homoserine lactone; 3O-C12-HSL) and RhlI (*N*-butanoyl-L-homoserine lactone; C4-HSL), respectively<sup>15</sup>. The importance of QS in controlling the behaviours of *P. aeruginosa* is emphasized by the fact that 6–10% of its genome is regulated by the *las* and *rhl* systems<sup>16</sup>.

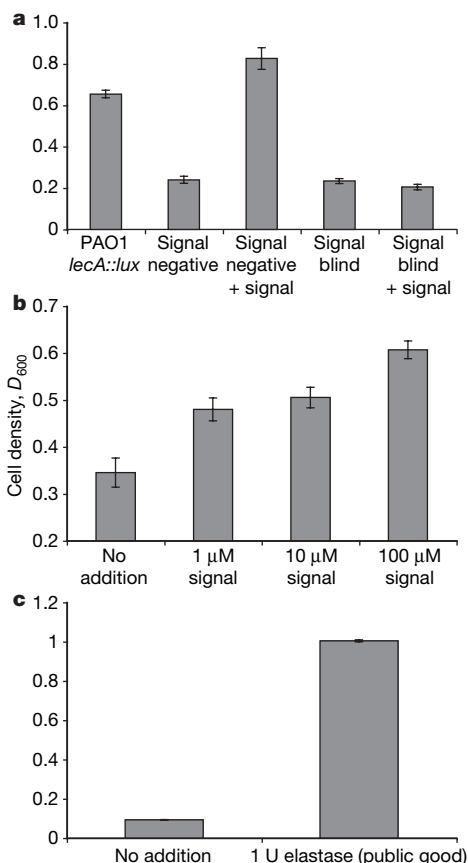
We examine the fitness consequences of QS and determine the possibilities for cheating. We focus on the *las* QS pathway because this system controls the *rhl* system hierarchically; a mutation in *las* QS results in a general abolition of QS in *P. aeruginosa*<sup>15,17,18</sup>. To examine the costs and benefits of QS, we used two types of cheating QS mutant. First, we used a ‘signal-negative’ mutant that does not produce the 3O-C12-HSL signal but still responds to signal—the *lasI* mutant (PAO1 *lecA::luxΔlasI*<sup>19</sup>). Second, we used a ‘signal-blind’ mutant that does not respond to extracellular signal—the *lasR* mutant (PAO1 *lecA::luxΔlasR*<sup>19</sup>). The signal-blind mutant does not increase signal production in response to extracellular signal and so a relatively small amount of signal is produced. Both mutants are found in natural populations, with the signal-blind strain being more common<sup>20</sup>. Furthermore, we experimentally altered the level of signal perceived by either the wild type or the signal-negative mutant by adding to cultures a synthetic signal chemically identical to that produced by *P. aeruginosa*<sup>21</sup>.

We first examined the fitness consequences of QS in an environment in which cooperation is favoured. Proteases are a group of exoproducts whose production is controlled by QS. *P. aeruginosa* produces a number of well-known proteases such as alkaline protease, the LasA protease, and the LasB protease more commonly known as elastase, which is completely dependent on a functional *las* QS system<sup>15</sup>. In a host, elastase is released from a bacterial cell to digest several proteins including elastin, fibrin and collagen. Digested

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protein can then be internalized by a bacterial cell and used as nutrition to promote growth. We examined the growth of the wild type and the *lasI* (signal-negative) and *lasR* (signal-blind) mutants in a medium in which the ability to make proteases is required for growth; we term this the quorum-sensing growth medium (QSM).

We found that QS provides a benefit at the population level by increasing the production of cooperative exoproducts that can aid growth in certain environmental conditions. Both the signal-negative and the signal-blind mutants showed significantly poorer growth than the parental wild-type strain in the QSM (Fig. 1a;  $F_{(1,32)} = 710.92$ ,  $P < 0.0001$ ). To confirm that this difference in growth yield was caused by QS, we examined the roles of signalling and the production of public goods (protease) separately. Specifically, we found the following: first, the addition of synthetic signal (20  $\mu\text{M}$  3O-C12-HSL) to the signal-negative strain significantly improved growth yield (Fig. 1a;  $F_{(1,31)} = 9.57$ ,  $P < 0.01$ ), as would be expected because this will stimulate the production of proteases such as elastase; second, the signal-negative mutant grew more rapidly as higher concentrations of synthetic signal were added (Fig. 1b;  $F_{(1,22)} = 79.90$ ,  $P < 0.0001$ ,  $r^2 = 0.78$ ;  $n = 24$ ), because this will stimulate greater production of proteases; third, addition of the synthetic signal to the wild type resulted in a significant increase in the expression of the *lasB* elastase structural gene, confirming an increased production of the elastase public goods that break down proteins ( $F_{(1,18)} = 302.2$ ,



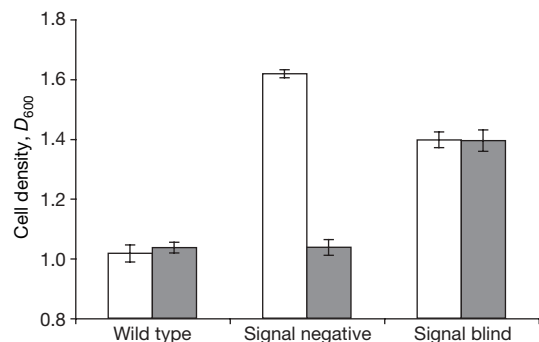
**Figure 1 | QS-controlled public goods provide a benefit at the population level in QSM.** **a**, The wild type grows (attenuance ( $D_{600}$ ) measurements after 24 h of growth) significantly more rapidly than the signal-blind and signal-negative mutants. Addition of synthetic signal improves the growth of the signal-negative mutant but not the signal-blind mutant (seven independent replicates per treatment). **b**, Increasing synthetic signal concentration improves cooperation and growth in a signal-negative mutant. The graph shows  $D_{600}$  measurements after 30 h of growth; six replicates per treatment. **c**, Addition of synthetic elastase significantly increases the growth of the signal-blind mutant after 20 h in QSM. All results are shown as means  $\pm$  s.e.m, five replicates per treatment.

$P < 0.0001$ ,  $n = 20$ ); fourth, addition of signal to the signal-blind strain resulted in no improvement in growth (Fig. 1a;  $F_{(1,30)} = 2.3$ ,  $P > 0.1$ ), as would be expected because the cells do not respond to the signal; and fifth, addition of purified elastase (porcine elastase) to the signal-blind strain increased growth (Fig. 1c;  $F_{(1,8)} = 116,800$ ,  $P < 0.0001$ ,  $n = 10$ ), by directly providing the public goods that allow proteins to be broken down.

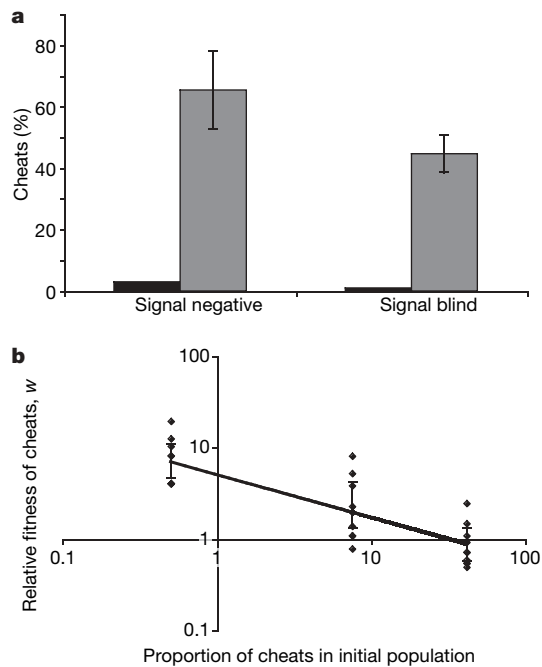
We also found that the production of the QS signal molecules and QS-dependent exoproducts (public goods) are metabolically costly. We compared the growth yield of the mutants and the wild type in nutrient-rich Luria–Bertani (LB) broth, in which the exoproducts produced by QS are not needed for growth. In these conditions, 22 h after inoculation, the QS mutants had grown to a higher density than the wild type (Fig. 2;  $F_{(1,34)} = 257.1$ ,  $P < 0.0001$ ). The cost of the response to QS molecules is further shown by the fact that addition of synthetic signal to the signal-negative mutant resulted in a decreased growth yield (Fig. 2;  $F_{(1,32)} = 1,043.32$ ,  $P < 0.0001$ ); it was significantly lower than that obtained with the signal-blind strain with or without signal (Fig. 2;  $F_{(1,33)} = 29.33$ ,  $P < 0.01$ ). Regulation of public goods production by QS generally occurs in the transition from exponential to stationary phase, and one would therefore expect fitness costs to occur primarily in the stationary phase. To test this, we examined growth of the wild type and QS mutants over time and found that growth rates were similar during exponential growth and that fitness differences occurred in the stationary phase (see Supplementary Fig. 3).

We then tested whether QS cheats can spread in a population, because they benefit from the signal or exoproducts produced by others without paying the production costs themselves<sup>1,2,11,14</sup>. We initiated populations of the wild type with a small proportion (1–3%) of one of the two mutants and followed their growth in QSM over 48 h. During this time, the signal-blind cheats increased in frequency from 1% to 45% and the signal-negative mutant increased from 3% to 66% (Fig. 3a). Consequently, over 48 h of growth, in mixed cultures, both the signal-blind and the signal-negative mutants had a significantly higher fitness than the parental wild type (in both cases,  $P = 0.02$ ,  $n = 6$ ; the relative fitness of the two mutants were 81-fold and 63-fold that of the wild type, respectively). Social evolution theory also predicts that the fitness of cheats in microbial populations should be frequency dependent, with relative fitness decreasing as cheats become more common<sup>22,23</sup>. As cooperators become more common, there is greater population growth and hence more opportunity for cheats to exploit cooperators<sup>22</sup>. In support of this prediction, we found that the relative fitness of the signal-blind cheat decreased significantly when it was more common (Fig. 3b;  $F_{(1,28)} = 57.15$ ,  $P < 0.0001$ ,  $n = 30$ ,  $r^2 = 0.671$ ).

Our results show that QS is a social trait, susceptible to exploitation and invasion by cheats. Given this, how is QS maintained in



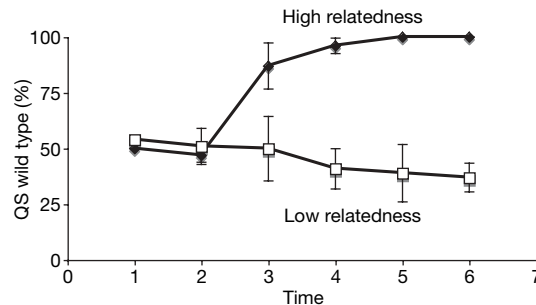
**Figure 2 | QS-dependent cooperation is costly.** Final cell density of wild type and mutants in a rich (LB) growth medium in the absence (open columns) and presence (filled columns) of signal (5  $\mu\text{M}$  3O-C12-HSL). All results are shown as means  $\pm$  s.e.m. for six independent measurements per treatment.



**Figure 3 | QS is subject to cheating.** **a**, QS signal-negative and signal-blind cheats invade populations of wild-type cooperators over 48 h of growth in QSM. All results are shown as means  $\pm$  s.e.m. (estimated assuming binomial errors and logit transformation) for six independent measurements per treatment. Black and grey bars represent the starting and final percentage of cheaters in the population, respectively. **b**, Invasion and relative fitness of signal-blind cheats are frequency dependent over 48 h of growth in QSM, with cheats having a higher fitness when they are less common. Relative fitness is the estimated growth rate of cheats relative to that of cooperators. The curve is a power regression line fitted to the raw data ( $n = 30$ ). All results are shown as means  $\pm$  s.e.m.

natural populations? The most likely explanation is kin selection<sup>8</sup>—if neighbouring cells tend to be close relatives they will have a shared interest in communicating honestly, and cooperating<sup>2,14</sup>. Kin selection is likely to be highly important in microbial social behaviours such as QS because of clonal reproduction and relatively local interactions<sup>11</sup>. We tested the importance of kin selection for QS with an experimental evolution approach. We maintained populations under conditions of relatively high and relatively low relatedness<sup>24</sup>, and examined how this influenced the relative fitnesses of the wild type and the signal-blind *lasR* mutant (see Supplementary Fig. 2). As predicted by kin selection theory<sup>14</sup>, QS was favoured at a relatively high relatedness (Fig. 4;  $F_{(1,4)} = 553.6$ ,  $P < 0.0001$ ,  $n = 6$ ). In the high-relatedness treatment, the wild type and the signal-blind mutant tended to be in different subpopulations, preventing exploitation of the wild type by cheats. In contrast, in the relatively low-relatedness treatment, subpopulations could contain both QS cooperators and the signal-blind cheats, allowing the signal-blind cheats to exploit the production of public goods (proteases) by the cooperative QS individuals and hence to increase in frequency within the population.

Our results and social evolution theory can help explain the prevalence of QS mutants in natural populations. First, in both environmental and clinical isolates, QS cheats are often found that do not perform normal QS behaviours<sup>20,25,26</sup>. The vast majority of these are signal-blind mutants that do not respond to signal. This is to be expected because signal-blind mutants avoid the cost of producing both the signal and exoproducts regulated by QS, whereas signal-negative mutants avoid only the cost of producing signal, which is likely to be far less metabolically costly than producing the exoproducts. Second, it has frequently been observed that such QS mutants are able to invade and spread in clinical settings, such as the lungs of



**Figure 4 | Kin selection and QS.** The proportion of quorum-sensing individuals (wild type) is plotted against time (rounds of growth). QS is favoured by higher relatedness: open squares, low relatedness; filled diamonds, high relatedness. All results are shown as means  $\pm$  s.e.m. for three independent replicate experiments per treatment.

humans with cystic fibrosis<sup>25,27</sup>. Populations of *P. aeruginosa* in the human lung are subject to low levels of dispersal in and out of the lung<sup>28</sup>, leading to relatively local competition within the lung. Such local competition reduces kin selection for cooperative behaviours such as QS, even if there are small numbers of lineages within the lung<sup>29</sup>. Consequently, kin selection theory predicts the observed pattern that QS cheats should invade over time in lung infections. More generally, we would predict a higher prevalence of QS cheats in any situation in which the average relatedness between interacting cells was lower or in which there was a greater intensity of local competition.

## METHODS SUMMARY

**Strains and growth conditions.** The *P. aeruginosa* strains PAO1 *lecA::lux* or PAO1 were used as QS-positive cooperating strains; *lasI* (signal-negative) and *lasR* (signal-blind) mutants were used as QS cheats. Strains were routinely grown in LB broth. For growth competition assays we used QSM. This consisted of OS minimal medium<sup>30</sup> supplemented with 1% BSA (Sigma) as the sole carbon source. For growth-curve experiments we grew strains in 300  $\mu$ l of LB broth or QSM in a combined automated luminometer–spectrometer (GENios Pro; Tecan Group). The QS experimental evolution study to test whether selection for QS could be influenced by indirect fitness benefits was based on the global competition treatments of a previous experiment<sup>24</sup>.

**Full Methods** and any associated references are available in the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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**Supplementary Information** is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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## METHODS

**Bacterial strains and growth conditions.** We grew bacteria in LB broth or on *Pseudomonas* isolation agar (Difco). The *P. aeruginosa* strains PAO1 *lecA::lux<sup>+</sup>* or PAO1 were used as QS-positive cooperating strains. The construction of *lasI* (signal-negative) and *lasR* (signal-blind) cheating mutants in PAO1 *lecA::lux* is described elsewhere<sup>19</sup>. Although the *lasI* mutant (PAO1 *lecA::luxΔlasI*) is unable to produce the 3O-C12-HSL signal<sup>19</sup>, it still responds to the presence of signal, as shown by the fact that in the presence of the 3O-C12-HSL signal (20 μM), but not in its absence, it significantly expresses the QS-dependent *lecA* gene (which codes for a galactophilic lectin)<sup>31</sup> ( $n = 12$ ,  $P < 0.0001$ ) and produces the QS-dependent protease elastase ( $n = 6$ ,  $P < 0.0001$ ). The *lasR* mutant (PAO1 *lecA::luxΔlasR*) produces minimal amounts of signal<sup>19</sup> but does not respond to extracellular signal as shown by the fact that *lecA* expression ( $n = 12$ ,  $P = 0.919$ ) and elastase production ( $n = 6$ ,  $P = 0.072$ ) are not restored on the provision of signal.

We routinely grew all strains at 37 °C in 5 ml of LB broth in 20-ml tubes (Sterilin) with shaking at 200 r.p.m. QSM was made by using OS minimal medium<sup>30</sup> supplemented with 1% BSA (Sigma) as the sole carbon source. For growth curve experiments, we grew strains in 300 μl of LB broth or QSM in a combined automated luminometer–spectrometer (GENios Pro; Tecan Group). Growth was followed by the determination of  $D_{600}$  values, and viable counts (colony-forming units) were obtained by plating suitable dilutions made in saline (0.9% NaCl) onto LB agar. Where required, we added synthetic AHL signal<sup>21,32</sup> at the concentrations indicated.

Unless stated otherwise, we performed all analyses by model simplification to the minimum adequate model, using generalized linear modelling techniques implemented in GLMStat 6.0 (Kagi Shareware). When analysing the effect of adding signal on the signal-negative mutant, we used  $\log_e(\text{signal} + 1)$  logging to linearize values and then added 1 to account for zeros.

**Growth competition assays.** We determined that protease production is an appropriate public-goods cooperative trait for our experiments. We measured the growth rates of a wild-type (cooperator) strain that produces QS-dependent proteases and two mutant strains (cheats) that either do not make QS signal but can respond to signal and thus make proteases (signal-negative) or do make minimal amounts of signal and do not respond (signal-blind). For growth competition assays, we first grew PAO1 (QS positive cooperator) and PAO1 *lecA::luxΔlasI* and PAO1 *lecA::luxΔlasR* (cheats) overnight separately in LB broth. Attenuance ( $D_{600}$ ) readings were performed, and PAO1 and the relevant cheat were then mixed together in the desired ratio (namely 99:1). To determine the exact starting ratio, a sample was then immediately taken and we performed serial dilutions to obtain single colonies on LB agar plates. These were incubated overnight at 37 °C and the ratio of cheats was determined with a light camera (E. G. & G. Berthold): cheats contained the *lux* genes and were therefore bioluminescent, whereas PAO1 was not. From the initial inoculum we transferred 20 μl to 200 μl of QSM and this was incubated for 48 h at 37 °C. Samples were then taken, serial dilutions were performed to obtain single colonies, and the cheat ratio was determined as above.

These data were analysed with a non-parametric sign test, because relative fitness ( $w$ ) is non-normally distributed (Shapiro–Wilkinson test; signal-negative,  $P = 0.001$ ; signal-blind,  $P = 0.02$ ; Fig. 3a). In this experiment, the fitness of the signal-blind mutant was about 81-fold that of the wild type, and the fitness of the signal-negative mutant was about 63-fold that of the wild type. Furthermore, our estimates of the relative fitness of the QS mutants are underestimates because they must also pay the metabolic cost of expression of the *lux* reporter. We tested for frequency dependence by examining how the relative fitness of the signal-blind mutant changed as its initial starting frequency in the population was varied from about 1% to 50% (Fig. 3b). For frequency-dependence experiments, ratios of about 1%, 10% and 50% cheats (signal-blind) were mixed with PAO1. From these inocula we transferred 20 μl to 200 μl of QSM and incubated for 48 h at 37 °C. Samples were then taken, serial dilutions were performed to obtain single colonies, and the cheat ratio was determined as above.

**Selection experiment.** We performed an experimental evolution study to test whether selection for QS can be influenced by kin selection. Our design is based

on the global competition treatments of a previous experiment<sup>24</sup> (see Supplementary Fig. 2). We used the signal-blind *lasR* mutant because this is the mutant most commonly observed in nature<sup>20,25–27</sup>. Our experiment involved two treatments: relatively high relatedness and relatively low relatedness<sup>24</sup>. Each treatment was replicated three times. Within each replicate we split each population into 12 subpopulations. We initiated each subpopulation with a 1:1 mix of the wild type and the signal-blind mutant. We did this by growing PAO1 wild type (QS positive cooperator) and the signal-blind strain (cheat) individually overnight in LB medium, taking attenuation measurements to measure cell density, and then mixing the strains in a single tube at a 1:1 ratio. We then added 20-μl aliquots of the 1:1 mix into 12 subpopulations in wells of a 96-well plate, each containing 200 μl of QSM, and incubated this plate for 48 h at 37 °C. We left these subpopulations to grow in QSM for 48 h, after which the populations were pooled and used to initiate 12 new subpopulations, and the process was repeated. We measured the ratio of cooperators to cheats in the mixed population by serially diluting the mixed tube, plating on LB agar plates to obtain single colonies, incubating these plates for 24 h, and viewing with a light camera. We manipulated relatedness by varying how the mix of the subpopulations was used to initiate the 12 new subpopulations. We achieved relatively high relatedness by initiating each subpopulation with a single colony, taken at random from a sample of the subpopulation mix. Specifically, we inoculated single colonies into 12 wells containing LB medium, incubated these at 37 °C for 24 h and then used 20 μl of culture from each well to inoculate a new subpopulation. For low relatedness we conducted the same procedure, except that the initial inoculation was performed with 20 μl of the pooled sample (about  $1.2 \times 10^9$  cells), rather than a single colony. We then incubated these subpopulations for 48 h at 37 °C, as described above, and repeated this procedure for a total of six rounds of growth in subpopulations. Before data analysis, we arcsin square root transformed the proportion of cooperators to account for the non-normality of proportion data.

**Time and cell-density-dependent measurement of bioluminescence.** We determined bioluminescence as a function of cell population density with a combined automated luminometer–spectrometer (GENios Pro; Tecan Group). We diluted overnight cultures of *P. aeruginosa* 1:1,000 (in LB medium) or 1:20 (in QSM), and 0.2-ml cultures were grown in 96-well microtitre plates. Luminescence and turbidity were automatically determined every 30 min. Luminescence was measured in relative light units. Where required, we supplied AHL signal at the concentrations indicated. AHLs were synthesized as described previously<sup>21</sup>. AHL was dissolved in methanol before it was added to growth medium at the indicated concentrations.

**Calculation of relative fitness ( $w$ ) of strains.** We calculated the relative fitness of cheating mutants ( $w$ ) by comparing the frequency of cheats at the beginning and end of the experiment. Specifically,  $w$  is given by  $[x_2(1 - x_1)]/[x_1(1 - x_2)]$ , where  $x_1$  is the initial proportion of cheats in the population and  $x_2$  is their final proportion<sup>22</sup>. For example,  $w = 2$  would correspond to the mutant's growing twice as fast as the wild-type cooperator.

**Assay for elastolytic activity.** We determined the elastolytic activity of bacterial supernatants by using the elastin Congo red (ECR; Sigma) assay as described previously<sup>33</sup>. We added a 100-μl aliquot of bacterial supernatant to 900 μl of ECR buffer (100 mM Tris-HCl pH 7.5, 1 mM CaCl<sub>2</sub>) containing 20 mg of ECR and incubated the mixture with shaking at 37 °C for 3 h. We removed insoluble ECR by centrifugation, and the  $A_{495}$  of the supernatant was measured. LB medium was used as a negative control.

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- Chhabra, S. R. *et al.* Autoregulation of carbapenem biosynthesis in *Erwinia carotovora* by analogs of *N*-(3-oxohexanoyl)-L-homoserine lactone. *J. Antibiot. (Tokyo)* **46**, 441–454 (1993).
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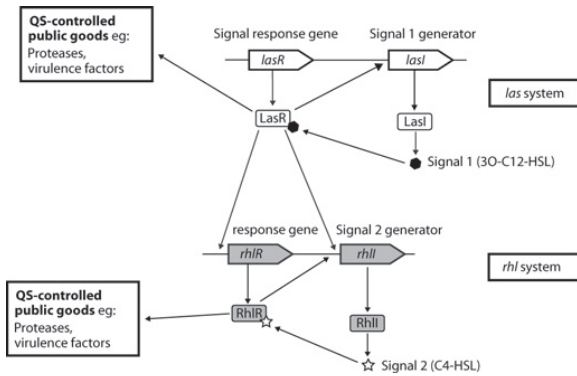
**SUPPLEMENTARY DATA:****Growth rates of wild type and QS mutants are similar in a rich growth medium.**

Previously it has been reported that QS mutations resulted in a decreased competitiveness and growth yield of *P. aeruginosa* in co-culture with *Agrobacterium tumefaciens*, suggesting that in mixed cultures, QS gives a fitness advantage, possibly due to enhanced exoproduct release<sup>1</sup>. In contrast, our results in Fig. 2 (main manuscript) suggest that in mono-culture, QS mutants have a fitness advantage with a significantly increased growth yield compared to the wild type in stationary phase cultures. In a previous long term evolution study, increased fitness of

1 An, D. D., Danhorn, T., Fuqua, C., & Parsek, M. R. Quorum sensing and motility mediate interactions between *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens* in biofilm cocultures. *P. Natl. Acad. Sci. USA* **103**, 3828-3833 (2006).

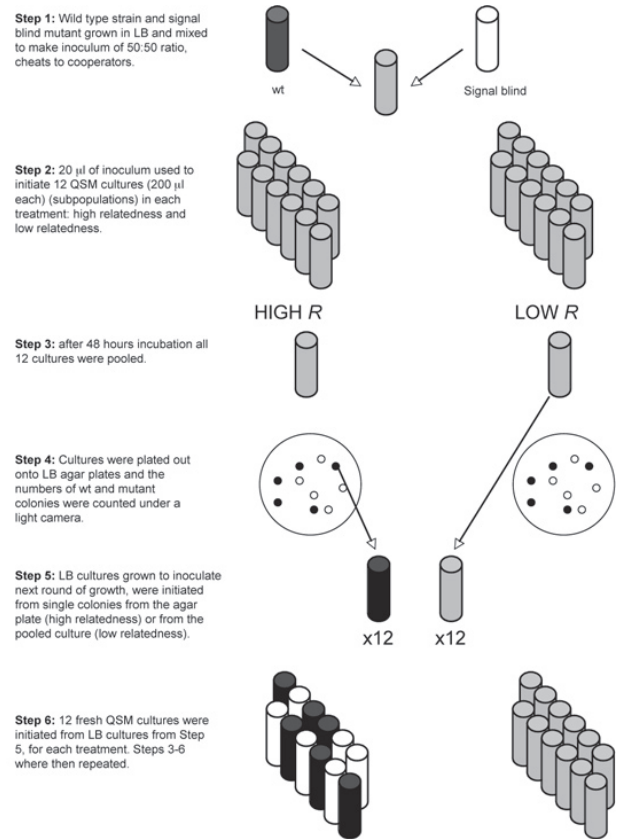
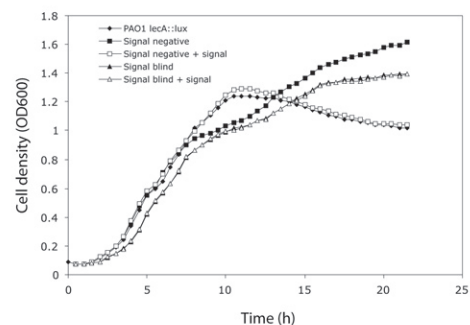
evolved clones was suggested to be due to either (i) a higher maximal growth rate, (ii) a reduced mortality in stationary phase, or (iii) a combination of these<sup>2</sup>. In order to determine whether QS mutants have a higher growth rate than the wild type, we measured growth of the wild type and QS mutants over a 22 h period (see Supplemental Fig. 3). Growth rates of the cultures was determined using the exponential portion of the growth curve (between 4.5 and 9h). Growth rates are similar for all strains in exponential phase (PAO1 *lecA::lux* 0.2225; signal negative 0.1875; signal negative + signal 0.2013; signal blind 0.2641; signal blind + signal 0.262). This suggests that the major fitness cost of quorum sensing occurs in stationary phase cultures.

2 Lenski, R. E., Rose, M. R., Simpson, S. C., & Tadler S. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. *Am. Nat.* **138**, 1315-1341 (1991).

**Figure S1**

**Figure S1:** The hierarchical quorum sensing (QS) system of *P. aeruginosa*. The QS cascade is induced at high population cell densities when within the cell, the LasR response protein binds to a critical concentration of 3O-C12-HSL signal which has been produced by neighboring cells and taken up from the surrounding environment. This results in activation of the *las* QS system and the production of a number of QS-regulated public goods such as the proteases. Activation of the *las* system is also important in the induction of the *rhl* QS system which is also required for the production of proteases and a number of other *rhl*-controlled public goods.

**Figure S2:** Experimental design. We varied relatedness between interacting individuals by initiating each subpopulation with either a single bacterial clone (relatively high *r*) or a mixture of bacterial clones (relatively low *r*). We use black to symbolize quorum sensing cooperators, white to symbolize signal blind cheaters, and gray for a mixture of cooperators and cheaters.

**Figure S2****Figure S3**

**Figure S3:** Kinetic growth of wild type and QS mutants. Growth of wild type and mutants over time in a rich (LB) growth medium in the absence and presence of signal (5  $\mu$ M 3O-C12-HSL).