How do types of public goods mediate microbial cooperation

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Abstract

There are ubiquitous interactions between microorganisms. The black queen hypothesis states that individuals selectively lose genes for the production of public goods when they can obtain public goods from other producers. We refer to this behavior as "cheating." The strong black queen hypothesis suggests that mutually beneficial interactions between some microorganisms may be achieved through mutual cheating rather than cooperation. Ultimately, each individual in the microbial community only needs to produce a minimum amount of public goods, and these individuals rely on each other and cheat each other. The purpose of this experiment is to explore the properties of public goods that mediate interactions between microorganisms. The experimental process will be mainly implemented through an agent-based model. The expectation of the experiment is to explore the characteristics of public goods and facilitate the testing of the strong black queen hypothesis. The significance of this study is to fill the gap in related experiments and promote the understanding of the properties of public goods and the interactions between microorganisms they mediate.

Background

In microbial communities, interactions between microorganisms are very common. (Cordero & Polz, 2014; Morris et al., 2013) Whether in microbial communities in nature or in humans such as those in the gut, complex interactions are prevalent to maintain and regulate cross-feeding or chemical cycling between microorganisms. (Meijer et al., 2020; Germerodt et al., 2016; Kent et al., 2020) Many times, the manifestations of this interaction are interdependent, and studies have proven that this interaction is very important in maintaining the stability of the microbial community. (Pande et al., 2016; Pande et al., 2014) The purposes of this experiment are to explore the formation process of these interdependent interactions and the public goods that mediate and maintain this relationship.

The introduction of public goods.

The key words for the purposes are "the formation process of microbial interactions" and "public goods". This is the definition of public goods. The essential products produced by one individual in a community which can also be used by other individuals in the community are public goods. For example, catalase-peroxidase, an iron-dependent, large enzyme, is the main defense mechanism of cyanobacteria against hydrogen peroxide in the environment. (Perelman et al., 2003; Martin & Wim, 1999) Most Synechococcus genomes contain the genes that can produce the catalase-peroxidase. (Scanlan et al., 2009) However, none of the Prochlorococcus genomes that have been sequenced contains the genes that can produce catalase-peroxidase. (Scanlan

et al., 2009) Prochlorococcus cannot produce catalase-peroxidase, but it can use the catalase-peroxidase produced by Synechococcus to resist hydrogen peroxide in the environment. In other words, the catalase-peroxidase produced by Synechococcus in the community is not only used by the Synechococcus that produce the catalase-peroxidase. In this community, catalase-peroxidase is a public good produced by microorganisms.

The introduction of Black Queen hypothesis.

In 2012, Morris et al. published their research and insights into the interactions between microbes. (Morris, Lenski & Zinser., 2012) Their research suggests that the process of evolution is not necessarily a progression from simplicity to complexity. (Morris, Lenski & Zinser., 2012; Weijer., 1997) For example, the lack of a digestive tract in tapeworms and the inability of some Lactobacillus species to synthesize essential metabolites such as amino acids are examples of "reductive evolution" in nature. (Pechenik., 2000; Callanan et al., 2008; Guchte et al., 2006) On this basis, they proposed the Black Queen hypothesis. (Morris, Lenski & Zinser., 2012) In the Black Queen hypothesis, individuals in the microbial community will tend to lose genes that produce costly public goods and instead use public goods produced by individuals in the community that have not lost these genes. Individuals who have lost this part of the gene can use the energy originally used to produce public goods for growth or reproduction. Relevant information is the schematic diagrams of the Black Queen's initial state and classic Black Queen hypothesis, which show that the process in the Black Queen hypothesis whereby they stop producing public goods and instead use

public goods produced by other individuals in the community, even if these public goods are necessary for them as well as for the producers. (Figure1a, Figure1b) And public goods in the community are only supplied by a small number of producers. (Figure1b) In this experiment, we call this process "cheating", and these individuals are the “cheaters” in the community. Individuals who retain the genes for producing public goods in the microbial community and continue to produce public goods in the community are called "producers". Producers produce public goods in a community, but public goods are not only used by producers. And these producers usually only account for a minority of individuals in the community.

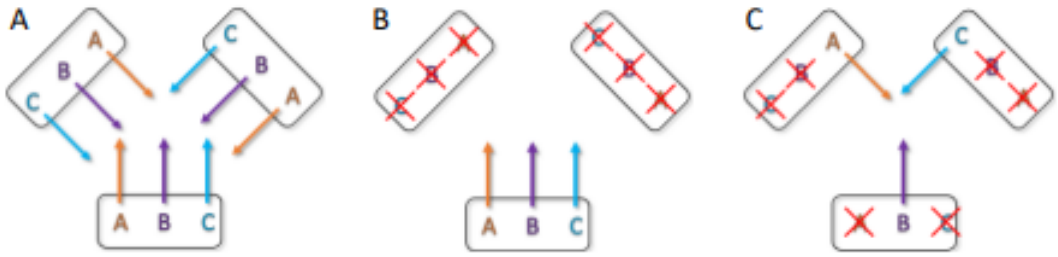
The Prochlorococcus mentioned above is such a cheater in the Black Queen hypothesis. Morris et al. believe that Prochlorococcus has selectively lost the gene for producing catalase-peroxidase and uses the catalase-peroxidase produced by Synechococcus in the community to resist hydrogen peroxide in the environment. (Morris, Lenski & Zinser., 2012) The iron and energy that are not used by Prochlorococcus to produce catalase-peroxidase are used for growth and other purposes.

The introduction of Strong Black Queen hypothesis.

In 2015, Fullmer et al. proposed the Strong Black Queen hypothesis, expanding on the Black Queen hypothesis proposed by Morris's team. (Fullmer, Soucy & Gogarten., 2015) Relevant information is the schematic diagram of the Black Queen’s initial and the Strong Black Queen hypothesis. (Figure1a, Figure1c) In the strong black queen hypothesis, all three types of cells in the community also need to use the public goods "A", "B", and "C". (Figure1c) But each of these three types of cells only needs

to produce one of the three types of public goods to meet the needs of the three types of cells for the three types of public goods. (Figure1c) Judging from the results, these three types of cells formed a mutually beneficial situation. The strong black queen hypothesis holds that the mutually beneficial outcome of these three types of cells is not necessarily achieved through a cooperative process. It is cell 1 that gradually lost the genes for producing public goods "B" and "C" in the evolution process, and only retained the gene for producing public goods "A". (Figure1c) The same goes for cells 2 and 3, which retain only the gene for producing public good "B" and the gene for producing public good "C" respectively. (Figure1c) The energy originally used to produce the other two public goods is used by the three cells for growth and reproduction. This process is the process in which three types of cells in the community stop producing some public goods in the evolution process in order to produce fewer public goods. It is a typical cheating process. Instead of three types of cells cooperating to evolve the ability to produce public goods "A", "B", and "C" because of their needs for three public goods. The result is the same as mutual benefit in form, but the process is completely different.

Figure 1. Black Queen Original, Classic Black Queen and Strong Black Queen schematic.



From Fullmer 2022, unpublished.

The rectangle represents the cell.

The A, B, and C in the cell represent the genes that produce public goods.

In summary, in the strong black queen hypothesis, the formal mutual benefit between individuals in the microbial community is not necessarily achieved through the process of cooperation, but through the process of cheating. And unlike the Black Queen hypothesis, due to the existence of multiple public goods and the same cheating ability of individuals in the community, the interaction of microorganisms in the community is not a simple relationship between deceiving and being deceived. After a long-term cheating process, each individual in the community plays the role of "cheater" and “producer” at the same time, resulting in mutual cheating and interdependence. Each microbial individual in the community ultimately produces a minimum amount of public goods, and uses public goods produced by other individuals. If the strong black queen hypothesis can be verified and proven, it will play an important

role in future research on the formation process of microbial interactions.

Experimental purpose

To test the Strong Black Queen hypothesis, my supervisor, Dr. Fullmer, developed and extended an agent-based, "bottom-up" model. However, the initial model is not enough to completely prove the strong black queen hypothesis. Some factors that may affect or even destroy the dynamic strong black queen need to be added, such as horizontal gene transfer, bottleneck effects and the characteristics of public goods. And my main work and goal is to explore the characteristics of public goods. The public goods have their own product characteristics and diffusion methods, which will greatly affect the occurrence and maintenance of strong black queen dynamics in nature, so it is very important for this experiment.

The introduction of the characteristics and types of public goods.

There are various types of public goods, such as bacteriocins and phenazines that can reduce bacterial intraspecific and interspecific competition, and siderophores that increase bacterial acquisition of iron. (Riley & Gordon., 1999; Pierson, L. S. I. & Pierson, E., 1996) In this experiment, the types of public goods are divided into three categories: intracellular enzymes, secreted metabolites and secreted enzymes. The peroxidase mentioned above is a typical intracellular enzyme. The main reason why the characteristics of these public goods affect the dynamic strong black queen is that different public goods have different functions and diffusion ranges. The lower the diffusion scope and diffusion rate of a public good, the higher the cost for cheaters to lose the production of the public good, and the less conducive it is to the formation and

maintenance of the strong Black Queen dynamic. The role of public goods is the same. The greater and more important the role of public goods, which means that the greater the costs and risks faced by cheaters when they stop producing such public goods, and the worse their ability to form and maintain a strong Black Queen dynamic. What follows is the introduction to typical public goods in each type of public goods.

Intracellular enzyme

Enzymes that play a catalytic role in cells are called intracellular enzymes, and most biological processes are related to them. Since intracellular enzymes usually work within cells, how are they used in communities as public goods? I'll illustrate with examples. The first is catalase-peroxidase, the main defense mechanism of cyanobacteria mentioned above to resist hydrogen peroxide in the environment. (Perelman et al., 2003; Martin & Wim, 1999) Catalase-peroxidase is a typical intracellular enzyme. The reason why catalase-peroxidase is used as a public good in the community is mainly related to their target “hydrogen peroxide” and the environment in which the microbial community is located. Hydrogen peroxide is formed in the ocean through the photooxidation of dissolved organic carbon. (Cooper et al., 1988) Since the membrane permeability of hydrogen peroxide is almost the same as that of water, Synechococcus containing the ability to produce catalase-peroxidase protects the interior of its own cells by reducing intracellular hydrogen peroxide, which will ultimately also reduce the level of hydrogen peroxide within a certain range outside its own cells. (Petasne & Zika, 1997; Seaver & Imlay, 2001) Within this range where hydrogen peroxide concentrations are reduced by Synechococcus, Prochlorococcus can

neither pay for the energy and iron required to produce catalase-peroxidase nor pay the price of a lack of defense against hydrogen peroxide. Under the influence of environment and membrane permeability of hydrogen peroxide and water, catalase-peroxidase becomes a public product in the community.

In addition to catalase-peroxidase, nitrogenase is also a typical public good of intracellular enzymes. The biological nitrogen fixation process involved in nitrogenase is an important source of nitrogen in terrestrial and marine ecosystems and is also of great significance to microbial communities. (Morris, Lenski & Zinser., 2012; Zheng et al., 2019; Raymond et al., 2004) However, the energy requirements of biological nitrogen fixation are also among the highest among metabolic functions, limiting the productivity of microbial communities in many environments. (Vitousek & Howarth, 1991) However, as a public good in the community, such important nitrogenases are produced by only a small number of individuals in the community. For example, nitrogen-fixing bacteria in the ocean account for less than one percent of the total cyanobacteria. (Church et al., 2005) Because nitrogen-fixing bacteria that produce nitrogenase can use nitrogenase to convert nitrogen in the air or seawater into nitrogen-containing compounds for storage in the environment. Non-nitrogen-fixing bacteria within this range can also utilize these nitrogenous compounds. In summary, the reason why intracellular enzymes can become public goods is that they can change the environment around the producer. To this extent, cheaters will also benefit from public goods. Whether intracellular enzymes can form and support dynamic strong black queens is related to whether the cost of synthesizing them is high, the way the effects

are diffused, and the surrounding environment.

Secreted enzymes

Secreted enzymes are usually enzymes that are synthesized within cells and then act outside the cells. ß-lactamase is one of the typical secreted enzymes. It is multi-resistant to β-lactam antibiotics such as cephalosporins, monomycins and penicillins. Because β-lactam antibiotics are effective against a broad spectrum of Gram-positive and Gram-negative bacteria, many bacteria require β-lactamases to resist the various antibiotics mentioned above. As secreted enzymes, how are β-lactamases used as public goods in the community? A 1969 study showed that some Gram-negative bacteria produce β-lactamases and that they secrete these β-lactamases when β-lactam antibiotics are present in the environment. (Neu, 1969) In this community containing β-lactamases, individual microorganisms can enjoy the protection of public goods β-lactamases. Therefore, secreted enzymes such as β-lactamases are different from intracellular enzymes in that secreted enzymes are usually directly secreted by individual cells in the form of enzymes and affect the surrounding environment. In summary, the main factor affecting the dynamics of secreted enzymes and intracellular enzymes is the same as the value of the public good itself, the energy required to produce this public good and the surrounding environment. The difference is that extracellular enzymes also need to consider how such public goods diffuse in the environment and across cell membranes.

Secreted metabolites

Siderophore is an important member of the public goods of secreted metabolite

type. In the microbial community, many microorganisms require iron for their growth. (Dale et al., 2004) However, in an aerobic environment and a suitable pH value, iron will exist in the form of trivalent cations and form precipitates, making it difficult for bacteria to effectively absorb iron. (Dale et al., 2004; Andrews, Robinson & Rodríguez-Quiñones, 2003) It is even more difficult for some bacteria in the host body to absorb iron. For example, mammals have glycoproteins with high affinity for iron. These glycoproteins can dissolve and transport iron in animals, further reducing the iron content outside the cells. (Weinberg, 1999) Some bacteria can secrete siderophores with high affinity for iron that capture and bind biologically available iron. The aspect of siderophores as public goods is that the iron they bind can be used not only by individuals who produce siderophores, but also by individuals with siderophore deficiencies. (D'Onofrio et al., 2010; Trick, 1989) To the extent that siderophore-bound iron is included, cheaters who are unable to produce siderophores can also exploit siderophore-bound iron. In summary, secreted metabolites are somewhat similar to secreted enzymes. They are both public goods that are first secreted out of individual cells and then affect the surrounding environment. Similarly, whether secreted metabolite can form and maintain a strong Black Queen dynamic is related to the public good itself, the way the public good diffuses in the environment and on the cell membrane, and the surrounding environmental conditions.

Experimental method

Experiments are mainly conducted based on models. In the experiment, the model I will use is an agent-based "bottom-up" model. (Fullmer, 2022, unpublished) In our model, cells are agents with internal variables, each agent having its own subroutine, to represent the cell's phenotypic and genetic background. Because in the strong black queen hypothesis, the result of a cell's increased fitness is usually related to the behavior and properties of neighboring cells. In order for each cell to maximize its ability to seek and improve its own fitness, each cell in the model is semi-independent of its neighbor cells and has its own rules. The above design is ideal for the dynamic characteristics of the strong black queen hypothesis.

As mentioned earlier, this experiment divides public goods into three categories. The first step of the experiment is to find and classify typical public goods, and to conduct a preliminary analysis of the characteristics of these public goods and related microorganisms through literature. Record and summarize the mode of action of these public goods, the environment in which they act, the speed and scope of diffusion in the environment and on cell membranes (secreted metabolites and secreted enzymes), the value of the public goods themselves and the costs required for production.

The second step is to use the above model to simulate the cooperation and competition of microorganisms in the natural environment and simulate the public goods in the environment. Variables are set within the cell that serve as the genetic background of the cell and determine the production of a variety of common products.

Variables within the cell are then examined, making some suitable variables available public goods in the environment. And set costs and penalties for producing public goods and stopping the production of public goods respectively.

The third step is to include in the model the strong black queen public goods characteristics recorded and summarized in the first step that affect the dynamics in the environment. By changing the value of public goods in the model, the production cost of public goods, the diffusion speed and scope of public goods, the mode of action of public goods, and the action environment of public goods, the performance of public goods with different characteristics in different environments is simulated. Finally, examine and determine which public goods are best or least suited to forming and sustaining a strong Black Queen dynamic.

Discussion

Before I started this experiment, my supervisor had developed a preliminary model. (Fullmer, 2022, unpublished) The preliminary model is a very exciting experimental result, but it does not completely test the strong black queen hypothesis. There are also factors that are significant enough to alter the model and disrupt the dynamics of the Strong Black Queen that need to be included and integrated into the model. Such as horizontal gene transfer, bottleneck effects and characteristics of public goods. Therefore, the purpose of this experiment to explore the characteristics of public goods is to incorporate the characteristics of public goods into the model, thereby promoting

a thorough test of the Black Queen hypothesis.

Regarding the mutual cheating between individual microorganisms, the words "cost" and "benefit" are mentioned in many papers I read. (Morris, Lenski & Zinser., 2012; Driscoll & Pepper, 2010; Van Vliet et al., 2022; Antonovics et al., 2015) I believe that the costs and benefits faced by cheaters who form a strong black queen dynamic are mainly determined by two factors: the environment and the characteristics of public goods. The so-called "benefits" are very easy to understand, that is, the energy saved by cheaters after they stop producing public goods. The more energy required to produce the type of public good, the greater the benefits to cheaters and the easier it is to form a dynamic strong black queen. In the same way, the less energy in the environment, the more difficult it is for microorganisms in the community to obtain energy, which will also lead to an increase in benefits and promote the formation of a dynamic strong black queen in the environment.

In summary, the characteristics of public goods and the environment directly determine the benefits and costs of cheaters, which in turn determine whether strong black queen dynamics will be formed and maintained in the environment. For example, the intracellular enzymes mentioned in the background section function as public goods by affecting the surrounding environment through their own characteristics. For example, the way catalase works is that bacteria synthesize catalase inside the cell and reduce the concentration of hydrogen peroxide inside the cell. But since the membrane permeability of hydrogen peroxide is about the same as that of ambient water, these catalase-producing individuals will inevitably reduce the concentration of hydrogen

peroxide in the surrounding environment. Such a public product mode of action results in the action range of intracellular enzymes being relatively fixed and small. This would increase the cost for cheaters to lose the gene that produces catalase. Cheaters would need to live in close proximity to producers to avoid the costs of catalase deficiency. Once such costs increase again, for example, the membrane permeability of water in the environment changes or the concentration of hydrogen peroxide in the environment increases, cheaters will be unable to survive in the environment and it will be difficult to form a dynamic strong black queen.

Secreted enzymes and Secreted metabolites may behave differently because their mode of action is to influence and change the surrounding environment outside the cell after being secreted by cells. Then once the environmental conditions are suitable for the proliferation of these Secreted enzymes or Secreted metabolites in the environment, they may spread to locations farther away from the producers. This greatly reduces the cost for cheaters to stop producing such public goods. Although cheaters still need to survive next to producers, this scope will be expanded, and it will be easier for cheaters to obtain the benefits of public goods. In addition, assuming that the energy required to synthesize public goods is the same, the producers of secreted enzymes or secreted metabolites also need to pay for the additional energy required to transport the public goods outside the cell. This invisibly increases the benefits for cheaters to stop producing such public goods, that is, they can save more energy for their growth and reproduction. In this way, the probability of forming and maintaining a dynamic strong black queen in the environment will be greatly increased. In summary, further

experiments in the future need to consider how to quantify in the environment the changes in benefits and costs incurred by cheaters who stop producing public goods caused by different characteristics of public goods.

Conclusion

This experiment explores the characteristics of different public goods through models, and introduces the strong black queen hypothesis and the typical public goods among the three types of public goods. And in the discussion part, some types of public goods are briefly compared from the perspective of costs and benefits. Since the interactions between microorganisms are often mediated by public goods. And different types of public goods usually have different diffusion methods and modes of action and other factors that affect the formation and maintenance of dynamic strong black queens in the environment. Therefore, the explore of the characteristics of public goods in this experiment has great significance for testing the strong black queen hypothesis and understanding the interactions between microorganisms. This experiment will also provide sufficient background and experience for future research on public goods.

Reference

Andrews, S. C., Robinson, A. K., & Rodríguez-Quiñones, F. (2003). Bacterial iron homeostasis. *FEMS Microbiology Reviews*, *27*(2), 215–237. https://doi.org/10.1016/S0168-6445(03)00055-X.

Antonovics, J., Bergmann, J., Hempel, S., Verbruggen, E., Veresoglou, S., & Rillig, M. (2015). evolution of mutualism from reciprocal parasitism: more ecological clothes for the Prisoner's Dilemma. *Evolutionary Ecology*, *29*(5), 627–641. https://doi.org/10.1007/s10682-015-9775-6.

Callanan, M., Kaleta, P., O'Callaghan, J., O'Sullivan, O., Jordan, K., McAuliffe, O., Sangrador-Vegas, A., Slattery, L., Fitzgerald, G. F., Beresford, T., & Ross, R. P. (2008). Genome Sequence of Lactobacillus helveticus, an Organism Distinguished by Selective Gene Loss and Insertion Sequence Element Expansion. *Journal of Bacteriology*, *190*(2), 727–735. https://doi.org/10.1128/JB.01295-07.

Church, M. J., Jenkins, B. D., Karl, D. M., & Zehr, J. P. (2005). Vertical distributions of nitrogen-fixing phylotypes at Stn ALOHA in the oligotrophic North Pacific Ocean. *Aquatic Microbial Ecology : International Journal*, *38*(1), 3–14. https://doi.org/10.3354/ame038003.

Cooper, W. J., Zika, R. G., Petasne, R. G., & Plane, J. M. C. (1988). Photochemical formation of H2O2 in natural waters exposed to sunlight. *Environmental Science & Technology*, *22*(10), 1156–1160. https://doi.org/10.1021/es00175a004.

Cordero, O. X., & Polz, M. F. (2014). Explaining microbial genomic diversity in light

of evolutionary ecology. *Nature Reviews. Microbiology*, *12*(4), 263–273. https://doi.org/10.1038/nrmicro3218.

D'Onofrio, A., Crawford, J. M., Stewart, E. J., Witt, K., Gavrish, E., Epstein, S., Clardy, J., & Lewis, K. (2010). Siderophores from Neighboring Organisms Promote the Growth of Uncultured Bacteria. *Chemistry & Biology*, *17*(3), 254–264. https://doi.org/10.1016/j.chembiol.2010.02.010.

Dale, S. E., Doherty-Kirby, A., Lajoie, G., & Heinrichs, D. E. (2004). Role of Siderophore Biosynthesis in Virulence of Staphylococcus aureus: Identification and Characterization of Genes Involved in Production of a Siderophore. *Infection and Immunity*, *72*(1), 29–37. https://doi.org/10.1128/IAI.72.1.29-37.2004.

Driscoll, W. W., & Pepper, J. W. (2010). THEORY FOR THE EVOLUTION OF DIFFUSIBLE EXTERNAL GOODS. *Evolution*, *64*(9), 2682–2687. https://doi.org/10.1111/j.1558-5646.2010.01002.x.

Fullmer, M. S., Soucy, S. M., & Gogarten, J. P. (2015). The pan-genome as a shared genomic resource: Mutual cheating, cooperation and the black queen hypothesis. *Frontiers in Microbiology*, *6*, 728–728. https://doi.org/10.3389/fmicb.2015.00728.

Germerodt, S., Bohl, K., Lück, A., Pande, S., Schröter, A., Kaleta, C., Schuster, S., & Kost, C. (2016). Pervasive Selection for Cooperative Cross-Feeding in Bacterial Communities. *PLoS Computational Biology*, *12*(6), e1004986–e1004986. https://doi.org/10.1371/journal.pcbi.1004986.

Guchte, M. van de, Penaud, S., Grimaldi, C., Barbe, V., Bryson, K., Nicolas, P., Robert,

C., Oztas, S., Mangenot, S., & Couloux, A. (2006). complete genome sequence of Lactobacillus bulgaricus reveals extensive and ongoing reductive evolution. *Proceedings of the National Academy of Sciences - PNAS*, *103*(24), 9274–9279. https://doi.org/10.1073/pnas.0603024103.

Kent, A. G., Vill, A. C., Shi, Q., Satlin, M. J., & Brito, I. L. (2020). Widespread transfer of mobile antibiotic resistance genes within individual gut microbiomes revealed through bacterial Hi-C. *Nature Communications*, *11*(1), 1–9. https://doi.org/10.1038/s41467-020-18164-7.

Martin Tichy, & Wim Vermaas. (1999). In Vivo Role of Catalase-Peroxidase inSynechocystis sp. Strain PCC 6803. *Journal of Bacteriology*, *181*(6), 1875–.

Meijer, J., van Dijk, B., & Hogeweg, P. (2020). Contingent evolution of alternative metabolic network topologies determines whether cross-feeding evolves. *Communications Biology*, *3*(1), 401–401. https://doi.org/10.1038/s42003-020-1107-x.

Morris, B. E. ., Henneberger, R., Huber, H., & Moissl-Eichinger, C. (2013). Microbial syntrophy: interaction for the common good. *FEMS Microbiology Reviews*, *37*(3), 384–406. https://doi.org/10.1111/1574-6976.12019.

Morris, J. J., Lenski, R. E., & Zinser, E. R. (2012). The black queen hypothesis: Evolution of dependencies through adaptive gene loss. *mBio*, *3*(2). https://doi.org/10.1128/mBio.00036-12.

Neu, H. C. (1969). Effect of beta-lactamase location in Escherichia coli on penicillin synergy. *Applied Microbiology*, *17*(6), 783–786.

https://doi.org/10.1128/aem.17.6.783-786.1969.

Pande, S., Kaftan, F., Lang, S., Svato, A., Germerodt, S., & Kost, C. (2016). Privatization of cooperative benefits stabilizes mutualistic cross-feeding interactions in spatially structured environments. *The ISME Journal*, *10*(6), 1413–1423. https://doi.org/10.1038/ismej.2015.212.

Pande, S., Merker, H., Bohl, K., Reichelt, M., Schuster, S., De Figueiredo, L. F., Kaleta, C., & Kost, C. (2014). Fitness and stability of obligate cross-feeding interactions that emerge upon gene loss in bacteria. *The ISME Journal*, *8*(5), 953–962. https://doi.org/10.1038/ismej.2013.211.

Pechenik, J. A. (2000) *Invertebrates.* Vol. 193. Singapore." McGraw Hill.

Perelman, A., Uzan, A., Hacohen, D., & Schwarz, R. (2003). Oxidative Stress in Synechococcus sp. Strain PCC 7942: Various Mechanisms for H2O2 Detoxification with Different Physiological Roles. *Journal of Bacteriology*, *185*(12), 3654–3660. https://doi.org/10.1128/JB.185.12.3654-3660.2003.

Petasne, R. G., & Zika, R. G. (1997). Hydrogen peroxide lifetimes in south Florida coastal and offshore waters. *Marine Chemistry*, *56*(3), 215–225. https://doi.org/10.1016/S0304-4203(96)00072-2.

Pierson, L. S. I., & Pierson, E. . (1996). Phenazine antibiotic production in Pseudomonas aureofaciens: role in rhizosphere ecology and pathogen suppression. *FEMS Microbiology Letters*, *136*(2), 101–108. https://doi.org/10.1111/j.1574-6968.1996.tb08034.x.

Raymond, J., Siefert, J. L., Staples, C. R., & Blankenship, R. E. (2004). The Natural History of Nitrogen Fixation. *Molecular Biology and Evolution*, *21*(3), 541–554. https://doi.org/10.1093/molbev/msh047.

Riley, M. A., & Gordon, D. M. (1999). The ecological role of bacteriocins in bacterial competition. *Trends in Microbiology*, *7*(3), 129–133. https://doi.org/10.1016/S0966-842X(99)01459-6.

Scanlan, D. J., Ostrowski, M., Mazard, S., Dufresne, A., Garczarek, L., Hess, W. R., Post, A. F., Hagemann, M., Paulsen, I., & Partensky, F. (2009). Ecological Genomics of Marine Picocyanobacteria. *Microbiology and Molecular Biology Reviews*, *73*(2), 249–299. https://doi.org/10.1128/MMBR.00035-08.

Seaver, L. C., & Imlay, J. A. (2001). Hydrogen Peroxide Fluxes and Compartmentalization inside Growing Escherichia coli. *Journal of Bacteriology*, *183*(24), 7182–7189. https://doi.org/10.1128/JB.183.24.7182-7189.2001.

Trick, C. G. (1989). Hydroxamate-siderophore production and utilization by marine eubacteria. *Current Microbiology*, *18*(6), 375–378. https://doi.org/10.1007/BF01571131.

Van Vliet, S., Hauert, C., Fridberg, K., Ackermann, M., & Co, A. D. (2022). Global dynamics of microbial communities emerge from local interaction rules. *PLoS Computational Biology*, *18*(3), e1009877–e1009877. https://doi.org/10.1371/journal.pcbi.1009877.

Vitousek, P. ., & Howarth, R. . (1991). Nitrogen limitation on land and in the sea: How

can it occur? *Biogeochemistry*, *13*(2), 87–115. https://doi.org/10.1007/BF00002772.

Weijer, C. (1997). Full House: The Spread of Excellence from Plato to Darwin. *BMJ*, *314*(7082), 761–761. https://doi.org/10.1136/bmj.314.7082.761a.

Weinberg, E. D. (1999). Iron loading and disease surveillance. *Emerging Infectious Diseases*, *5*(3), 346–352. https://doi.org/10.3201/eid0503.990305.

Zheng, M., Zhou, Z., Luo, Y., Zhao, P., & Mo, J. (2019). Global pattern and controls of biological nitrogen fixation under nutrient enrichment: A meta-analysis. *Global Change Biology*, *25*(9), 3018–3030. https://doi.org/10.1111/gcb.14705.