Signals in the Dark: What factors select for the evolution of cooperation controlled by quorum sensing?

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Abstract

Quorum sensing is a ubiquitous strategy in which bacteria are able to sense the presence of others via the density of a secreted molecule. Vibrio harveyi is one such bacterial species that uses quorum sensing to control a public goods cooperation strategy. As with all cooperative strategies, this strategy is at risk of cheating organisms ousting cooperators. Using the platform Empirical, we first replicated the results from a wetlab experiment and then determined the effects of population structure and resource availability on the de novo evolution, short-term, and long-term stability of a quorum sensing-controlled public goods strategy. We found that environments that enabled pre-existing cooperators to remain stable were not always the same environments in which cooperation could evolve de novo. Specifically, cooperation was able to persist in the short term in semi-structured populations with low resource levels, but not maintained over long evolutionary time scales.

Introduction

Quorum sensing is a primary mechanism for coordination in many species of organisms, including various bacteria (Waters and Bassler 2005; Bruger and Waters 2015). It occurs when individual cells produce a small amount of a signal molecule – called an autoinducer – and detect the amount of that molecule in their surroundings. By sensing the concentration of autoinducer, cells can get a rough estimate of how many related organisms are likely to be around them. If the size of the colony is large enough (i.e. it has reached a quorum), the organisms will start performing an action that is only beneficial when there are a large number of cooperating cells. In this way, a colony is able to cooperate to perform expensive actions like bioluminescence (Brown and Johnstone 2001), digest resources too large for individuals (Drescher et al. 2014), or wait to become virulent until they can overwhelm an immune system (Barnard et al. 2007).

Like most forms of cooperation, quorum sensing is susceptible to cheating. Cheating organisms do not contribute to the costly cooperative behavior, but they gain from the rest of the cells’ cooperation (West et al. 2007). For example, cooperators may produce an enzyme that cheaters can gain from by taking in freed resources without paying the cost of producing the enzyme themselves. Some especially insidious cheaters may even produce the autoinducer signal that makes other organisms believe that a quorum has been reached and that they should start the costly behavior (Bruger and Waters 2015).

Vibrio harveyi is an example of a marine bacterium that uses quorum sensing to control the production of a public good (Bruger and Waters 2015). The public good is an extracellular protease that can break down proteins outside of the cell, but must be excreted in large amounts to be useful. The restricted nutrients secreted when the proteins are broken down are then generally available in the environment for the surrounding bacteria to ingest. There is a known defector strain that produces negligible amounts of autoinducer and protease, but still intakes the nutrients freed by surrounding cooperators.

Bruger et al. has found in their experiments that their wildtype V. harveyi, that induces protease production at high cell density via quorum sensing, is resistant to defectors while an unconditional cooperator is not, indicating that the quorum-sensing control gives the cooperators the ability to repel invading defectors (Bruger and Waters 2016). Their experiments, however, are necessarily limited in time due to the nature of wetlab experiments. Therefore, the question is: what factors select for the origin and maintenance of cooperation controlled by quorum sensing, thereby preventing the known defector from invading the population? Further, are the factors that select for the origin of the trait the same as those that select for the maintenance of the trait?

We hypothesized that population structure and the availability of unrestricted resources are possible factors to select for cooperation in a quorum sensing system. We also hypothesized that the conditions that stabilize the cooperative trait in the short term will not necessarily be the conditions under which the trait can evolve de novo or persist over long evolutionary time scales. We tested the effects of these factors in an agent-based computer system called Empirical and found that increased population structure and decreased unrestricted resources both select for cooperation to evolve de novo and stabilize pre-existing cooperation. We
Figure 1: Relative fitness of wildtype and defector across quorum threshold values.

also found that while cooperation may be able to evolve in a semi-structured environment, it may not necessarily be maintained in that environment over long evolutionary time scales.

Methods
To investigate the factors that select for and stabilize quorum-sensing-controlled public goods, we created an agent-based system in the artificial life platform Empirical. While more complex artificial life systems, like Avida (Ofria et al., 2009; Beckmann et al., 2012), have been used previously for similar work, the questions we are addressing are more focused and will benefit from initial investigations with a targeted simulation. Therefore, we implemented the following key features: 1) a system with the fundamental components necessary for evolution (mutation, inheritance, and differential fitness), 2) organisms that can sense their neighbors, identify quorum, and conditionally produce a restricted good, 3) a population with varying spatial structures, and 4) rewards that can be set independently for unrestricted and restricted resources

Specifically, our system creates a toroidal world of organisms that are able to engage in quorum sensing and public goods production that frees an otherwise restricted resource, but all organisms, even non-cooperators, can utilize the unrestricted resource. Each organism has a simple genome consisting of one value: the probability of attempting to cooperate. The quorum threshold – the proportion of neighbors producing an autoinducer necessary to produce a public good – is user-configurable and set to 60% by default. This default value was determined empirically, by testing a range of possible values, as seen in Figure 1, determining that 60% would capture the general trends across most parameter values. Whether an organism will produce the autoinducer is determined at birth by its probability of attempting to cooperate. As such, we make cooperation and autoinducer production pleiotropically linked and we remove the possibility of a defector that ‘lies’ by producing autoinducer. At every ‘update’ each organism is allowed to try to cooperate by testing its probability of cooperation. If an organism tries to cooperate, its neighbors within a specified radius (10 by default) are checked for autoinducer production. If enough neighbors are producing an autoinducer compared to the set quorum threshold, the organism pays the cost to produce the public good (by default 9 units of resource) and all organisms within a one-cell radius receive a portion of the restricted resource freed by the public good, including the producer, all cooperators, and any cheaters fortunate enough to be part of the group. By default there are 45 resource units split among up to nine organisms round-robin starting with the producer.

After the public goods benefits are determined, each organism is tested for reproduction. If an organism has reached 50 or more units of resource, it is allowed to reproduce. Both the parent and the offspring are mutated (essentially creating two offspring and replacing the parent). If mutations are enabled, the ‘probability to cooperate’ value has a user-defined chance of mutating, 0.001 by default, and if it mutates, a new value is pulled from a uniform distribution between 0 and 1.

We started all experiments with an initial population of 1% of the spaces filled, randomly distributed across the world. An initial starting amount of unrestricted resource (i.e. a resource that does not need to be freed via public goods production) of 80,000 is distributed evenly throughout the world. This unrestricted resource is necessary to allow the wildtype to increase to sufficient density to trigger quorum and subsequent public goods production.

Most experiments included regular ‘bottleneck’ events, simulating a daily transfer in a wet lab. The population is randomly reduced back to 1% of the world and 80,000 more units of unrestricted resource are distributed evenly. These bottleneck events occur every 100 updates, which is approximately how long it takes for the population to fill the world in monocultures, in agreement with standard wetlab protocol. Finally, we created three hand-coded organism types that can be injected at varying proportions into the starting population. The ‘wildtype’ simulates quorum-sensing-controlled public goods production by having a cooperation probability of 1.0 and a quorum threshold of 0.6 (i.e. 60% of neighbors producing autoinducer within 10 spaces). The ‘unconditional’ type simulates an unconditional or constitutive cooperator by having a cooperation probability of 1.0 and a quorum threshold of 0.0 (i.e. no neighbors need to produce autoinducer). Finally, the ‘defector’ type simulates a defecting organism by a cooperation probability of 0.0 and it would have the set default quorum threshold if it were to mutate. Spaces in the world can be empty, but only one organism can occupy a space at a time. All experiments included 30 replicates. The code for the quorum sensing module is at https://github.com/devosoft/quorum-orga-for-Empirical and the base Empir-
Figure 2: Monocultures of wildtype, unconditional cooperator, and defector in the *Vibrio harveyi* system (top, used with permission from (Bruger and Waters, 2016) and our simulation (bottom). The qualitative similarity between the two systems verifies that our simulation is correctly calibrated.

Results and Discussion

We determined how factors such as population structure and units of unrestricted resources affected the *de novo* evolution of the cooperation trait from a non-cooperating phenotype and the stability of pre-established populations of cooperators when challenged with defectors.

**Verification of Simulation Accuracy**

Before starting experiments with our simulation, we calibrated the parameters with the goal of qualitatively matching the *Vibrio harveyi* system that we aimed to simulate. In Figure 2, the *V. harveyi* growth of each phenotype in monoculture is shown on the right and the simulation is shown on the left.

**Does population structure increase selection for quorum-sensing-controlled public goods?**

We first hypothesized that population structure plays a large role in the initial evolution and continued stability of public goods cooperation controlled by quorum sensing due to kin selection (Foster et al., 2006). The fitness gained from the public good is determined by how many surrounding organisms are producing the public good, therefore the more cooperators are able to isolate themselves from defectors, the more successful the cooperators will be, as we found in previous work (Johnson et al., 2014).

As shown in Figure 3, population structure does enable the unconditional cooperator to outcompete the defector, whereas in the well-mixed environment, the unconditional cooperator quickly loses. Further, the wildtype is also able to outcompete the defector only when spatial structure is applied. Notably, the wildtype is never able to grow to a density to activate public goods production in a well-mixed environment (and therefore each replicate drifts to one dominate phenotype, averaged in the figure).

The final population structure we implemented is a set of subpopulations in the form of pools similar to a 96-well plate setup. Each subpopulation was a 10 x 10 well-mixed pool, and organisms from one subpopulation were only able to move to another subpopulation during bottleneck events when the surviving 1% of organisms were randomly distributed. It is in this structure that the effect of quorum-sensing can be seen most strongly. The unconditional cooperator is quickly driven to extinction. However, the wildtype is able to eventually outcompete the defector, despite these pools having well-mixed internal structures. The wildtype finding is the same result seen in 96-well plates with *V. harveyi* (Bruger and Waters, 2016) and is a demonstration of Simpson’s Paradox (Penn et al., 2012), where a dynamic is observed in subpopulations, but that dynamic disappears when those populations are combined. Bruger *et al.* found, however, that the unconditional cooperator was also able to outcompete the defector in 96-well plates (Bruger and Waters, 2016). This difference is likely due to the timing of the bottleneck event and will be explored in future work.

After verifying that we were seeing the same ecological results as the wetlab experiments, we proceeded to evolu-
tionary time scales that are more difficult to achieve in the wetlab. By enabling the cooperation probability to mutate, we were able to determine what effect population structure may have on evolving populations of *V. harveyi* in conditions such as free living compared to a biofilm.

As seen in Figure 4, when the populations start with the cooperative trait at 0% probability, i.e. a defector population, only in a spatially-structured environment does the cooperative trait evolve to significantly higher than the control value. The control variable is a number that is mutated at the same rate as the cooperative trait but is not under selection and therefore shows how the cooperative trait would evolve if it were not under selection, positive or negative. When compared to the previous ecological results, it is noteworthy that while the wildtype could invade the defectors in a pools environment when there were no mutations, the cooperative trait is not similarly adaptive when mutations are enabled. This result suggests that cooperation of this type would need to first emerge in a spatially structured environment though it could then persist in well-mixed pools. As such, this result is an example of where observing the conditions under which a trait is maintained is not necessarily sufficient for determining the origin of that trait.

The final stage of evolution is the long-term maintenance of a trait. Due to the ancient nature of the many natural systems with quorum-sensing-controlled public goods cooperation, it is clear that such a trait can be maintained for extended time scales. However, it is not guaranteed that the same factors that provide a short-term competitive advantage will also hold for long-term evolution. Therefore, we started populations with full wildtype cooperators (i.e. they had 100% chance to cooperate, though still determined by quorum sensing) and enabled mutations to assess the stability of the cooperative trait over evolutionary time. As shown in Figure 5, spatial structure is still the most beneficial environment for the cooperative trait, maintaining cooperation at near 100% and significantly higher than the control value (Wilcoxon rank sum pairwise \( p - value < 2.2e - 16 \)). Furthermore, when the environment is structured into well-mixed pools, the percent of cooperation stabilizes at around 75%, higher than the 61% when evolving from a non-cooperative population, though similar. The cooperation probability in the well-mixed pools is significantly higher than the control value, showing that the value of 75% is adaptive (Wilcoxon rank sum pairwise \( p < 0.005 \)). Finally, the cooperative trait is not significantly different than the control in the well-mixed environment (Wilcoxon rank sum pairwise \( p - value = 0.09512 \)). These results indicate that while a population may achieve high cooperation in a pools environment, they may not be able to persist at that high level of cooperation long term.

Figure 4: When the populations start with no cooperative trait, only the spatially structured population evolves a cooperative trait value significantly different from the control value. The variation in the control variable is due to the varying genetic drift resulting from different population structures.
Does the availability of unrestricted resources decrease the benefit of public-goods cooperation?

Because our specific cooperative behavior is focused around restricted resources (i.e. freed by a public good), the relative distribution of unrestricted resources and restricted resources is likely to influence what strategy is most successful (Gardner and West [2010]). There is a small amount of unrestricted resource in all of our experiments because it is required for a population of purely wildtype to be able to start producing the public good and freeing the restricted resource. However, we hypothesized that increasing amounts of unrestricted resource would destabilize cooperation and allow defectors to more easily invade because defectors would be less reliant on being able to use restricted resources freed by cooperators.

We created a new set of experimental treatments where unrestricted resources were introduced after each bottleneck event. The treatments differed in the amount of unrestricted resource provided and allowed us to explore the effects of varying levels of unrestricted resources on the evolution of public-goods cooperation. As shown in Figure 6, in well-mixed and spatially structured environments, the resource levels we tested – 80k, 100k, 120k, 140k, 160k, 180k and 200k – did not have a significant effect on the final proportion of cooperators. This result disputes our hypothesis that increased unrestricted resource would destabilize cooperation, indicating that spatial structure has a larger effect than the amount of unrestricted resource.

However, in the intermediately structured pools environment, increased unrestricted resources prevented cooperators from out-competing defectors. This result confirms that when there is enough unrestricted resource, defectors are able to avoid their lower yield weakness and prevent the cooperators from reaching quorum.

For a trait to be successful in ecological competitions, a genome must first arise that possesses the trait and then increase in frequency in a population. To determine the likelihood of the cooperative trait evolving from a fully defector background across resource levels, we initiated experiments where the population started with the defector phenotype of 0% chance of cooperating and allowed for mutations upon reproduction. As shown in Figure 7, we again compared the value of the cooperation probability to a control variable to determine whether the trait was under selection. We found that in well-mixed environments, cooperation is not under positive selection. However, in spatially-structured environments, the cooperative trait quickly invades the population of defectors at all resource levels. This result indicates that spatial structure is sufficient to enable cooperators to overcome defectors even when the defectors do not face an unrestricted resource limitation. Finally, at resource levels 80-140k in the pools environment, cooperation is able to evolve de novo at the same resource levels in which cooperators were able to outcompete defectors ecologically. This result
Further, we found that whether there is an effect and the direction of selection can vary depending on whether the trait is evolving de novo, maintaining short term, or maintaining long term. The final cooperation values of the population are not guaranteed to stabilize at the same value regardless of the stage of evolution.

As we begin to explore using evolving populations in medical and agricultural interventions, we must identify how that cooperative trait may evolve over the long term. Digital systems such as the one presented here are ideal for testing such scenarios and making more informed decisions about matters relating to human health and safety.

**References**


Figure 7: **Cooperation evolving de novo across resource values and spatial structure.** In spatial environments, the resource levels do not affect the evolution of cooperation. In the pools environment, lower levels of resource select for cooperation whereas higher levels do not.

Figure 8: **Resource values do not significantly affect the long-term stability of the cooperative trait, with the exception of 80k unrestricted resource units in the pools environment, where cooperation is maintained at a value significantly above the control.**


