

Quorum Sensing and the Social Evolution of Bacterial Virulence

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Summary

The ability of pathogenic bacteria to exploit their hosts depends upon various virulence factors, released in response to the concentration of small autoinducer molecules that are also released by the bacteria [1–5]. In vitro experiments suggest that autoinducer molecules are signals used to coordinate cooperative behaviors and that this process of quorum sensing (QS) can be exploited by individual cells that avoid the cost of either producing or responding to signal [6, 7]. However, whether QS is an exploitable social trait in vivo, and the implications for the evolution of virulence [5, 8–10], remains untested. We show that in mixed infections of the bacterium *Pseudomonas aeruginosa*, containing quorum-sensing bacteria and mutants that do not respond to signal, virulence in an animal (mouse) model is reduced relative to that of an infection containing no mutants. We show that this is because mutants act as cheats, exploiting the cooperative production of signal and virulence factors by others, and hence increase in frequency. This supports the idea that the invasion of QS mutants in infections of humans [11–13] is due to their social fitness consequences [6, 7, 14] and predicts that increased strain diversity will select for lower virulence.

Results and Discussion

We examined the social nature and virulence consequences of QS, the process whereby bacterial cells use small autoinducer molecules to regulate certain behaviors [1–5]. The diffusion of

autoinducer molecules into cells has two consequences. First, they may stimulate the production of many products, including exoproducts such as extracellular enzymes, nutrient-scavenging molecules, and toxins, which facilitate the growth of both free-living and pathogenic bacteria. In pathogenic bacteria, these exoproducts are often termed “virulence factors,” because they lead to an increase in damage to the host, either directly or through facilitating bacterial growth [1–5]. Second, the diffusion of autoinducer molecules into cells leads to an increase in the production of signaling molecules themselves (autoinduction). At high cell densities, this can lead to a positive feedback that markedly increases the production of virulence factors and other exoproducts [1–5]. The idea here is that QS turns on the cooperative production of exoproducts when it is most useful to do so: at high cell density.

Quorum Sensing and Virulence

We examined the virulence of *Pseudomonas aeruginosa* in the mouse burn model by using a number of QS mutants. *P. aeruginosa* is an opportunistic pathogen, capable of causing disease in plants and animals, including humans [15]. *P. aeruginosa* pathogenesis in human burn wounds has been extensively examined with the thermally injured mouse (burn) model, which closely resembles human burn wound sequelae. In this acute wound model, a third-degree scald burn is produced and a low infecting dose (10^2 colony-forming units [CFU]) of *P. aeruginosa* causes up to 100% mortality within 48 hr [16]. *P. aeruginosa* regulates production of a number of virulence factors via a complicated hierarchical QS cascade [15]. We mutated (separately) four key QS genes in PA14, a human clinical isolate of *P. aeruginosa* that is also capable of causing disease in mice, plants, nematodes, and insects [17]. We constructed two signal-negative strains that do not produce their cognate autoinducer molecules—one that does not produce *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL; PA14Δ*lasI*) and one that does not produce *N*-butanoyl homoserine lactone (C4-HSL; PA14Δ*rhII*). We also constructed two signal-blind strains—one that does not respond to each of the autoinducer molecules (PA14Δ*lasR* and PA14Δ*rhIR*).

We tested whether infections initiated with only QS mutants, which either do not produce or do not respond to autoinducer molecules, leads to reduced virulence, as has been found previously [16]. We infected mice with either one of our four different mutants or the wild-type from which they originated. We found that the virulence of the mutants, as measured by the rate of host mortality, was significantly lower for the QS mutants (Figure 1). These results confirm the group benefit to the infection success provided by QS. QS provides a benefit at the group level and leads to an increase in virulence because it allows bacteria to better spread within the host [16]. It should be noted that this does not suggest that QS in certain organisms evolved as an infection strategy, just that it is a trait that can be beneficial in certain environments, including hosts.

We then tested whether the presence of mutants reduced the virulence in mixed infections that were initiated with a mutant and the wild-type. We initiated infections with either

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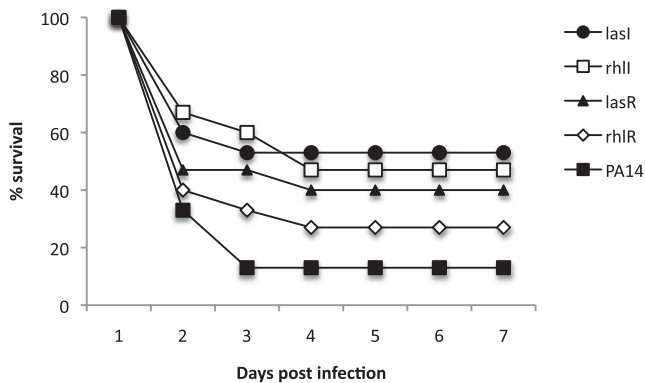


Figure 1. The Virulence of QS Mutants

The survival rate for mice (burn model) infected with either a normal PA14 wild-type or QS mutants, plotted against time (days after burn/infection). Fifteen mice per treatment. The death rate does not vary significantly between the different mutants ($\chi^2_3 = 3.43$, $p > 0.3$) but is significantly lower in the mutants compared with the wild-type ($\chi^2_1 = 6.54$, $p = 0.01$).

100% PA14 wild-type, 100% QS (*las*) mutant, or a 50:50 mixture of the two, repeating this experiment with both the *lasR* (signal-blind) and *lasI* (signal-negative) mutants. We focused from here onward on the *las* QS mutants, because they are the most commonly detected QS mutant in natural infections of humans [11–13]. In addition, the *las* QS pathway controls the *rhl* system hierarchically (a mutation in *las* QS results in a general abolition of QS in *P. aeruginosa*) [15]. In both cases, the virulence of the 50:50 mix was significantly lower than that of the 100% wild-type, and not significantly different from the 100% mutant infections (Figure 2). The effect on virulence was large, with the addition of mutants to the wild-type infections approximately halving the mortality rate over the course of our experiments. This result was not due to the lower numbers of wild-type cells used to infect the mixed infections, because if the number of cells used to inoculate an infection was halved, when infecting with only the wild-type, this did not lead to a significant reduction in mortality ($\chi^2_1 = 1.00$, $p = 0.32$, see Supplemental Data available online).

Social Interactions In Vivo

We then tested whether the reduced virulence in mixed infections could be explained by social interactions between the wild-type and the mutants. Specifically, we tested whether the reduced virulence was due to the mutants exploiting the cooperative behavior of the wild-type, to their benefit, but at the cost of the overall infection. If this is the case, then we can make two predictions. First, in mixed infections, containing both mutants and the wild-type, the mutants should increase in frequency because they are able to benefit from (exploit) the QS behavior of others, while avoiding the cost of either signaling or responding to signal. Second, mutants will have a higher fitness when they are rarer (negative frequency dependence), because they will be better able to exploit those that perform QS [18].

We found support for both of these predictions, in both the acute burn and the chronic wound animal models. We carried out experiments in both models because, whereas mortality is high and rapid in the acute model, the chronic wound model allows us to follow infections over a longer timespan. In addition, the models are representative of different types of human *P. aeruginosa* infection such as burn versus chronic diabetic

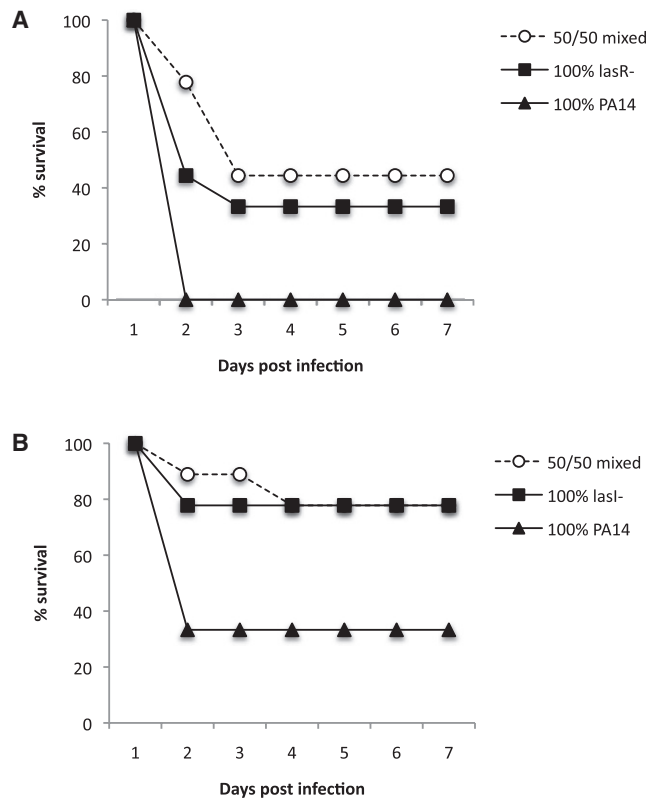


Figure 2. The Virulence of Mixed Infections

The survival curves for mice (burn model) infected with either the PA14 wild-type, a QS mutant, or a 50:50 mixture of the two, plotted against time (days after burn/infection). Shown are data for (A) the signal-blind (*lasR*) and (B) the signal-negative (*lasI*) mutants. Nine mice per treatment. In both cases, the survival rate of mice infected with the 50:50 mixture of wild-type and mutant is significantly greater than that of the wild-type (*lasR*: $\chi^2_1 = 13.91$, $p = 0.0002$; *lasI*: $\chi^2_1 = 6.24$, $p = 0.02$) and not significantly different from the mutant (*lasR*: $\chi^2_1 = 0.23$, $p > 0.6$; *lasI*: $\chi^2_1 = 0.0015$, $p > 0.9$).

wounds [19]. We first tested whether QS mutants increase in frequency in mixed infections. We initiated infections of the wild-type with approximately 1% of either the signal-blind (*lasR*⁻) or signal-negative (*lasI*⁻) mutants. In the chronic wound model, over a period of 7 days, the signal-blind mutant increased in frequency from 1.3% to 32.4%, and the signal-negative mutant increased in frequency from 1.0% to 13.4% (Figure 3), with the relative fitness of the two mutants being 47-fold and 16-fold that of the wild-type, respectively. In the acute burn model, over a period of 2 days, the signal-blind mutant increased in frequency from 1.4% to 14.3%, and the signal-negative mutant increased in frequency from 0.1% to 2.0% (Figure 3), with the relative fitness of the mutant being 13-fold and 28-fold that of the wild-type, respectively. Consequently, in mixed infections, in both the acute burn and the chronic wound animal models, both the signal-blind and the signal-negative mutants had a significantly higher fitness than did the parental wild-type.

We then tested whether the success of the mutants showed frequency dependence, with mutants having a lower relative fitness when they are more common. Social evolution theory predicts that if the mutants and wild-type are cheaters and cooperators, respectively, then the relative fitness of the mutants will be greatest when they are rarer, because then they will be better able to exploit the cooperators [18, 20, 21]. This is

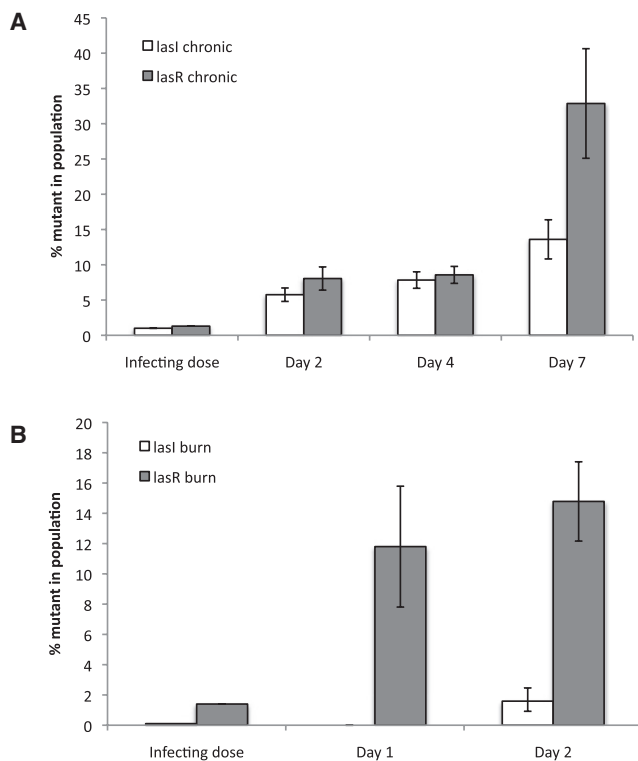


Figure 3. QS Mutants Invade Populations

QS signal-negative (*lasI*) and signal-blind (*lasR*) mutants invade populations of wild-type cooperators during infections of mice. Shown are data for signal-blind and signal-negative mutants in the chronic wound model (A) (six mice sacrificed per day for *lasR* and 3 mice per day for *lasI*, on each of days 2, 4, and 7 after infection) and burn wound model (B) (signal-blind: six mice sacrificed per day, on each of days 1 and 2 after infection; signal-negative: six mice sacrificed on day 2 after infection). In all cases, the proportion of cheats significantly increased compared with the initial starting frequency of approximately 1% in the infecting dose (chronic *lasR*: $p < 0.0001$, $n = 18$; chronic *lasI*: $p = 0.004$, $n = 9$; burn *lasR*: $p < 0.0001$, $n = 18$; burn *lasI*: $p = 0.031$, $n = 6$, starting frequency 0.1%; Wilcoxon ranked sign tests). Results shown are mean \pm SEM, comparing the initial infection with that on the final day measured (back transformed after arcsine square root transformation).

because (1) a higher proportion of cooperators will lead to greater population growth, allowing more time for cheats to exploit cooperators, and (2) population structuring will reduce the extent to which the cheats and cooperators interact, which penalizes cheats more at higher frequencies [18]. Both of these factors are likely to be important within hosts. We initiated infections of the wild-type, in both the acute burn and chronic wound model, with 0.1%, 1%, or 20% of the signal-blind (*lasR*⁻) mutant and 0.1% or 20% of the signal-negative (*lasI*⁻) mutant. In all cases, as predicted, we found that the relative success of the signal-blind and signal-negative cheats was lower when they were more common (Figure 4). Although the mutant increased in frequency from all starting frequencies, their relative fitness was greater when at lower starting frequencies. With both the signal-blind and the signal-negative mutant, stronger frequency dependence (higher relative fitness) was observed in the chronic wound model, as would be expected, because that allows a greater period of growth [18].

We also found that the spread of mutants to new areas of the mouse host can be facilitated by the presence of the wild-type. It has previously been shown that QS mutants are inhibited in

their ability to cause systemic infections and thus reach the liver [16]. We examined the spread of bacteria to the liver in the 18 burned mice we had infected with mixed inocula of both the wild-type and the signal blind (*lasR*) mutant. We found that, after 24 hr, *P. aeruginosa* was present in the livers of 39% of the mice (7/18) and that these liver infections were entirely wild-type bacteria (0% signal-blind [*lasR*] mutant). By 48 hr, 100% of mice (18/18) had *P. aeruginosa* in their livers. However, in these mice, all of the livers had been invaded also by the signal-blind (*lasR*) mutant, which had risen from 0% to an average of 11.7% (95% C.I.: 8.1%–15.7%) of the bacteria in the liver infections. Given that infections initiated with only mutants reach only a small fraction of livers [16], this suggests that once the liver is colonized by wild-type bacteria, the mutants are then able to invade and significantly increase in frequency ($F_{(1,23)} = 51.47$, $p < 0.0001$), presumably because of their ability to exploit the cooperative behavior of the wild-type. This could be further tested by a simultaneous comparison of the spread of mutants in mixed and single infections.

Overall, our results support the hypothesis that QS mutants spread within natural infections, because they are cheats that are able to exploit the cooperative signaling and exoproduct production of the wild-type. QS mutants, especially signal-blind (*lasR*) mutants, are commonly found to arise and spread in clinical settings, such as the lungs of humans with cystic fibrosis [11–13]. The alternative explanation for the spread of such QS mutants is that they are better adapted to the host environment [22]—i.e., a direct rather than social benefit (although both are possible). However, if this was the case, then we would expect (1) infections of mutants to spread better within hosts and be more virulent than infections of the wild-type, and (2) the invasion of mutants in mixed infections to lead to increased growth and virulence. In contrast to these predictions, in the acute burn model, the opposite patterns occur, with mutants leading to a reduction in both the within host spread (see above) and virulence (Figures 1 and 2) of infections. In the chronic wound model, there was no significant difference in the bacterial density (CFU) per gram of host tissue, between infections initiated with the wild-type and the two (signal-blind [*lasR*⁻] and signal-negative [*lasI*⁻]) mutants ($F_{(1,11)} = 2.26$, $p = 0.16$; trend was in the direction of less growth in the mutants). In addition, if the spread of mutants within hosts was due to adaptation to the host environment, then we would not predict the observed pattern of frequency dependence (Figure 4). More generally, our results confirm that QS can be a social trait in a natural environment and that signaling between cells is not just an artifact of laboratory culture methods, such as artificially high densities [23, 24].

Conclusions

Our results support the idea that parasite virulence in bacteria can be driven by cooperative interactions, which may explain the lack of a consistent pattern in the influence of strain diversity (relatedness) on parasite virulence [8, 9, 25–27]. It is usually assumed that a higher relatedness between the bacteria infecting a host (lower strain diversity) will lead to more prudent exploitation of the host, and hence lower virulence [28–30]. However, if host exploitation is limited by the extent of cooperation, then a higher relatedness will favor higher levels of cooperation that in turn allows the host to be exploited more efficiently, and hence a higher virulence [8, 10]. Even more complicated patterns can be expected if interference competition occurs between strains, through the production of

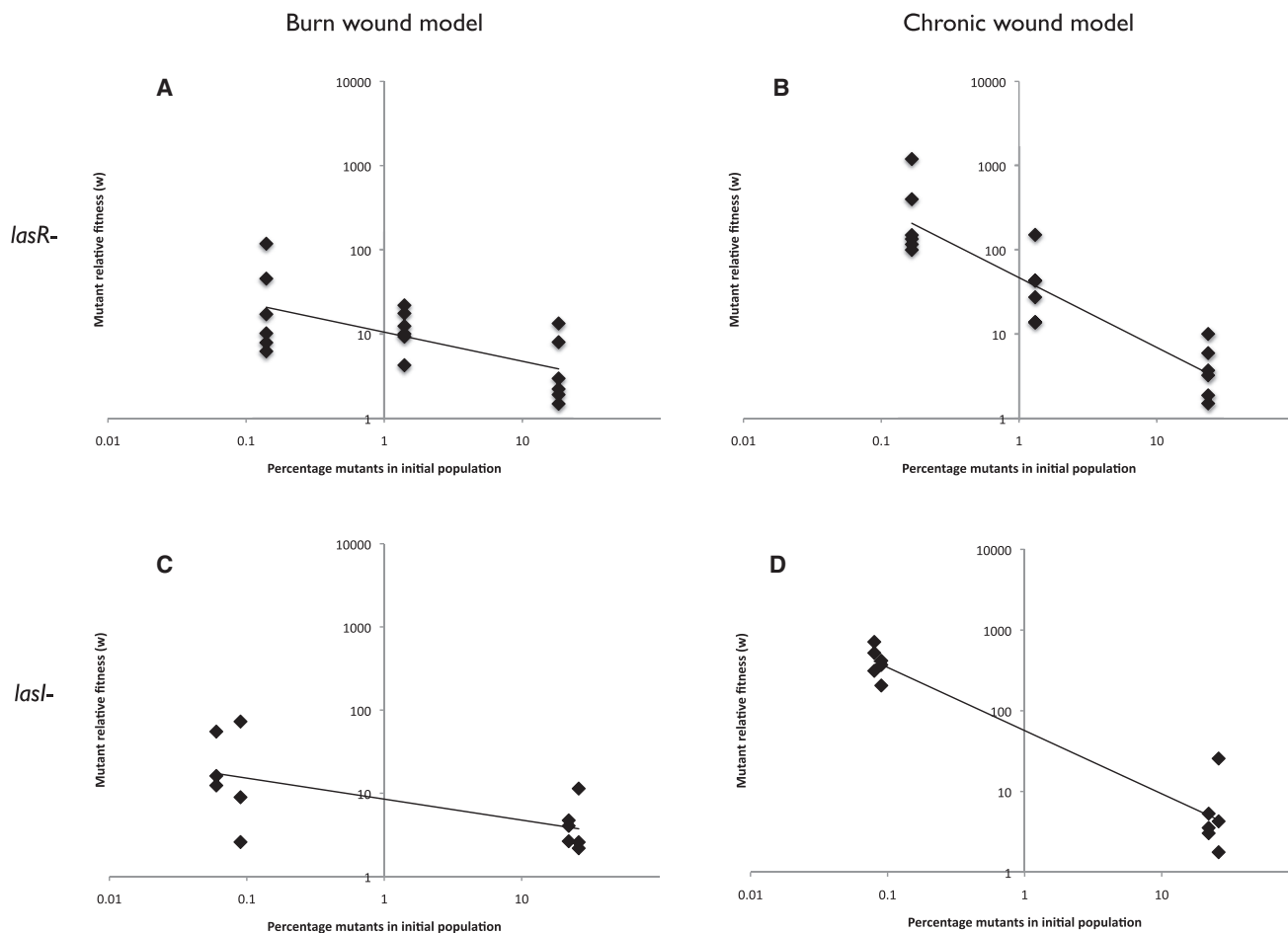


Figure 4. Mutant Fitness Is Negatively Frequency Dependent

The relative fitness of signal-blind (*lasR*⁻; [A] and [B]) and signal-negative (*lasI*⁻; [C] and [D]) mutants is plotted against the proportion of mutants used to inoculate the infection, for both burn wound (A and [B]) and chronic wound (B and D) mouse models. In all cases, mutants have a higher fitness when they are less common (*lasR*⁻: burn wound: $F_{(1,16)} = 11.20$, $p = 0.004$; chronic wound: $F_{(1,16)} = 72.58$, $p < 0.0001$; *lasI*⁻: burn wound: $F_{(1,10)} = 6.42$, $p = 0.030$; chronic wound: $F_{(1,10)} = 64.63$, $p < 0.0001$). Furthermore, in all cases, the mutant frequency increased over time, as we have already illustrated for a subset of starting frequencies in Figure 3. Relative fitness is the estimated growth rate of mutants relative to that of the wild-type (see [Experimental Procedures](#)). In all cases, the same qualitative pattern of an increase in mutant frequency over time was also observed at all starting frequencies in our experiment shown in this figure, where the frequency of cheats in the infection dose was varied from 0.1% to 20%.

factors such as bacteriocins [31]. The fact that biological details can alter the qualitative nature (direction) of the prediction contrasts with related areas of social evolution theory that have been applied to parasites, such as sex ratio adjustment in response to competition between related males, where the biological details do not have a strong influence on the predictions of theory [32]. Finally, our results raise the potential for social interactions to be exploited in medical interventions. Cheats that do not perform cooperative behaviors could be introduced into hosts to outcompete wild-type cooperators. As well as directly reducing virulence, this could drive down the bacterial population size, which may benefit other intervention strategies such as treatment with antibiotics.

Experimental Procedures

Bacterial Growth and Inoculum

P. aeruginosa strains were grown in Luria-Bertani (LB) medium [33]. Overnight cultures were subcultured in fresh LB broth and grown at 37°C for 3 hr to an optical density of approximately 0.8 at 600 nm. Cultures were serially diluted in sterile phosphate-buffered saline (PBS). To generate *lasI*, *lasR*, *rhlI*, and *rhlR* mutants in *P. aeruginosa* PA14, pSB219.8A (pRIC380

carrying *lasI*::Gm), pSB219.9A (pRIC380 carrying *lasR*::Gm), pSB224.12B (pRIC380 carrying *rhlI*::Tc), and pSB224.10A (pRIC380 carrying *rhlR*::Tc) were conjugated into PA14, resulting in PA14::*lasI*, PA14::*lasR*, PA14::*rhlI*, and PA14::*rhlR*, respectively. Mutations were confirmed by PCR analysis (data not shown). To distinguish between wild-type and mutant after in vivo competition assays, Mini-CTX*lux* was transformed into PA14 wild-type resulting in PA14*lux*. This provided a background level of bioluminescence that could be detected under a light camera and so the wild-type was bioluminescent whereas the QS mutants were not. We examined the fitness cost of introducing Mini-CTX*lux* to PA14 by performing growth experiments on PA14 and PA14*lux* in 300 μ l of LB broth in a combined automated luminometer-spectrometer (GENios Pro; Tecan Group). Over 19.5 hr, the *lux*-tagged strain showed no significant difference in population growth (yield: $F_{(1,30)} = 1.19$; $p = 0.284$; rate: $F_{(1,30)} = 0.4$; $p = 0.532$; see [Supplemental Data](#)). It would be useful if future work tested the generality of this result across a greater range of conditions, including in vivo, and/or also carried out complementary experiments in which the wild-type was tagged.

Acute and Chronic Wound Models

Female Swiss Webster (SW) mice were obtained from Charles River Laboratories (Wilmington, MA). Mice used in experiments were 6 to 8 weeks old and weighed 20–25 g. Mice were anesthetized by intraperitoneal injection of Nembutal at 100 mg/kg (5% sodium pentobarbital; Abbott Laboratories, North Chicago, IL), and their backs were shaved. The acute third degree

burn wound was induced as previously described, with infection doses of approximately 200 bacteria [34, 35]. Chronic wounds were induced by the surgical removal of a 1.5 × 1.5 cm full-thickness patch of skin from the shaved back. The wounds were covered with a transparent, semipermeable polyurethane dressing that allowed for daily inspection of the wound, wound size determination, topical application of bacteria onto the wound, and protection from other contaminating bacteria. 10⁴ CFU *P. aeruginosa* were applied under the dressing, on top of the wound. Mice were treated humanely and in accordance with the protocol approved by the Animal Care and Use Committee at Texas Tech University Health Sciences Center (Lubbock, TX).

Quantitation of Bacteria within the Skin and Livers

At indicated times, mice were euthanized by intracardial injection of 0.2 ml of Sleepaway (sodium pentobarbital-7.8% isopropyl alcohol euthanasia solution; Fort Dodge Laboratories, Inc., Fort Dodge, IA). Skin and liver sections from wounded mice were extracted, weighed, placed in 2 ml PBS, and homogenized. Homogenates were serially diluted and plated on LB agar plates to determine the number of bacterial CFU, which was calculated per gram of tissue.

Statistical Analyses

Unless stated otherwise, we carried out all analyses by model simplification to the minimum adequate model, with generalized linear modeling techniques implemented in GLMStat 6.0 (Kagi Shareware, Ken Beath, Australia). The mouse survival data were analyzed by parametric survival analysis in S-Plus 8.0 (Insightful Corp, Seattle, WA), assuming a Weibull distribution (although qualitatively identical results were obtained assuming an exponential distribution). We calculated the relative fitness of mutants (*w*) by comparing the frequency of mutants 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 mutants in the population and *x*₂ is their final proportion [18]. For example, *w* = 2 would correspond to the mutant growing twice as fast as the wild-type cooperator.

Supplemental Data

Supplemental Data include two figures and can be found with this article online at [http://www.current-biology.com/supplemental/S0960-9822\(09\)00622-8](http://www.current-biology.com/supplemental/S0960-9822(09)00622-8).

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References

- Keller, L., and Surette, M.G. (2006). Communication in bacteria: an ecological and evolutionary perspective. *Nat. Rev. Microbiol.* 4, 249–258.
- 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? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 362, 1241–1249.
- Henke, J.M., and Bassler, B.L. (2004). Bacterial social engagements. *Trends Cell Biol.* 14, 648–656.
- Williams, P., Winzer, K., Chan, W.C., and Camara, M. (2007). Look who's talking: communication and quorum sensing in the bacterial world. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 362, 1119–1134.
- Brown, S.P., and Johnstone, R.A. (2001). Cooperation in the dark: signalling and collective action in quorum-sensing bacteria. *Proc. Biol. Sci.* 268, 961–965.
- Diggle, S.P., Griffin, A.S., Campbell, G.S., and West, S.A. (2007). Cooperation and conflict in quorum-sensing bacterial populations. *Nature* 450, 411–414.
- Sandoz, K.M., Mitzimberg, S.M., and Schuster, M. (2007). Social cheating in *Pseudomonas aeruginosa* quorum sensing. *Proc. Natl. Acad. Sci. USA* 104, 15876–15881.
- Brown, S.P., Hochberg, M.E., and Grenfell, B.T. (2002). Does multiple infection select for raised virulence? *Trends Microbiol.* 10, 401–405.
- Foster, K.R. (2005). Biomedicine. Hamiltonian medicine: why the social lives of pathogens matter. *Science* 308, 1269–1270.
- West, S.A., and Buckling, A. (2003). Cooperation, virulence and siderophore production in bacterial parasites. *Proc. Biol. Sci.* 270, 37–44.
- Smith, E.E., Buckley, D.G., Wu, Z., Saenphimmachak, C., Hoffman, L.R., D'Argenio, D.A., Miller, S.I., Ramsey, B.W., Speert, D.P., Moskowitz, S.M., et al. (2006). Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proc. Natl. Acad. Sci. USA* 103, 8487–8492.
- Schaber, J.A., Carty, N.L., McDonald, N.A., Graham, E.D., Cheluvappa, R., Griswold, J.A., and Hamood, A.N. (2004). Analysis of quorum sensing-deficient clinical isolates of *Pseudomonas aeruginosa*. *J. Med. Microbiol.* 53, 841–853.
- Denervaud, V., TuQuoc, P., Blanc, D., Favre-Bonte, S., Krishnapillai, V., Reimmann, C., Haas, D., and van Delden, C. (2004). Characterization of cell-to-cell signaling-deficient *Pseudomonas aeruginosa* strains colonizing intubated patients. *J. Clin. Microbiol.* 42, 554–562.
- West, S.A., Griffin, A.S., Gardner, A., and Diggle, S.P. (2006). Social evolution theory for microorganisms. *Nat. Rev. Microbiol.* 4, 597–607.
- Venturi, V. (2006). Regulation of quorum sensing in *Pseudomonas*. *FEMS Microbiol. Rev.* 30, 274–291.
- Rumbaugh, K.P., Griswold, J.A., Iglewski, B.H., and Hamood, A.N. (1999). Contribution of quorum sensing to the virulence of *Pseudomonas aeruginosa* in burn wound infections. *Infect. Immun.* 67, 5854–5862.
- Rahme, L.G., Stevens, E.J., Wolfort, S.F., Shao, J., Tompkins, R.G., and Ausubel, F.M. (1995). Common virulence factors for bacterial pathogenicity in plants and animals. *Science* 268, 1899–1902.
- Ross-Gillespie, A., Gardner, A., West, S.A., and Griffin, A.S. (2007). Frequency dependence and cooperation: theory and a test with bacteria. *Am. Nat.* 170, 331–342.
- Bodey, G.P., Bolivar, R., Fainstein, V., and Jadeja, L. (1983). Infections caused by *Pseudomonas aeruginosa*. *Rev. Infect. Dis.* 5, 279–313.
- MacLean, R.C., and Gudelj, I. (2006). Resource competition and social conflict in experimental populations of yeast. *Nature* 441, 498–501.
- Turner, P.E., and Chao, L. (1999). Prisoner's dilemma in an RNA virus. *Nature* 398, 441–443.
- D'Argenio, D.A., Wu, M., Hoffman, L.R., Kulasekara, H.D., Deziel, E., Smith, E.E., Nguyen, H., Ernst, R.K., Larson Freeman, T.J., Spencer, D.H., et al. (2007). Growth phenotypes of *Pseudomonas aeruginosa* lasR mutants adapted to the airways of cystic fibrosis patients. *Mol. Microbiol.* 64, 512–533.
- Redfield, R.J. (2002). Is quorum sensing a side effect of diffusion sensing? *Trends Microbiol.* 10, 365–370.
- Hense, B.A., Kuttler, C., Muller, J., Rothballer, M., Hartmann, A., and Kreft, J.U. (2007). Does efficiency sensing unify diffusion and quorum sensing? *Nat. Rev. Microbiol.* 5, 230–239.
- Read, A.F., and Taylor, L.H. (2001). The ecology of genetically diverse infections. *Science* 292, 1099–1102.
- Buckling, A., and Brockhurst, M.A. (2008). Kin selection and the evolution of virulence. *Heredity* 100, 484–488.
- Frank, S.A., and Schmid-Hempel, P. (2008). Mechanisms of pathogenesis and the evolution of parasite virulence. *J. Evol. Biol.* 21, 396–404.
- Herre, E.A. (1993). Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* 259, 1442–1445.
- Frank, S.A. (1996). Models of parasite virulence. *Q. Rev. Biol.* 71, 37–78.
- de Roode, J.C., Pansini, R., Cheesman, S.J., Helinski, M.E., Huijben, S., Wargo, A.R., Bell, A.S., Chan, B.H., Walliker, D., and Read, A.F. (2005). Virulence and competitive ability in genetically diverse malaria infections. *Proc. Natl. Acad. Sci. USA* 102, 7624–7628.
- Gardner, A., West, S.A., and Buckling, A. (2004). Bacteriocins, spite and virulence. *Proc. Biol. Sci.* 271, 1529–1535.
- Nee, S., West, S.A., and Read, A.F. (2002). Inbreeding and parasite sex ratios. *Proc. Biol. Sci.* 269, 755–760.
- Ausubel, F., Brent, R., Kingston, R., Moor, D., Seidman, J., Smith, J., and Stahle, K. (1988). *Current Protocols in Molecular Biology* (New York: Wiley Intersciences).
- Haynes, A., 3rd, Ruda, F., Oliver, J., Hamood, A.N., Griswold, J.A., Park, P.W., and Rumbaugh, K.P. (2005). Syndecan 1 shedding contributes to *Pseudomonas aeruginosa* sepsis. *Infect. Immun.* 73, 7914–7921.
- Schaber, J.A., Triffo, W.J., Suh, S.J., Oliver, J.W., Hastert, M.C., Griswold, J.A., Auer, M., Hamood, A.N., and Rumbaugh, K.P. (2007). *Pseudomonas aeruginosa* forms biofilms in acute infection independent of cell-to-cell signaling. *Infect. Immun.* 75, 3715–3721.

Supplemental Data

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Kendra P. Rumbaugh, Stephen P. Diggle, Chase M. Watters, Adin Ross-Gillespie, Ashleigh S. Griffin, and Stuart A. West

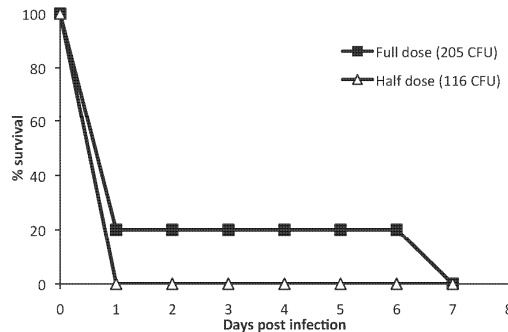


Figure S1. Inoculum dose and virulence. We have shown that infections of a mixture of wild type and QS mutant have a lower virulence than infections of pure wild type. Our aim here was to test that this could not be explained by the fact that the mixed infections were initiated with only half as many wild type cells, and that this was below a threshold number required for successful infection. We found that inoculations with the normal number of cells (mean CFU = 205) did not lead to significantly different host mortality rates than infections with approximately half as many cells (mean CFU = 116; n=5 mice per treatment). No effect of inoculum dose on the mortality rate has also been observed in a previous experiment with higher inoculum doses, comparing when burned mice were infected with 5×10^3 versus 5×10^5 CFU PA14 [1].

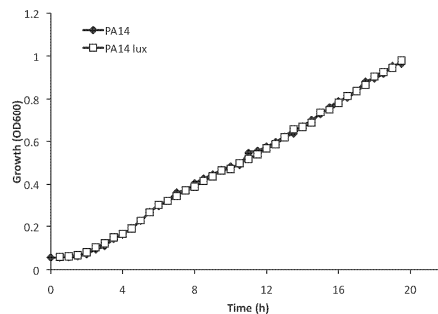


Figure S2. PA14 growth curves. To examine the fitness cost of carrying the Mini-CTXlux marker, we compared the growth profiles of PA14 and PA14lux in an automated spectrophotometer/luminometer (GENios PRO, Tecan Group) for 18 h. The lux marker did not significantly affect growth, either in terms of final OD yield (F1,30 = 1.19; P = 0.284) or growth rate (F1,30 = 0.4; P = 0.532; slopes of log(OD) were extracted from fitted Baranyi curves using R v2.0 (www.r-project.org).

Supplementary References

1. Hendrickson, E.L., Plotnikova, J., Mahajan-Miklos, S., Rahme, L.G., and Ausubel, F.M. (2001). Differential roles of the *Pseudomonas aeruginosa* PA14 rpoN gene in pathogenicity in plants, nematodes, insects, and mice. *J Bacteriol* *183*, 7126-7134.