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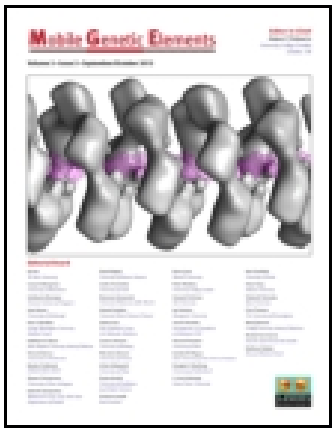

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Mobile genetic elements are involved in bacterial sociality

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Mobile genetic elements are involved in bacterial sociality

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Mobile genetic elements in bacteria are enriched in genes participating in social behaviors, suggesting an evolutionary link between gene mobility and social evolution. Cooperative behaviors, like the production of secreted public good molecules, are susceptible to the invasion of non-cooperative individuals, and their evolutionary maintenance requires mechanisms ensuring that benefits are directed preferentially to cooperators. In order to investigate the reasons for the mobility of public good genes, we designed a synthetic bacterial system where we control and quantify the transfer of public good production genes. In our recent study, we have experimentally shown that horizontal transfer helps maintain public good production in the face of both non-producer organisms and non-producer plasmids. Transfer spreads genes to neighboring cells, thus increasing relatedness and directing a higher proportion of public good benefits to producers. The effect is the strongest when public good genes undergo epidemics dynamics, making horizontal transfer especially relevant for pathogenic bacteria that repeatedly infect new hosts and base their virulence on costly public goods. The promotion of cooperation may be a general consequence of horizontal gene transfer in prokaryotes. Our work has an intriguing parallel, cultural transmission, where horizontal transfer, such as teaching, may preferentially promote cooperative behaviors.

Keywords: bacterial cooperation, genetic relatedness, horizontal gene transfer, mobile genetic elements, plasmid transfer, public good production, social evolution, synthetic biology

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Introduction

Plasmids and other mobile elements frequently bear genes involved in social interactions between bacteria.[1] Particularly, they carry genes participating in the production of public goods, molecules that are accessible to other organisms than the producing ones. For instance, β-lactamases, secreted proteins that degrade antibiotics extracellularly, are a cooperative mode of antibiotic resistance,[2] predominantly located on plasmids.[3] More generally, a bioinformatic analysis of *Escherichia* genomes revealed that genes predicted to code for secreted proteins, likely to be involved in social interactions, are over-represented on plasmids or mobile regions of the chromosome,[4] suggesting a link between genes involved in social interactions and gene mobility.

The maintenance of cooperative behaviors is an important topic in evolutionary biology. Costly public goods can be exploited by cheaters, individuals that benefit from them without contributing to their production, leading to extinction of cooperators (**Fig. 1A**). Social evolution theory predicts that cooperation can be maintained when its benefits are directed preferentially to cooperative organisms (**Fig. 1B**), as summarized by Hamilton's rule[5]: a behavior will be selected when $r\,b > c$, with c being its direct fitness cost for the actor, b its indirect fitness benefit to recipients (all individuals benefiting from it), and r the relatedness between actors and recipients. Relatedness is a measure of the statistical association between cooperators, and high relatedness effectively means that recipients of cooperation benefits are likely to be cooperators themselves. High relatedness arises from actors and recipients sharing the cooperative alleles, which usually happens among kin, due to vertical gene transmission. However, horizontal transfer in bacteria can modify this pattern. Particularly, the genes responsible for social behaviors can spread in a population,[6] modifying relatedness at the social locus.[4] It has thus been proposed that

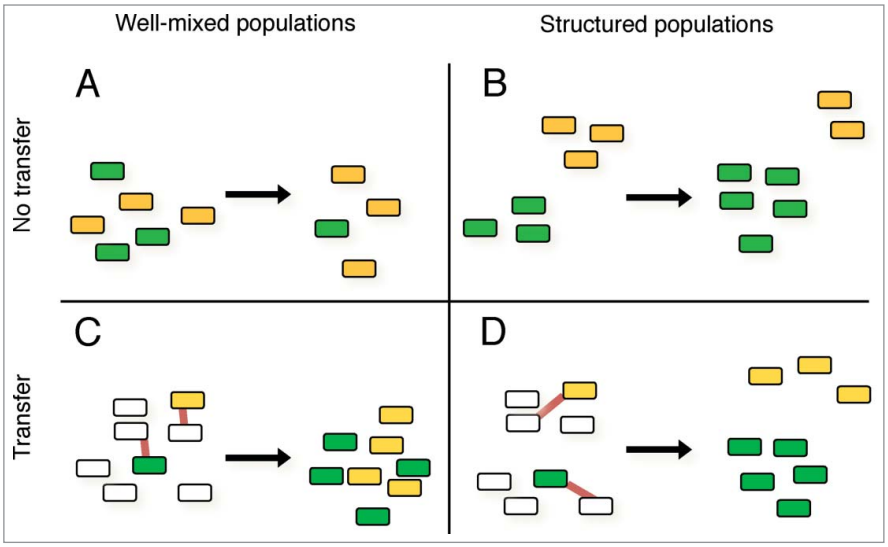

Figure 1. Scenarios for public good maintenance and horizontal transfer in bacteria. Producer cells (green) produce a public good that benefits growth of neighboring cells. Non-producer cells (yellow) benefit from the public good but do not produce it. Well-mixed populations are shown in (**A**) and (**C**) and structured populations where producers interact mainly with other producer cells are shown in (**B**) and (**D**). In (**C**) and (**D**) populations, producer and non-producer alleles can be transferred by conjugation (red pili). Non-producers outcompete producers in competition in a well-mixed population (**A**), but not in structured populations where public good benefits can be restricted to producers (**B**). In well-mixed populations transfer can promote public good production because of the infectious spread of the producer allele, but can spread the non-producer allele as well (**C**). In structured populations, transfer promotes public good production by increasing relatedness (**D**).

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horizontal gene transfer helps maintain cooperation in bacteria.[4,6]

Two explanations for the effect of transfer on cooperation have been put forward. The first hypothesis relies on the enforcement of cooperation in previously non-producing cells by horizontal transfer (**Fig. 1C**).[6] A public good gene, encoded on a transferable plasmid, converts non-producer cells into producers and thus compensates for the competitive disadvantage of the producing cells compared to cells that do not bear the production allele (**Fig. 1C**, green cells). However, cheater plasmids bearing a non-producing allele are likely to appear quickly, for instance by deletion of the production allele from a producing plasmid. If cheater plasmids have the same or higher transfer rate compared to cooperative plasmids, cooperation would again be threatened by the spread of cheaters, now at the plasmid level [4] (**Fig. 1C**, yellow cells). The second hypothesis addresses this issue of cheating plasmids by focusing on the effect of horizontal transfer on genetic relatedness in a structured population (**Fig. 1D**), rather than direct cooperation enforcement. Models show that horizontal transfer increases relatedness in a patch-structured metapopulation, increasing the probability that 2 individuals in a subpopulation carry the same allele by spreading this allele within the subpopulation.[4] As high relatedness favors cooperation, horizontal transfer should then promote cooperation.

Testing the effects of horizontal gene transfer with a synthetic approach

The mechanisms described here suggested 2 different ways in which plasmid transfer could promote cooperation, but both lacked experimental validation. More generally, mathematical models provide conclusions on the mechanisms by which a given factor can influence a system's outcome. However, they often do not tell if the parameter's values of living organisms and environments are in the range where the described effect is possible or relevant. Neither of the models here identifies the rates of transfer that would be sufficient to maintain cooperation, a key parameter. Moreover, models make numerous assumptions, that are not necessarily true or realistic, and can also overlook basic or complex processes occurring in living organisms that would affect the conclusions. In our case, important constraints influencing conjugation in natural systems, such as physical ones, could have been missed in the equations.

We thus experimentally tested the 2 hypotheses and used numerically solved analytical models with experimentally determined parameters to help understanding population dynamics and explore a wider parameter range.[7] This combination allowed us to benefit from the advantages of both modeling and experimental methodologies. In order to rigorously test for the 2 mechanisms by which transfer could promote cooperation, we used synthetic biology techniques, enabling independent control of public good production and plasmid transfer. The synthetic approach aims at constructing and studying simpler systems where defined components are modified in a controlled way, reducing the complexity of the system.[8] Indeed, natural systems are typically complex, with multiple interactions shaped by evolution and selection. These interactions can be adaptive in natural environments, but will be confounding factors in an analysis where the goal is to precisely measure the effect of individual parameters. Synthetic systems are powerful tools, trading some complexity for control over factors, and can generally be viewed as intermediates between models and natural biological systems.[9]

We constructed a synthetic system that combines 2 well-studied natural components: public good production genes from *Pseudomonas aeruginosa* quorum sensing, and the transfer control system of *Escherichia coli* F plasmid (**Fig. 2**). In our case, working with a synthetic system reduced the risk of unwanted interactions between the components being tested. Both public good production[10] and plasmid transfer[11] are extremely regulated in natural systems and sensitive to multiple environmental factors. The relevant genes could thus be regulated by similar or antagonistic signals because of previous co-evolution of transfer and cooperation or could simply be affected by the same environmental conditions. Distinguishing between and

controlling for such co-regulation is difficult, even under laboratory conditions. However, with our synthetic system, we could compete strains or plasmids that differ purely in public good production or transfer ability, allowing us to directly link the fitness effects with the transfer of specific alleles. Moreover, fluorescence markers allow us to precisely follow competing strains and plasmid transfer, quantifying the effect of transfer in our experiments. By decoupling transfer and its control, we can focus on the transfer of specific genes (here producer/non-producer alleles), excluding potential effects linked not only to the transfer of other genes involved in plasmid mobilisation and pili production, but also the entry exclusion or toxin-antitoxin systems. Finally, in order for all cells to be able to receive plasmids, we use an F plasmid with no entry exclusion genes to control for transfer.

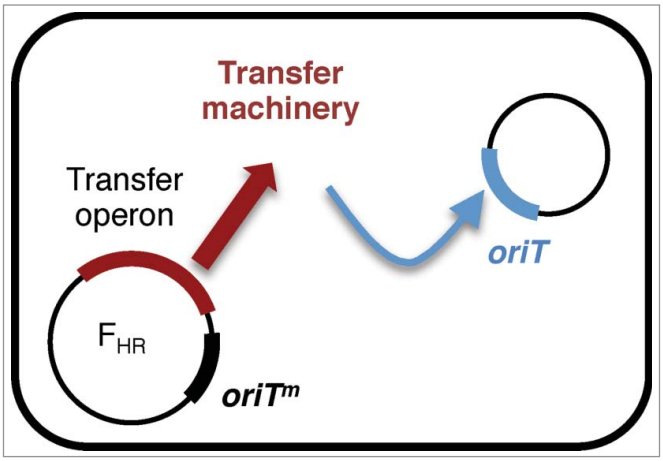

Figure 2. Synthetic control of horizontal gene transfer. F_{HR} plasmid is a helper F plasmid bearing *oriTm*, a mutant *oriT* not recognized by the F conjugation machinery coded by F transfer operon. The transfer machinery acts only in *trans*, and mobilizes (blue arrow) the plasmids bearing the wild-type *oriT*.

Horizontal transfer promotes the maintenance of public good production

We started by experimentally showing that transfer can efficiently promote public good production purely by enforcement of gene expression in recipient cells. Transfer lead to allele frequency changes that largely counteracted the cost of public-good production. We thus demonstrated the validity of the first hypothesis proposing a link between transfer and cooperation,[6] in the short-term: enforcement through transfer can be a very efficient strategy for cooperative alleles to spread in the absence of population structure (**Fig. 1C**, when considering that only green cells spread). However, when we competed producer and non-producer alleles that are both mobile, producers decreased in frequency even more than in the total absence of transfer (**Fig. 2C**, when both green and yellow cells spread). We thus must conclude that enforcement can explain short-term maintenance and even invasion of public good genes, but not long-term dynamics. Indeed, mobile non-producer alleles will appear rapidly over evolutionary timescales, for instance simply by knockout of the producer gene in a mobile plasmid, and will then outcompete the mobile producer alleles.

In the second part of the study, we addressed the hypothesis that transfer promotes cooperation by increasing relatedness [4] (**Fig. 1D**). In order to do so, we used a simple metapopulation consisting of 2 subpopulations that differ in the frequency of producer cells. Each subpopulation is well mixed, but on average, producers encounter more producer than non-producer cells at the metapopulation scale. We showed that public-good production was more favored at the metapopulation level when both producer and non-producer alleles are able to transfer, compared to a situation without any transfer. The effect of transfer was based on between-population dynamics: within subpopulations, the producer allele frequencies did not increase, but among populations growth differences were amplified by transfer. The outcome is analogous to the one arising from the Simpson's paradox, a scenario that was already shown to allow for cooperation maintenance by biasing its benefits toward cooperators.[12] Despite the fact that producers decline within each subpopulation, they outcompete non-producers at the metapopulation scale because populations enriched in producers are more successful and represent a greater proportion of the total population. With transfer, selection among populations was increased, which in turn favored producers at the metapopulation level.

The synthetic approach allowed us to test and confirm modeling predictions, showing that our models sufficiently captured the crucial aspects of real biological systems. The key insight of our study is that the effect of transfer can take place within real organisms, and with realistic parameters concerning transfer, benefits and costs, and population structure. For example, the plasmid transfer rate in our experiments was well within, if on the high end, the range for the transfer rates in nature. On the other hand, some of the features of our system are actually likely to be less favorable for either transfer or public good production than the natural ones. Most prominently, the costs of transfer and secretion are probably stronger than in nature, due to the absence of natural regulations decreasing such costs[11,13]: transfer is derepressed and public good production is constitutive in our system, maximizing the cost to the cell. Finally, we focused on conditions where transfer is likely because plasmid-free cells are initially abundant, and avoided the case where most of the cells have plasmids. In natural systems with entry exclusion, there will be little opportunity for transfer in such situation; more complex phenomena like superinfection and virulence toward the host cell could also occur.[14] Our work confirms that maintenance of public good production through horizontal transfer is

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possible and likely in nature, at least with relatively high transfer rates and availability of plasmid-free cells. Reversely, the maintenance of cooperation could itself explain the existence of high rates of transfer in some natural isolates,[15] alternatively to the selection for a purely parasitic plasmid spread. Still, the full range of parameter values in nature remains unknown, motivating further research on this topic.

Horizontal transfer acts through a gene-specific increase in relatedness

Our experiments have shown that the stable promotion of public good production relies on the increase in relatedness created by horizontal transfer in structured populations: the association between public good producers increases at the metapopulation level. We got further insight into the dynamics and effect of genetic relatedness by studying it with simulations. We observed that soon after the plasmid invades, relatedness increases strongly (**Fig. 3A**). Because transfer happens at a local scale, it homogenizes local allele content in the cell's neighbors, resulting in relatedness increase. We examined the importance of the interplay between the population structure and the transfer dynamics by modeling horizontal transfer across - and not within – subpopulations. Our results have shown that when transfer is not local, plasmid invasion still occurs, but its effects on relatedness and in turn cooperation, disappear (**Fig. 3B**). We concluded that the effect of horizontal transfer on the maintenance of cooperative behaviors fundamentally relies on infectious transmission specifically at short spatial scales. It is only when transfer is local that the infectious transmission spreads alleles at a local scale while creating stronger assortment at a higher scale.

Maintenance of cooperative behaviors by horizontal mobility in other systems

In our work we focused on the case of plasmid conjugation, which naturally happens at a local scale, between neighboring cells. A number of other horizontally transferred mobile elements, such as integrative conjugative elements,[16] also use conjugation and should thus follow a similar dynamics. On the other hand, there are horizontally transferred elements, including bacteriophages, with an extracellular phase that may make their transmission less spatially constrained. Still, they are likely to infect cells close to the ones that they originated from, and in a patch-structured metapopulation, the infection will also disseminate genes mostly within patches. Another important factor, the cost of transfer, can also vary depending on the particular mobile element. The spread of phages by lysis makes them into virulent parasites, strongly increasing the cost of transfer. But some bacteriophages, such as the filamentous phages involved in *Vibrio cholerae* virulence, spread by secretion, without cell lysis,[17] and are likely to confer similar costs to the host as the plasmids do. Generally, we expect that our conclusions, derived for plasmid conjugation, extend to any costly, locally spread mobile genetic elements: the promotion of cooperation may be considered a general consequence of horizontal gene transfer in prokaryotes.

In the study we consider here we have used a prokaryotic system, but can more generally wonder whether our conclusions would apply to mobile elements in eukaryotes. We believe the effect of transfer would be reduced since mobile elements in eukaryotes are spread through sexual reproduction.[18] Without fast horizontal transmission, the epidemic dynamics of the type we described will be strongly limited and cooperative genes would benefit less from the increase in relatedness arising from gene mobility. There is however another type of phenomenon with dynamics that matches our bacterial system quite well, namely cultural transmission.

Cultural transmission generally refers to the way humans, or animals, learn and pass information within their societies. A given behavior can be transferred many times within a single generation, much faster than genetic transmission. When it happens within groups, cultural learning has been shown to increase variation among groups and promotes cooperative behaviors.[19] Early models of the effect of horizontal gene transfer on relatedness borrowed some of the cultural transmission formalism,[4] highlighting the similarities of both phenomena. Horizontal gene transfer in bacteria and cultural transmission in humans share a speed of transmission that is higher than the one for respectively vertical and genetic transmission, and increase assortment when they happen at a local scale. Both processes also have a high specificity: only some of the genes or behaviors are transmitted horizontally. The transferred entities will, on average, experience higher relatedness than others, and will be favored due to their social aspects. Finally, in cultural transmission as well as in plasmid conjugation, the benefits of horizontal spread of

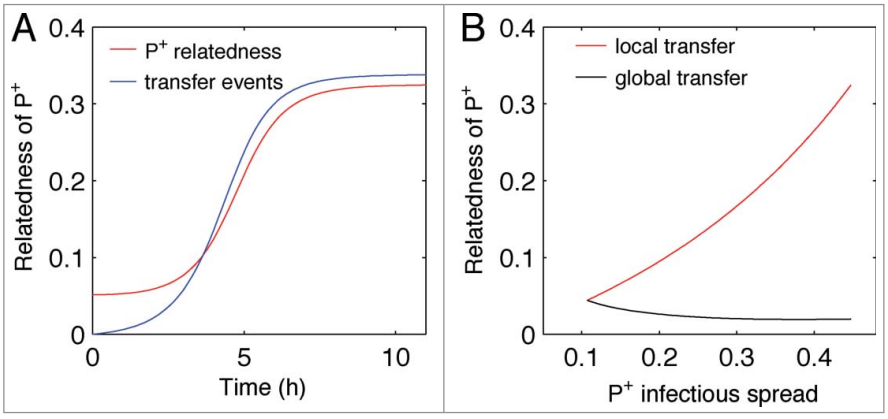

Figure 3. Relatedness dynamics. (**A**) Genetic relatedness at the producer (P^+) locus (red) and the proportion of cells in the population that arose by transfer (transfer events, blue) are shown as a function of time. (**B**) Relatedness is shown as a function of the producer allele infectious spread, with local transfer (similar to the experiments, red line) and global transfer (simulating mixing of transferred plasmids across subpopulations, black line).

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cooperation may in turn select for the mobility itself, further coupling cooperation and transfer.

Transfer in natural environments and epidemic dynamics

A key parameter affecting the possibility of transfer itself and its effect on cooperation is the availability of plasmid-free cells. We focused experimentally on conditions where plasmid-bearing cells are initially rare, leading to many transfer events. Despite the lack of quantitative data for natural ecosystems, the very existence of plasmid transfer mechanisms demonstrates that plasmid-free cells occur frequently enough so that transfer can be selected. Additionally, many plasmids exhibit transitory derepression, their transfer genes being strongly upregulated shortly after entering a new recipient cell, enabling invasive spread in the new host populations.[20] Such regulatory mechanism also suggests that plasmids indeed often encounter new, plasmid-free host populations and undergo epidemic spread. Our simulations showed that the effect of transfer on public good production is the strongest precisely during the epidemic plasmid spread, because the spread leads to a very high number of transfer events. With high transfer rates, sufficient relatedness results simply from the stochastic frequency variations among subpopulations when plasmid-bearing cells are initially rare. The transfer effect will thus be strongest when a few plasmid-bearing individuals repeatedly encounter populations of plasmid-free cells. This could be particularly relevant for the case of pathogens whose growth and virulence are promoted by secreted toxins,[17,21,22] or resistance enzymes.[2] The transfer of toxin or resistance genes to the host microbiome would amplify public good production upon infection, directly increasing fitness and promoting cooperation.

Conclusion

In our recent study we have shown experimentally that horizontal transfer, via its effect on relatedness, favors cooperation. Moreover, our simulations suggest that epidemic spread of mobile elements is essential for this dynamics. Further, based on controlled lab experiments and numerical simulations, we expect our findings to apply to pathogenic bacteria, which regularly encounter new hosts and whose virulence and resistance is often based on secreted molecules encoded on mobile elements. The dynamics we describe could be an important force driving their ecology and evolution. Treatments countering plasmid conjugation, in addition to directly slowing the spread of antibiotic resistance could reduce virulence and resistance benefits. Moreover, because transfer acts on a social, cooperative behavior, resistance to such treatments may be less easy to evolve than resistance to more classical antibiotics.[23] Finally, transfer may be a cooperative behavior in and of itself, and also susceptible to cheating.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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