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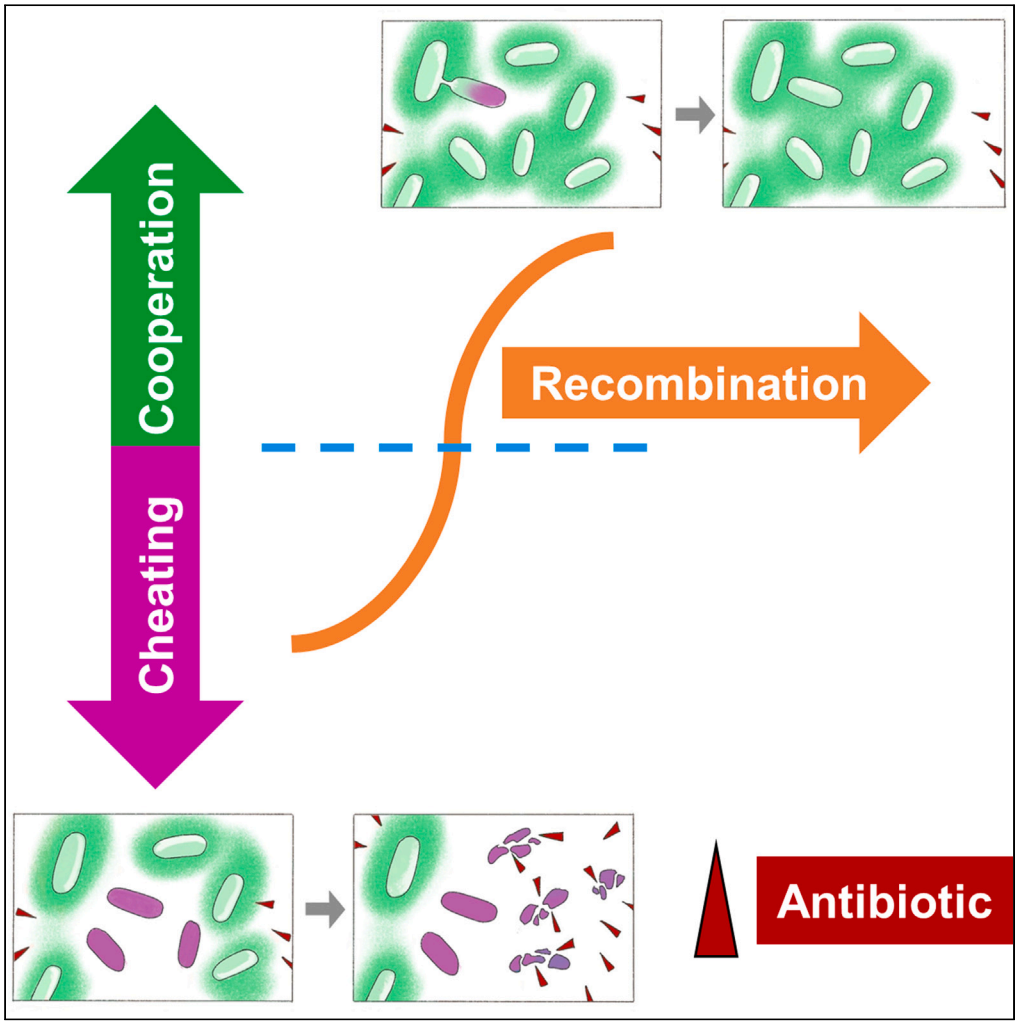

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Highlights

Homologous recombination provides a mechanism to enforce cooperation in bacteria

An agent-based model is used to study cooperation in a public goods game

Increased recombination rate allows cooperators to outcompete freeloaders

Benefits of cooperation, viscosity, and population size can also promote cooperation

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Recombination as an enforcement mechanism of prosocial behavior in cooperating bacteria

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SUMMARY

Prosocial behavior is ubiquitous in nature despite the relative fitness costs carried by cooperative individuals. However, the stability of cooperation in populations is fragile and often maintained through enforcement. We propose that homologous recombination provides such a mechanism in bacteria. Using an agent-based model of recombination in bacteria playing a public goods game, we demonstrate how changes in recombination rates affect the proportion of cooperating cells. In our model, recombination converts cells to a different strategy, either freeloading (cheaters) or cooperation, based on the strategies of neighboring cells and recombination rate. Increasing the recombination rate expands the parameter space in which cooperators outcompete freeloaders. However, increasing the recombination rate alone is neither sufficient nor necessary. Intermediate benefits of cooperation, lower population viscosity, and greater population size can promote the evolution of cooperation from within populations of cheaters. Our findings demonstrate how recombination influences the persistence of cooperative behavior in bacteria.

INTRODUCTION

Many bacterial species are social, with the fate of their populations dependent on coordination and cooperation within the collective. This is typically achieved by the secretion of molecules shared as public goods between cells,[1] which greatly influences the survival of the entire collective.[2,3] In bacteria, public goods include a variety of secreted molecules, such as exopolysaccharides to form biofilms, toxins to kill competitors, digestive enzymes, biosurfactants for group motility, detoxifying proteins, and siderophores to sequester limited nutrients.[1,4] Cooperation is susceptible to exploitation because the costly public goods are freely available to benefit all individuals, regardless of their contribution. These freeloaders thereby gain a local fitness advantage over cooperative neighbors, undermining the stability of cooperation[2] and potentially leading to the collapse of the entire population.[5,6] For example, cooperative iron uptake in some populations of *Pseudomonas aeruginosa* can be compromised by cheaters to the point that the pyoverdine system is lost.[7] In *Vibrio*, quorum sensing can disappear from subpopulations overrun with cheaters, and the reestablishment of cooperation requires interventions from other ecological factors.[8] Referred to as the 'public goods dilemma', the provision of public goods is costly for individuals yet beneficial for all.[2,5,9] For cooperation to persist, enforcement mechanisms are needed to curtail cheating.[10] In bacteria, cooperative behavior can be enforced through quorum sensing, policing, partial privatization, spatial structure, and pleiotropic effects.[6,11] These measures ensure that the benefits of cooperation are disproportionately reaped by other cooperators. Whether through direct effects or by withholding the benefits of cooperation, the fitness of cheating is reduced.

Bacteria frequently import gene or gene fragments through horizontal gene transfer (HGT), which can result in either the replacement of existing homologous DNA through recombination or the addition of new DNA in their chromosome.[12,13] The process of HGT plays a central role in generating the variation upon which natural selection can act.[14] It is an important mechanism for bacteria to expand their ecological niches and endure environmental stresses such as toxic chemicals and antibiotics.[15] With HGT, the pace of adaptation in bacteria is dramatically hastened, readily radiating adaptations to neighbors, a process not limited by the standing genetic variation nor the slow rate by which adaptive genes are created through mutation and transmitted vertically.[13,16]

We hypothesize that HGT followed by homologous recombination acts as an enforcement mechanism for cooperative behavior in bacterial populations. When bacteria exhibit preferences for transfer partners,

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recombination can act as a homogenizing force that maintains and reinforces similarity among members of a group.[17] This is similar to how punishment functions to convert cheaters into cooperators, causing the local conformance of cooperation.[18] As an enforcement mechanism, transfer of a cooperative gene can therefore transform cheating cells into cooperating cells, thus effectively curtailing cheaters in the population.[19,20] Evidence supporting this has come from differential equation models, genomic data, and experimental work with genetic constructs.[21–24]

When a gene coding for a public good has both functional cooperative alleles and loss-of-function cheater alleles, homologous recombination can convert bacteria with cheater alleles and produce the conformance of cooperation in the collective. Here, we test our hypothesis by developing an agent-based model of homologous recombination in a population of bacteria playing a public goods game. Models that describe individual agents operating independently are particularly useful because they allow us to explore the behavior and interaction of individuals and the population-level outcomes that emerge from their interactions.[25] In our study, we model homologous recombination of a single gene transferred through a conjugative mobile genetic element (MGE), with the localized cell-to-cell interaction lending itself well to agent-based modeling. This conjugative MGE and its encoded allele for cooperation are transmitted both horizontally (with a set recombination rate) and vertically, allowing the model to be generalizable for the wide variety of conjugative MGEs.[26] Our results demonstrate the ecological conditions in which recombination can promote cooperation in a population of cheater strains. These conditions include intermediate relative benefits of cooperation (m), lower population viscosity (v), and higher population size (n). Outcomes from this study have profound implications to understanding how homologous recombination influences the persistence of cooperative behavior among bacteria. Furthermore, our results show that complex dynamics evolve when considering negative fitness effects from the act of gene transfer.

RESULTS

Characteristics of the bacterial population

A population consists of bacterial cells of the same species. The population can be partitioned into cooperating cells (cooperators) or cheater (selfish) cells. Each cooperator contributes a fitness benefit (public good) to the entire group, with the contribution multiplied by a value (cooperation multiplier m)[18] that is distributed equally among all group members, including the cooperators. In our model (Figure 1; Table S1), we set the cooperators to contribute one unit of fitness to this common pool. The effects of varying the multiplier in groups containing one cooperator are shown in Table 1, which illustrates how the multiplier reflects the benefit of public good production. This is to reflect on how the combined effort of a group can result in a benefit greater than the sum of what each individual would gain on their own.[18,27] A multiplier of one indicates that in a population of one cooperator, the fitness cost of public good production is the same as the fitness gained from it, effectively a net zero benefit. A multiplier of two indicates that the fitness gained from one unit of public good is double the fitness cost of producing one unit of public good. In a population consisting solely of cooperators, this means that any fitness spent producing this public good will be returned twofold. However, when there are cheaters in the population, the benefits are shared with them as well, resulting in a potential net loss of fitness. As m increases, the public goods have a greater positive effect on fitness. At sufficiently high values of m, cooperators can still have a net fitness gain even with cheaters in the population. The value of m thus reflects the fitness benefits of cooperation relative to the cost. While these benefits of cooperation are distributed to the whole group, the costs are borne only by the cooperators.[28]

Over time, the fitness of each cell decreases to account for the energy expenditure needed to maintain growth. In our model, this averages 1 fitness unit every two generations. Recombiners may also carry an additional constitutive fitness penalty (c) associated with maintaining the cellular apparatus for recombination. The fitness cost due to growth is constant throughout. Cells die when their fitness reaches zero. When cells die, other cells in the population reproduce to keep the population constant, with the relative reproduction rates proportional to their average fitness.

An individual cell is able to disperse from its place of origin. The number of generations it takes for this movement to happen is defined as the population viscosity (v). Increased population viscosity (i.e., limited dispersal) has been shown to promote cooperation.[29,30] An increase in population viscosity makes the benefits of cooperation preferentially available to other cooperators by limiting the dispersal of the cooperators themselves, thus selecting for increased cooperation.[31] In contrast, a decrease in population viscosity

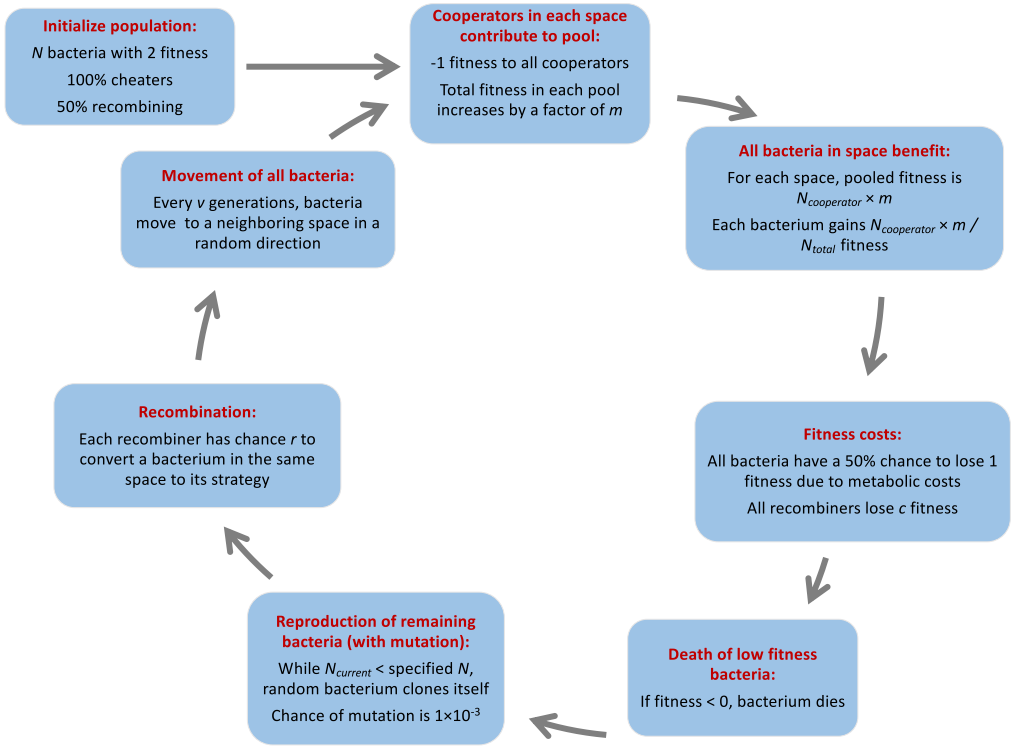

Figure 1. Graphical illustration of the agent-based model of bacterial recombination

The diagram shows the steps of the model used for our simulations. First, the population is initialized with a fixed size (*N*). All the initial bacteria are cheaters with a fitness value of 2, and half are recombining while the other half are non-recombining. For each patch, all the cooperators present then contribute 1 fitness to a common pool. This pool then multiplies its value by the specified multiplier (*m*). It is then equally distributed back to all of the bacteria in the patch. The bacteria then lose fitness due to constitutive metabolic costs. All bacteria have a 50% chance to lose 1 fitness. Recombiners always lose an additional *c* fitness. Bacteria with a fitness <0 then die off. The remaining bacteria then reproduce clonally until the population size reaches *N* again. Daughter cells take the characteristics of the mother cells, except in the case of mutations. These mutations change the strategy of the daughter at a rate of 1 every 1000. Recombiners then have a probability of *r* to change each cell in the same patch they are in. Bacteria may then move to a neighboring patch in a random direction based on population viscosity (i.e., every *v* generations). Detailed results are found in Table S1.

spreads the benefits of cooperation to others regardless of their contribution. Therefore, as the distribution of benefits and distribution of cooperators are decoupled, the fitness benefit of cooperation is reduced.[32,33] For bacteria grown in liquid or agar media or in natural habitats such as the viscous mucus in cystic fibrosis lung, *v* corresponds to the viscosity of the medium itself. A positive correlation between culture medium viscosity and the prevalence of cooperators has been experimentally demonstrated in *P. aeruginosa*.[34]

By default, daughter cells take the characteristics of their progenitors. Cooperators may also produce cheater cells and vice versa as a result of mutations that occur independently. However, recombiners always produce recombining daughters, while non-recombiners always produce non-recombining daughters. Recombining cooperators may convert another cell into a cooperator, while recombining cheaters may convert another cell into a cheater. This is measured by the recombination rate (*r*). Cells converted by recombiners also become recombiners themselves. This ability to convert reflects the transmission of conjugation and recombination machinery being carried by an MGE.

The collapsing of two processes, HGT by conjugation followed by recombination with chromosomal DNA, into a single parameter in our model simplifies its computational requirements and is more generalizable without compromising the outcome of the model. Transfer events that do not result in recombination would be lost in the succeeding generation, thereby not affecting the trajectory of the model. The singular

Table 1. Effect of m on fitness of bacteria in an interacting population

# of cooperators	# of cheaters	*Cooperation multiplier (m)*	Fitness pool	Additional fitness for each cell	Net fitness for each cooperator
a	b	m	$m \times a$	$\frac{m \times a}{a+b}$	$\frac{m \times a}{a+b} - 1$
a	0	m	$m \times a$	m	$m - 1$
1	0	1	1	+1	0
1	1	1	1	+0.5	−0.5
1	0	2	2	+2	+1
1	1	2	2	+1	0
1	0	3	3	+3	+2
1	1	3	3	+1.5	+0.5

The first row describes the general formula used, while the second row describes the scenario where there are no cheaters in the population. The succeeding rows show examples with varying m values.

rate shown, which would be the product of the rates of transfer and recombination, is based on observed recombination rates in bacterial genomes,[35,36] which in turn requires a previous transfer event. Our approach is realistic when the frequency of within-group transfer is high, which has been reported to occur in diverse bacterial taxa.[37–41] Using a single parameter in our model also covers a scenario where transfer alone forces cooperation (as in the case of plasmid-enforced recombination), increasing the generalizability of the model.

Lastly, we restricted our analysis to homologous recombination at a single locus and not other forms of HGT to simplify the scope of our model. While homologous recombination is only one of many mechanisms of HGT, it is a sufficient explanation for our one-locus model of genetically encoded cooperation. Furthermore, because the trait is binary (presence/absence), we do not account for copy number effects. Because of the prevalence of homologous recombination via conjugative elements in bacteria, our model will generate important insights relevant to microbial evolution.[12,14,26] Our model can also easily be modified to accommodate features of other mechanisms of HGT (e.g., transformation, transduction, non-canonical transfer mechanisms via vesicles, nanotubes and gene transfer agents).

Effect of varying recombination rate on the maintenance of cooperation

We first examined the outcomes whereby a cooperating mutant bacterium, i.e., one that is able to produce a public good, invades a population of non-producing cheaters. To model the invasion of cooperators in a population, we set the initial population consisting entirely of cheater cells, of which half were recombiners and the other half were non-recombiners. Thus, all the cooperators in the population arose from cheaters that mutated. The population therefore consisted of four types of bacteria: recombining cheaters, non-recombining cheaters, recombining cooperators, and non-recombining cooperators. There is no fitness cost associated with being a recombiner in the initial population.

We determined the effects of variation in population size (n) while keeping v constant at 1 (i.e., all cells move to a new location every generation). Regardless of the population size, cooperators were not able to invade a population of cheater strains when $v = 1$ and $m = 1$ (purple line in Figures 2A–2C). The ingress of cooperators occurred only when $m > 1$. At n = 3000 (Figure 2A), we observed increasing proportion of cooperators in the population when $m = 2$ and as r increases with cooperators dominating the population at $r \geq 0.05$. When $m = 3$, 4, and 5, cooperators dominated the population regardless of r with slightly higher proportions of cooperators for lower values of m. At n = 5000 (Figure 2B), we observed increasing proportion of cooperators at $m = 2$ and as r increases with cooperators dominating the population at $r \geq 0.07$. At $m = 3$, the proportion of cooperators increases as r increases with cooperation dominating at $r \geq 0.02$. When $m = 4$ and 5, cooperators dominated the population regardless of r with overlaps in the ranges of the two confidence intervals. At n = 7000 (Figure 2C), we observed increasing proportion of cooperators in the population when $m = 2$ and as r increases, with cooperators dominating at $r \geq 0.06$. We see the similar patterns when $m = 3$, 4, and 5. The proportion of cooperators in the population increases as r increases, with cooperators dominating at $r \geq 0.04$ ($m = 3$) and at $r \geq 0.02$ ($m = 4$). When $m = 5$, cooperators dominate regardless of r.

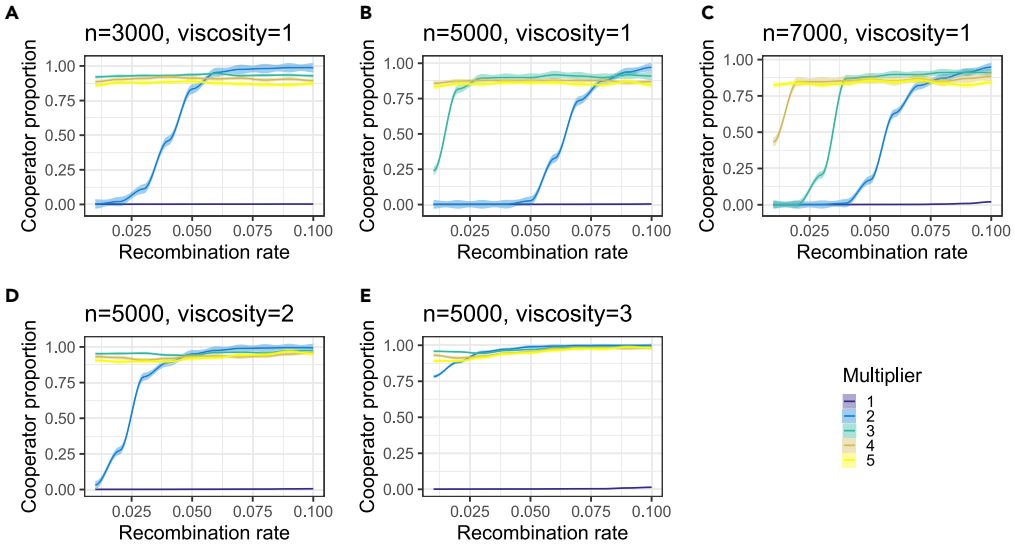

Figure 2. Effect of variable recombination rates on the population of cooperators

The graphs show the proportion of cooperators relative to the total population (y axis) at the end of 500 generations for each recombination rate (*r*) tested (x axis). Different colored lines show the results using each cooperation multiplier (*m*) and the bands show the 95% confidence interval.

(A–E) Multiplier of 1 (no fitness benefit of cooperation) acts as a negative control. The panels correspond to different population sizes (*n*; panels A-C) and population viscosities (*v*) tested (panels D and E). No recombination cost was included in these simulations. Each condition was replicated 100 times. For panel D, details of results when $r = 0.01$ and $r = 0.4$ are shown in Videos S1 and S2.

We next sought to determine the effects of variation in population viscosity on the viability of cooperation (Figure 2 panels B, D, and E). At $v = 2$ and 3 while maintaining $m = 5000$ (Figure 2 panels D and E), no cooperators invade the population at $m = 1$. At $v = 2$ and $m = 2$ (Figure 2D), we observed increasing proportion of cooperators as r increases, with cooperators dominating at $r \geq 0.03$. Videos S1 and S2 show the results when $r = 0.01$ and $r = 0.04$. When $m = 3$, 4, and 5, cooperators dominate the population regardless of r, with slightly higher proportions of cooperators for $m = 3$ compared to $m = 4$ and 5 across different values of r. At $v = 3$ and $m \geq 2$ (Figure 2E), cooperators dominate the population regardless of r.

Overall, these results show that the relationship between the prevalence of cooperators and the recombination rate approximates an S-shaped curve. Initial increases in r from 0 may result in only small increases in the proportion of cooperators in the population. As r increases, there is a transition with a rapid increase in the proportion of cooperators. Finally, we reach a plateau, with further increases in r no longer increasing the proportion of cooperators. This relationship is altered as a function of three variables. Higher values of cooperation multiplier or population viscosity result to curves that shift to the left, indicating an increase in the range of values of r in which cooperation dominates the population. In contrast, an increase in population size (and thus cell density) shifts the curve to the right, indicating a decrease in the range of r in which cooperation dominates. Videos S1 and S2 show how a change in r can lead to different population-level outcomes of cooperation.

Effect of varying recombination cost to the maintenance of cooperation

We next sought to determine the effect of varying the recombination rate (r) in conjunction with a constitutive recombiner fitness cost. Using an initial population size of 5000 and $v = 1$, we implemented a recombination fitness cost (c) of 0, 1, and 2 in each generation. Initial tests did not include a recombination fitness cost ($c = 0$) to simplify the model. However, given both the metabolic costs associated with carrying recombination machinery as well as mobile genetic parasites that reside in bacteria,[42] we opted to include a range of fitness penalties to account for this.

When $m = 1$ (Figure 3A), there is no benefit at all to cooperation, thus acting as a negative control, and the proportion of cooperators in the population remains low regardless of c. However, if there is no fitness cost

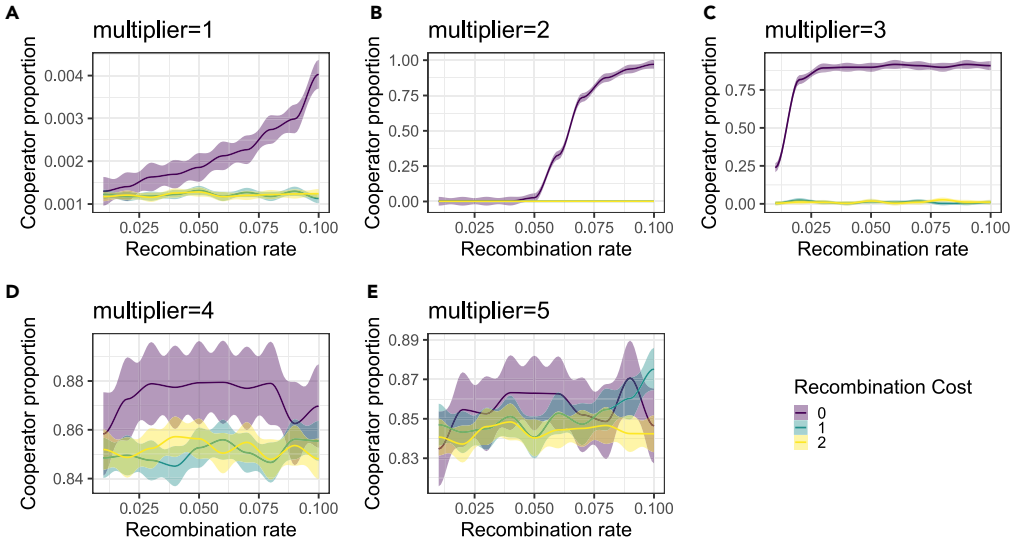

Figure 3. Effect of variable recombination costs on the population of cooperators

The graphs show the proportion of cooperators relative to the total population (y axis) at the end of 500 generations for *r* ranging from 0.01 to 0.10 in increments of 0.01 (x axis). We set n = 5000 and *v* = 1 for each run. Different colored lines show the results using each fitness recombination cost (*c*), with possible values of 0, 1, and 2. The bands show the 95% confidence interval. The panels correspond to different values of *m* ranging from 1 to 5 in increments of 1 (A–E). The panels correspond to different cooperation multipliers tested. Each condition was replicated 100 times.

to recombination, we observed a slightly higher proportion of cooperators as *r* increases, meaning the population is still capable of maintaining a very small population of cooperators. When $m = 2$ (Figure 3B) and $m = 3$ (Figure 3C), even though cooperation has a positive fitness effect on the population, the net effect to cooperators is still negative when $c > 0$. This is due to the extinction of recombinants that would otherwise enforce cooperation, allowing selfish cells to flourish. At $c = 0$ and $m = 2$, cooperators start to dominate at $r \geq 0.07$. At $c = 0$ and $m = 3$, cooperators start to dominate at $r \geq 0.02$, with the curve observed from Panel B shifting to the left. When $m = 4$ (Figure 3D) and $m = 5$ (Figure 3E), the multiplier is large enough for the net fitness effects of cooperation on cooperators to be positive even when there are cheaters. Cooperators thus dominate the population regardless of the value of *c*. However, we still observed an effect of *c* on the proportion of cooperators. At $m = 4$, there are more cooperators when $c = 0$ compared to populations when $c = 1$ and $c = 2$ from $0.08 \geq r \geq 0.02$. We also observed a shift in the proportion of cooperators at $m = 5$ when $r = 0.10$, shown in the $c = 1$ curve lying above the $c = 0$ curve. Overall, these results show that introducing fitness costs to recombinants can reduce the proportion of cooperators in the population given otherwise identical conditions. This is most notable for lower values of *m*. This is shown by the reduction in the parameter space, namely the range of *r*, in which cooperation dominates. We expected these results because fitness costs can lead to the extinction of recombiners, thus nullifying the benefits of recombination rate on cooperation.

We next determined the effect of variable recombination costs to the invasion of cooperation in a population of non-producing cheaters when cells are less able to disperse. In the previous section (Figure 3), we set the population viscosity at 1 (i.e., all cells move to a new location every generation). Now, we set $v = 2$, which means that all cells move to a new location only every two generations (Figure S1). Results show $c > 0$ can result in a higher proportion of cooperators than when $c = 0$ under some conditions, contrary to what would be expected. This only seems to happen under conditions already favorable to cooperators, such as higher *v* and *m* values. We found similar results when $v = 3$, which means that all cells move to a new location only every three generations, and greater resolution between the different values of *c* is observed (Figure S2).

Effect of varying recombination costs to the persistence of recombining cheaters

We also sought to determine the effect of variable recombination costs to the spread of cooperation when there are recombining cheater cells in the population (Figure 4). When $m = 1$ (Figure 4A), there is no benefit

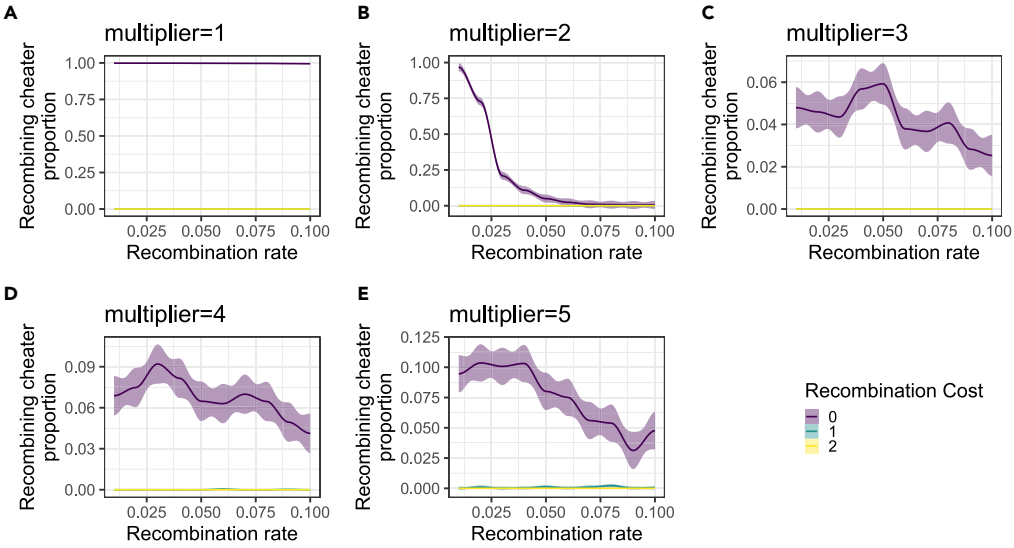

Figure 4. Effect of variable recombination costs on the population of recombining cheaters

The graphs show the proportion of recombining cheaters relative to the total population (y axis) at the end of 500 generations for *r* ranging from 0.01 to 0.10 in increments of 0.01 (x axis). The results are from the same runs carried out in Figure S1, with n = 5000 and *v* = 2. Different colored lines show the results from *c* values of 0, 1, and 2. The bands show the 95% confidence interval. The panels correspond to different values of *m* ranging from 1 to 5 in increments of 1 (A–E). Each condition was replicated 100 times. For panel E, details of results when *c* = 0 and *c* = 1 are shown in Videos S3 and S4.

to cooperation, and cooperators fail to invade the population. When $c = 0$, recombining cheaters dominate the population. However, when $c > 0$, recombining cheaters become extinct, leaving only the non-recombining cheaters. When $m = 2$ (Figure 4B), there are fitness benefits of cooperation to the overall population, but not enough of a benefit to cooperators for them to consistently dominate. When $c = 0$, we observed *r*-dependent dominance of cooperators once more. Recombining cheaters dominate only when $r < 0.03$. However, similar to Figure 3A, recombining cheaters become extinct when $c > 0$, leaving only the non-recombining cheaters.

When $m = 3$ (Figure 4C), the fitness payoffs of cooperation are sufficient for cooperators to dominate at all values of *c*. We thus observed very few recombining cheaters that remain in the population. Recombining cheaters become extinct when $c > 0$. However, recombining cheaters continue to persist in the population albeit at low proportions when $c = 0$. Notably, the maximum proportions of recombining cheaters can be observed when *r* range from 0.04 to 0.05, corresponding to when the $c = 0$ curve lies below the $c = 1$ and $c = 2$ curves in Figure S1C. When $m = 4$ (Figure 4D), the fitness payoffs of are sufficient for cooperators to dominate at all values of *c*. Again, recombining cheaters become extinct when $c > 0$. There is a maximum at $r = 0.03$, which is where the $c = 1$ and $c = 2$ curves lie above the $c = 0$ curve in Figure S1D. When $m = 5$ (Figure 4E), the fitness payoffs of are sufficient for cooperators to dominate at all values of *c*. Shared benefits of cooperation allow for a very small proportion of recombining cheaters when $c = 1$. When $c = 0$, there is a maximum from $r = 0.02$ to $r = 0.04$, corresponding to where $c = 0$ curve lies below the $c = 1$ and $c = 2$ curves in Figure S1E. Videos S3 and S4 show the results when $c = 0$ and $c = 1$, respectively.

To summarize, we show here that at $m = 4$; $r = 0.03$ and at $m = 5$; $0.02 \leq r \leq 0.04$, cooperation fares relatively worse when there is no fitness cost to recombination (Figure 4). These same conditions also correspond to the maxima for recombining cheater populations in the model. While the proportion of recombining cheaters in the population remain low, they are much higher than the extinction or near extinction values when $c > 0$. Videos S3 and S4 show how a change in only *c* can lead to different levels of persistence of cheating.

Effect of variable recombination costs to the spread of recombining cheaters when cells are less able to disperse (v = 3)

Lastly, we sought to determine how increasing the population viscosity affects the previously observed relationship between recombination costs and the prevalence of cooperation. At $m = 1$ (Figure 5A), there

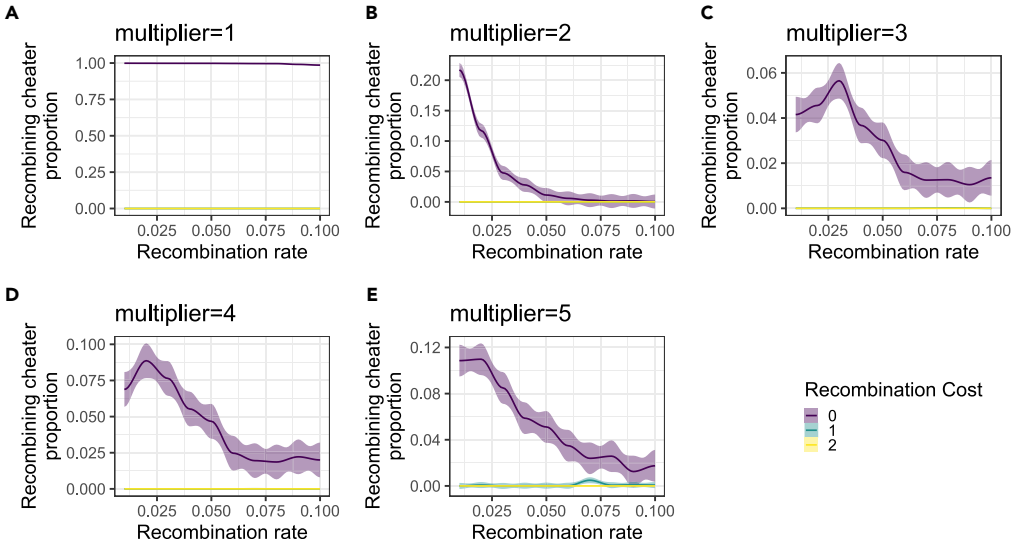

Figure 5. Effect of variable recombination costs on the population of recombining cheaters when cells are less able to disperse ($v = 3$)

The graphs show the proportion of recombining cheaters relative to the total population (y axis) at the end of 500 generations for r ranging from 0.01 to 0.10 in increments of 0.01 (x axis). The results are from the same runs carried out in Figure S2, with n = 5000 and $v = 3$. Different colored lines show the results from c values of 0, 1, and 2. The bands show the 95% confidence interval. The panels correspond to different values of m ranging from 1 to 5 in increments of 1 (A–E). Each condition was replicated 100 times.

is no benefit to cooperation, and cooperators fail to invade the population. When $c = 0$ recombining cheaters dominate the population, with a very slight dip when $r = 0.1$, corresponding to the increase of the cooperator population. However, when $c > 0$, recombining cheaters also go extinct, leaving only selfish non-recombiners. At $m = 2$ (Figure 5B), there are fitness benefits to cooperation to the overall population, but not enough of a benefit to cooperators for them to consistently dominate. At $c = 0$, the recombining cheater population remains low but dependent on r. As r increases, the recombining cheater population decreases, corresponding to the increase in cooperator population seen in Figure 5B. At $c = 1$ and at $c = 2$, recombining cheaters remain extinct.

At $m = 3$ (Figure 5C), the fitness payoffs of cooperation are sufficient for cooperators to dominate at all values of c. Again, recombining cheaters become extinct when $c > 0$. When $c = 0$ we see the recombining cheater population vary with r, with a peak at $r = 0.03$, corresponding to where the $c = 0$ curve dips below the $c = 1$ and $c = 2$ curves in Figure 5C. At $m = 4$ (Figure 5D), the fitness payoffs of cooperation are sufficient for cooperators to dominate at all values of c. The patterns resemble those of panel C, and recombining cheaters again become extinct when $c > 0$. When $c = 0$, there is a maximum from $r = 0.02$ to $r = 0.03$, corresponding to where the $c = 0$ curve dips below the $c = 1$ and $c = 2$ curves in Figure 5D. At $m = 5$ (Figure 5E), the fitness payoffs of cooperation are sufficient for cooperators to dominate at all values of c. At $r \leq 0.03$, the $c = 1$ and $c = 2$ curves lie above the $c = 0$ curve. Shared benefits of cooperation allow for a very small recombining cheater population when $c = 1$. When $c = 0$, the recombining cheater population remains low and decreases with increasing r. This result aligns with the $c = 0$ curve found below the $c = 1$ and $c = 2$ curves when $r \leq 0.03$.

To summarize, at $m = 3$; $r = 0.03$, at $m = 4$; $0.02 \leq r \leq 0.03$, and at $m = 5$; $r \leq 0.03$, cooperation fares relatively worse when there is no fitness cost to recombination (Figure 5). These conditions also correspond to the maxima for recombining cheater populations. This result echoes the trends we observed in Figure 4.

DISCUSSION

Cooperation is vulnerable to exploitation by cheaters that freeload on publicly produced goods while not sharing in the costs of their production; yet, paradoxically, cooperation is ubiquitous throughout the Tree

of Life. In our study, we show how increasing the recombination rate can result in a greater proportion of cooperators in a population. The fate of cheaters in the face of HGT followed by homologous recombination employed as an enforcement mechanism is different compared to other forms of bacterial enforcement (e.g., quorum sensing). Other enforcement mechanisms reduce the relative fitness of the cheater compared to cooperators, leading to cheaters being selected out of the population.[6,11] In contrast homologous recombination eliminates cheaters differently. By replacing a loss-of-function cheating allele with a functional allele for cooperation, a cheater simply stops being a cheater. In concert with other enforcement mechanisms, a bacterium may benefit from this change in strategy, as it gets to avoid the fitness penalties associated with these. While an increased recombination rate expands the parameter space in which cooperation can evolve, we note that recombination is neither sufficient nor necessary for the dominance of cooperation. It is only one of several parameters, such as population structure, that influences the evolution of prosocial behavior.

We also demonstrate that fitness costs to recombinants can have different effects on the evolution of cooperation depending on the other parameters. Prohibitively high fitness costs result in the collapse of recombination-driven cooperation due to the extinction of recombiners when the fitness benefits of cooperation are insufficient to maintain a cooperating population. When cooperation is already strongly dominant, fitness costs can result in either an increased or decreased prevalence of cooperators. The decreased prevalence of cooperators can be attributed to a similar decline in recombination-enforced cooperation. The increased prevalence of cooperators can be attributed to the extinction of selfish recombiners when recombination carries a fitness cost. Even in populations dominated by cooperators, selfish recombiners can persist in a population when $c = 0$. Although we modeled homologous recombination in this study, our results can be generalized to other forms of HGT due to similar dynamics.

Both the recombination rate and fitness costs to recombinants are parameters that in turn interact with population viscosity, resulting in complex population dynamics. When there is no fitness cost to recombination, population viscosity as a homogenizing force acts to increase the parameter space in which cooperation can dominate a population. This is in line with expectations of population viscosity promoting cooperation.[32,33] However, when recombinants are subject to a fitness penalty, then population viscosity no longer acts in concert with recombination rate to increase cooperation. The homogenizing forces of population viscosity and recombination within the subpopulation level are redundant, and the combined benefits may not be sufficient to overcome the fitness costs of recombinants.

Our study builds upon previous reports showing that HGT can promote cooperation,[22–24] but our model also shows both positive and negative interactions occurring between recombination rate and other ecological parameters. We highlight how increasing the recombination rate expands the parameter space in which cooperators dominate freeloaders. Homologous recombination can be a significant source of genetic diversity, sometimes exceeding the contribution of mutation by an order of magnitude over the length of the entire genome.[35,36] High rates of homologous recombination have been observed in *Streptococcus pneumoniae*,[43] *Neisseria gonorrhoeae*,[38] and *Helicobacter pylori*,[44] with some lineages within each species considered as hyper-recombinants.[45] Even in those species long considered as truly clonal (i.e., they do not experience recombination such as *Mycobacterium*), a signal of low recombination still revealed a weak effect on diversity compared with mutation,[46] thereby leading to questions whether clonality truly exists in bacterial species.[47] Nonetheless, future work focused on evaluating how heterogeneity in recombination, including hyper-recombining lineages, impacts cooperation is valuable. However, increasing recombination rate alone is neither sufficient nor necessary. Our study highlights other factors (intermediate benefits of cooperation, lower population viscosity, and higher population size) can promote cooperation in a population of cheater cells. Furthermore, our agent-based model can be extended to model small populations, taking founder effects into account,[25] as in the case of transmission bottlenecks.[48] Finally, although our model focuses on homologous recombination at a single locus, it can easily be modified to study other modes of infectious transfer.

Our results on the effect of recombination fitness costs on the prevalence of cooperation are especially relevant to bacteria considering that horizontally acquired exogenous DNA can be detrimental to their hosts.[49] The recombination cost function thus reflects the potential fitness burden of foreign DNA elements. This includes recombination events where the inserted DNA incurs fitness costs due to disrupting an existing gene at the point of insertion, transcribing or translating the acquired or modified gene if the

gene product has no useful function, or disrupting existing regulatory and physiological networks.[42] Different MGEs also directly burden their host cells, justifying a constitutive fitness cost for the conjugative MGE mediating recombination in our model. Plasmids are known to confer fitness costs to their host bacteria when they are in genetic conflict.[50,51] Prophages may require bacteria to carry costly immune mechanisms in addition to occasionally lysing their host cells.[52] Bacterial transformation carries fitness costs both from the physiological cost of the machinery and from the uptake of deleterious mutations.[53] Even short, randomly transferred DNA fragments introduced into a neutral position in the genome are also mildly deleterious in a variety of bacteria.[54] While the tradeoffs of these have been studied with respect to the benefits of acquiring adaptive genes, here we also introduce how individually maladaptive traits can promote cooperation, effectively benefiting the population. Similar to how animals use signals to communicate cooperation, where fitness costs of the signal indicate their honesty,[55,56] the homologous recombination machinery (and HGT machinery in general) may play a similar role in bacteria. When the fitness costs of recombination prevent it from proliferating in cheating populations while still allowing it to persist in cooperating ones, it can serve as an indicator for cooperation.

In conclusion, our model shows how different environmental and physiological conditions influence the evolution of cooperation in the public goods game. Under conditions ranging from unfavorable to slightly favoring cooperation, the higher costs of recombination consequently cause both recombination and cooperation traits to falter. Under conditions very favorable to cooperation, higher fitness costs associated with recombination increased the proportion of cooperators compared to conditions when recombination has no associated fitness cost due to the extinction of recombining cheaters. Homologous recombination may promote cooperation, working in tandem with other factors such as population viscosity and fitness costs to recombination. Our work provides a basis for future work in studying other enforcement strategies of cooperation and other mechanisms of HGT. It provides an essential framework for future investigations on bacterial sociobiology and game theory, including studies using experimental evolution to validate our results in real-life scenarios.

Limitations of the study

We recognize that our *in-silico* method does not fully reflect the various aspects and intricacies of bacterial cooperation found in nature. For instance, bacteria may exhibit variable recombination rates even within a population, and this can further change over time. Our model only contained recombiners and non-recombiners, but a population may have several degrees of recombination rates in between that further complicate the dynamics. The fitness effects of public good genes may also vary with environmental conditions and time, as in the case of resistance genes. We opted to not include temporal variations with these factors to keep the model computationally tractable. Nonetheless, this study provides important insights on the dynamics of recombination rate, benefits of cooperation, population viscosity, fitness cost, and population size in shaping bacterial cooperation.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107344.

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AUTHOR CONTRIBUTIONS

Conceptualization, C.P.A. and I.P.A.L.; Methodology, I.P.A.L.; Formal analysis, I.P.A.L.; Writing – Original Draft, C.P.A. and I.P.A.L.; Writing – Review and Editing, C.P.A., I.P.A.L., O.T.E., and J.P.G.; Supervision, C.P.A.; Funding Acquisition, C.P.A., I.P.A.L., and J.P.G.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Code and data for recombination modeling	This paper	https://datadryad.org/stash/dataset/doi:10.5061/dryad.9ghx3ffnc
Software and algorithms		
NetLogo v.6.1.1	Wilensky[57]	http://ccl.northwestern.edu/netlogo/
ggplot2 v.3.3.3	Wickham[63]	https://ggplot2.tidyverse.org
pals v.1.6	Wright[64]	https://kwstat.github.io/pals/
patchwork v.1.1.1	Pedersen[65]	https://patchwork.data-imaginist.com https://github.com/thomasp85/patchwork
R language v.6.3.3	R Core Team[66]	https://www.R-project.org/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the lead contact, Cheryl P. Andam (candam@albany.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All original code and data can be accessed in Dryad and are publicly available: https://datadryad.org/stash/dataset/doi:10.5061/dryad.9ghx3ffnc.
- Instructions for running the simulations are provided at the same Dryad site.
- Additional information required to reanalyze the data reported in this paper is available from the lead contact upon reasonable request.

METHOD DETAILS

Agent-based model of bacterial populations

We used the multi-agent programmable modeling environment NetLogo v.6.1.1 (http://ccl.northwestern.edu/netlogo/)[57] to simulate bacterial populations consisting of individual agents that move around and interact in a world composed of a grid of patches and shaped as a torus. Based on the standard public good game used in evolutionary game theory,[28,58] we consider an initial well-mixed population of N individuals consisting of bacterial cells that can be either cooperating or cheating, each of which can either be a recombiner or non-recombiner. Here, we only focus on homologous recombination, defined as the acquisition of exogenous DNA and subsequent replacement of DNA in the recipient genome with homologous sequence from a donor.[59] We further restricted our study on homologous recombination mediated by conjugative MGE. We did not consider other mechanisms of recombination that occur in microbes (e.g., illegitimate or nonhomologous recombination, homology-facilitated illegitimate recombination[60,61]) or other forms of HGT (e.g., transduction, transformation, noncanonical HGT[62]).

We define the following population variables.

Population viscosity (v)

Dispersal of individuals from its patch. In the model, this is the number of generations before a cell moves to an adjacent patch. *E.g.*, $v = 1$ all cells move every generation, $v = 2$ all cells move every two generations. This was included due to the known effects of population viscosity on the evolution of altruism.[32]

Cooperation multiplier (*m*)

Cooperators in the same location contribute fitness to the population that is distributed to all the cells present. Before the fitness effect is distributed, the total value of the contributions is multiplied by this value, signifying the benefit of cooperation. *E.g.,* if a subpopulation has two cooperators each contributing one value of fitness, there will be two values of fitness in the pool. If $m = 1$, then $2 \times 1 = 2$ values of fitness are distributed back to the population, so there is no benefit to cooperation (i.e., because the total distributed fitness is equal to the total contributed fitness). If $m = 2$, then $2 \times 2 = 4$ values of fitness are distributed back to the population. If the population is composed of only cooperators, any $m > 1$ allows cooperation to succeed, but as the number of cheaters increases, m also needs to increase.

Recombination rate (*r*)

In the model, bacteria only interact with other bacteria in the same location as themselves. Each generation, every recombining cell has a chance to change the strategy (i.e., to act either as a cooperator or cheater) of every cell in the same location to match its own. *E.g.*, if $r = 0.01$, every recombining cooperator cell has a 1% chance to turn its neighbors into a cooperator, even if the neighbor is already a cooperator. Every recombining cheater also has a 1% chance to turn its neighbors into a cheater. If there are no other cells in the same location, this does nothing because there are no other cells to change. For example, if there is one recombining cooperator and 100 non-recombining cheaters in the same location, we would expect one of those cheaters to be forced into cooperation in the next generation. The cooperator would not be converted to a cheater because none of the cheaters are recombiners. Converted cells always become recombiners themselves. This process of conversion models homologous recombination through conjugation, though our abstraction does not deal with conjugation events that fail to change the alleles of the recipient. This therefore describes the probability of two events happening in succession. However, we elect to model this as a single parameter since changing it to two separate parameters would only increase model complexity and thus computational requirements without additional results. This is because plasmid transfer without recombination will simply result in it being lost in the next generation. Population-level studies of genomes from a variety of bacterial taxa have shown that the impact of homologous recombination can sometimes considerably exceed that of mutation, and therefore greatly contributing to genetic diversity.[35,36] This is in line with our model's starting ratio ($r/\mu = 10$) of recombination rate ($r = 0.01$) to mutation rate ($\mu = 0.001$).

Fitness cost (*c*)

While the potential deleterious effects of frequent recombination in bacteria are known,[49,50,52,54] there have been no studies that directly quantified this. We thus tested multiple constitutive fitness costs for recombinants. For simplicity, there is no fitness cost associated with being a recombiner ($c = 0$) in the initial population.

All individual cells acquire a resource of the value R at the beginning of the simulation, which corresponds to their fitness. For our simulations, we set $R = 2$. This decays by 1 every two generations. To increase their fitness, individuals gather payoff by playing a public goods game whereby individuals can either cooperate or cheat. Cooperators cooperate by contributing this resource toward the rest of the population, while the cheaters keep the resource for themselves. At every generation, individuals move around the world, interact via the public goods game (i.e., cooperate or cheat), reproduce, recombine, and die. The size of the world is 32 by 32 patches in the shape of a torus. We also consider variation in population viscosity, with movement to a different location one unit away every 1, 2, or 3 generations. The direction of movement is random. Cells may also stay in the same location depending on the population viscosity.

Variation in recombination rates and cost of recombination

We implemented two different simulation modes. First, we used the model to examine the effect of varying recombination rates. Using initial population sizes of 3000, 5000, and 7000, we implemented a cooperation multiplier ranging from one to five in increments of one. These values were chosen because they showed variable population dynamics with changes in recombination rate while remaining computationally tractable. The recombination rates ranged from 0.1 to one in increments of 0.1, while the mutation rate was set to 0.001. To model the invasion of cooperators in a population, we set the initial population consisting entirely of cheater cells, half of which were recombiners and the other half were non-recombiners. There was no fitness cost associated with being a recombiner in the initial population. Each simulation ran for

500 generations, which was sufficient for the population to stabilize based on preliminary tests where the populations were run for 1000 generations. Each set of conditions was replicated 100 times. Second, we examined the effect of varying the recombination rate in conjunction with a constitutive recombiner fitness cost. This was done to account for both the metabolic costs associated with carrying the recombination machinery as well as genetic parasites that reside on bacteria with very mobile genomes.[42] Using an initial population size of 5000, we implemented a recombination fitness cost of 0, 1, and 2 in each generation. All other conditions were the same as above. For both simulation modes, the proportion of cooperating cells, cheater cells, recombining cheaters, and recombining cooperators in each final population was recorded.

Data were visualized using ggplot2 v.3.3.3,[63] pals v.1.6,[64] and patchwork v.1.1.1[65] implemented in R language v.6.3.3.[66] Detailed results are found in Table S1.

QUANTIFICATION AND STATISTICAL ANALYSIS

The curves in Figures 2, 3, 4, 5, S1 and S2 were generated using a local polynomial regression, taking into account 20% of the total data closest to each point for fitting each location. This was done using the stat_smooth function of ggplot2, with the parameters method = "loess" and span = 0.2. The bands around the curves indicate the 95% confidence interval, also from the stat_smooth function. The runs from each set of parameters were replicated 100 times.