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# The emergence of cooperation from a single mutant during microbial life cycles

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Cooperative behaviour is widespread in nature, even though cooperating individuals always run the risk of being exploited by free-riders. Population structure effectively promotes cooperation given that a threshold in the level of cooperation was already reached. However, the question how cooperation can emerge from a single mutant, which cannot rely on a benefit provided by other cooperators, is still puzzling. Here, we investigate this question for a well-defined but generic situation based on typical life cycles of microbial populations where individuals regularly form new colonies followed by growth phases. We analyse two evolutionary mechanisms favouring cooperative behaviour and study their strength depending on the inoculation size and the length of a life cycle. In particular, we find that population bottlenecks followed by exponential growth phases strongly increase the survival and fixation probabilities of a single cooperator in a free-riding population.

## 1. Introduction

Cooperative behaviour often provides a strong benefit for populations. But why are cooperators not undermined by non-cooperative individuals which take the benefit but save the costs for its provision [1–3]? For higher developed organisms, there are several ways to escape this *dilemma of cooperation*: for individuals, which are capable of recognizing other individuals, memorizing previous interactions and controlling their handling accordingly, reciprocity and punishment can promote cooperation [3,4]. However, those mechanisms cannot act in organisms of modest complexity like microbes or during the early course of life where memory and recognition were mostly lacking.

In such scenarios, cooperation might prevail owing to the structure of the population [1,3,5–8]; this idea has been studied both theoretically and experimentally in the context of kin-, group- and multilevel selection [1,6,9–24]. If cooperators more likely interact with other cooperators (positive assortment), they keep most of their benefit for themselves and are less exploited by non-cooperators. However, owing to the costs of cooperation, a fitness disadvantage compared with non-cooperators is still present: positive assortment supports cooperation, but is not necessarily sufficient to ensure its maintenance. Crucially, positive assortment can act only if cooperation is already established in the population such that cooperative individuals can successfully assort. Thus, the question remains how cooperation can emerge *starting with a single cooperating mutant*.

In this paper, we address this issue for a generic situation of microbial populations. Cooperative microbes typically produce public goods whose synthesis is metabolically costly [25–31]. For example, consider the proteobacteria *Pseudomonas aeruginosa* and siderophore production: when iron is lacking in the environment, cooperative strains produce iron-scavenging molecules (siderophores) [31,32]. Released into the environment these molecules can efficiently bind single iron molecules, and the resulting complex can then be taken up by surrounding bacteria. Microbial populations are highly structured: several colonies form one population [33,34]. New colonies arise owing to migration into new habitats or more actively owing to controlled life cycles triggered by environmental factors. For example, studies of *P. aeruginosa* [33,35] confirm
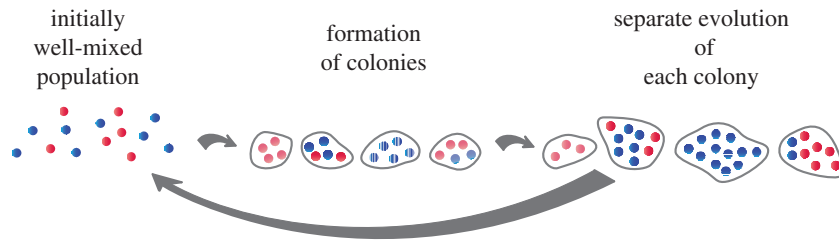
**Figure 1.** A simplified life cycle of microbial populations. Cooperators (blue) and free-riders (red) in an initially well-mixed population are randomly subdivided into groups of average size $n_0$. The groups then evolve separately following two main rules. First, groups with a higher fraction of cooperators grow faster as more public good is present in those populations. Second, in each group, cooperators reproduce more slowly than free-riders as the latter do not have to provide the public good; for detail, see main text. After a certain time $T$, all groups are merged and the cycle restarts. (Online version in colour.)

that typical life cycles pass through different steps with regularly occurring dispersal events ensued by the formation of new colonies. As the initial colony sizes are typically small, such dispersal events coincide with population bottlenecks. Emulating the dynamical colony formation, microbial cooperation has been studied experimentally by employing a life cycle where new colonies are regularly formed from old ones [36,37]. These experiments and theoretical work [1,3,11,38–40] show that such a restructuring mechanism can cause an increase in the level of cooperation.

Based on these observations, we here theoretically investigate if such a restructuring scenario can not only maintain cooperation, but also allow for the evolution of cooperation from a single mutant. Assuming a constant population size, the onset of traits from a single mutant and its fixation have been studied in the frameworks of population genetics and evolutionary game theory [41–49]. However, the consequences of ecological factors such as population growth and population restructuring remain unclear [19,20]. To tackle this question for the microbial scenario introduced before, we consider a life cycle model, consisting of three steps [11,39] (figure 1): (i) in the *group-formation step*, a population consisting of cooperators (C) and non-cooperating *free-riders* (F) is randomly assorted into different groups (colonies). (ii) In the ensuing *group-evolution step*, each group evolves separately according to generic growth laws of microbes. (iii) In the *group-merging step*, colonies are merged to one population again. Consecutively, the cycle starts anew with the next group-formation step. The synchronous reformation of groups via merging all individuals into a single population follows recent experiments [37] and is obviously a simplification of natural colony formation. However, it captures the essence of regular occurring bottlenecks, namely rearranging colonies which have an initially small population size. Furthermore, as it comprises a worst-case scenario for cooperation, it is suited to study the possible onset of cooperation in microbial populations starting with a single cooperative mutant.

Starting with a well-mixed population with a fraction $x_0$ of cooperators, $M$ groups (colonies) are formed during the group-formation step. Successively, each group $i$ is assigned $v_{0,i}$ randomly chosen individuals of the well-mixed population; group sizes $\{v_{0,i}\}$ are Poisson-distributed with mean $n_0$. The random assortment leads to a statistical variation in the initial fraction of cooperators, $\xi_{0,i}$. Approximately, it is of the order $n_0 \cdot x_0(1 - x_0)$. We investigate this unbiased random assortment of groups as it does not assume individuals to be distinguishable by the sorting mechanism and again constitutes a worst-case scenario for cooperators.

After assortment into groups, each group $i$ evolves and grows separately. The dynamics within groups is given by

a stochastic process based on birth and death events which are characterized by the corresponding *per capita* birth rates $\Gamma_{S,i}^+$ and death rates $\Gamma_{S,i}^-$, where $S \in \{C, F\}$ denotes the trait of the individual in group $i$ [50,51]. The birth rates of individuals depend on two factors, namely the trait of the individual and the composition of the colony the individual is living in. First, cooperators reproduce slower than free-riders in each colony as they have metabolic costs owing to the production of the public good, $\Gamma_{C,i}^+ < \Gamma_{F,i}^+$. Second, as more cooperative groups produce more public good, individuals in colonies containing a higher fraction of cooperators are better off, $\Gamma_{S,i}^+ < \Gamma_{S,j}^+$ for $\xi_i < \xi_j$. The death rates incorporate the effect of limited resources and, therefore, increase with an increasing population size, $\Gamma_{S,i}^- < \Gamma_{S,j}^-$ for $v_i < v_j$. For specificity, we assume the following birth and death rates which fulfil all conditions stated above:

$$\Gamma_{S,i}^+ = r(1 + p\xi_i)(1 - \delta_{S,C}c) \quad \text{and} \quad \Gamma_{S,i}^- = \frac{v_i}{K}, \qquad (1.1)$$

where $\delta_{s,C}$ is the Kronecker delta defined by $\delta_{C,C} = 1$ and $\delta_{F,C} = 0$. While $p$ sets the growth advantage of cooperators on the colony level, the parameter $c$ measures the metabolic costs of cooperation. The growth rate $r \equiv 1$ is assumed to be fixed setting the time scale of growth. The functional form of the growth rates assumed here reproduces the generically observed growth dynamics of microbial populations [52]: small colonies grow exponentially and their size is bounded by a maximal colony size which here scales with $K$. A more detailed description of the dynamics including a discussion of the deterministic equations can be found in [39,50]. Furthermore, in [39], the specific form of the rates (1.1) is justified by successful comparisons with experiments by Chuang *et al.* [37]. Note also that the qualitative results presented in this paper do not depend on the specific functional forms of the growth rates, but only on the rather generic conditions of population bottlenecks followed by growth.

After a *regrouping time T*, the separated groups are merged again into one well-mixed population with a then changed global population size $N = \sum v_i$, and a fraction of cooperators which is given by the weighted average

$$x = \frac{\sum_i \xi_i v_i}{\sum_i v_i}. \qquad (1.2)$$

The cycle then starts anew with the new fraction of cooperators, $x_0 \equiv x$. Although the fraction of cooperators within each group is expected to decrease during group evolution, an increase in the global fraction of cooperators is possible in principle: the disadvantage of cooperation within each
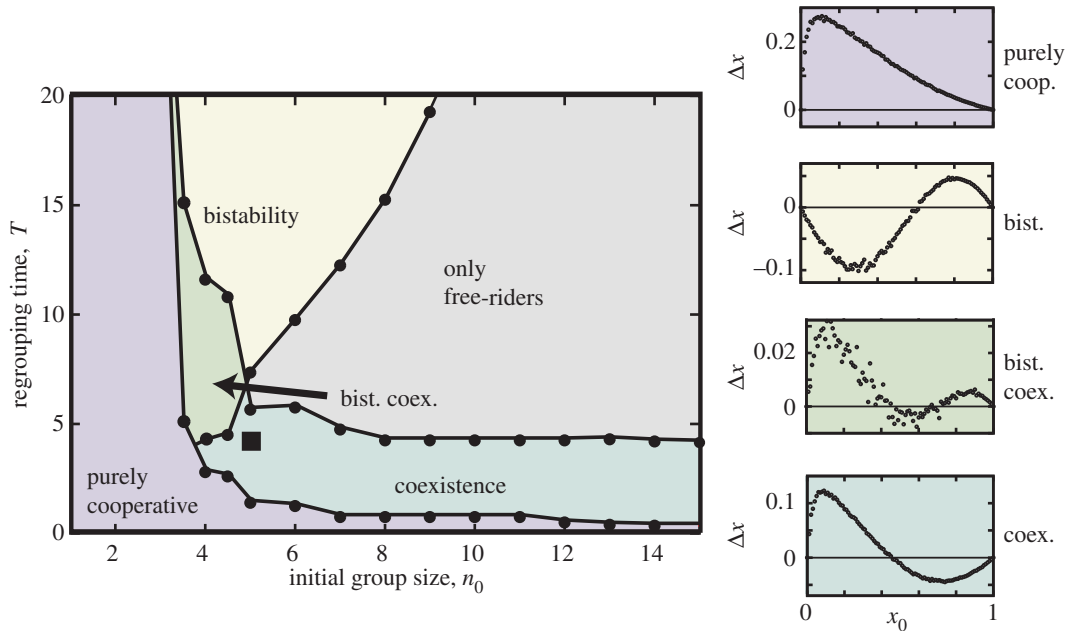
**Figure 2.** Parameter dependence of the different cooperation scenarios. Depending on $n_0$ and $T$ five different scenarios with different fixed point behaviours arise. For each scenario, we show an exemplary drift diagram on the right, where the change in the fraction of cooperators after one life cycle is shown depending on the initial fraction of cooperators, $\Delta x(x_0)$. Dots correspond to simulation results of the transition points and the lines are guides to the eye to separate the different scenarios: pure cooperation with a stable fixed point at 1, i.e. for a purely cooperative population ($n_0 = 4$, $T = 1.5$), coexistence with a stable fixed point at $0 < x^* < 1$ ($n_0 = 6$, $T = 1.8$), bistability with an unstable fixed point at $0 < x^* < 1$ ($n_0 = 5$, $T = 20$), bistable coexistence where both a stable and an unstable fixed point are present ($n_0 = 4$, $T = 5.5$) and only free-riders. The black square corresponds to the parameters studied in figure 3 ($n_0 = 5$, $T = 4$). Other parameters are $p = 10$, $K = 100$, and $c = 0.1$. (Online version in colour.)

group can be overcome by changing weights, $v_i/N$, in the total population. To achieve this, there must be a sufficiently high positive correlation between group size and cooperator fraction [3,53]. Such an increase of cooperation is an example of Simpson's paradox [3,37].

For the random assortment of groups considered here, two mechanisms promoting cooperation can be distinguished as previously studied [39]. First, for very small population bottlenecks, purely cooperative colonies might be formed where there is no conflict with free-riders (*group-fixation mechanism*). Second, more cooperative groups grow comparably fast and thereby compensate for the selection advantage of free-riders (*group-growth mechanism*). As those mechanisms are crucial for the understanding of our results concerning single mutants spreading in the population, we first repeat some arguments from [39] and additionally introduce analytical calculations and a study of the key parameters to support them. The second part of this article is devoted to the main question of the paper namely whether a single cooperative mutant which cannot rely on benefits provided by other cooperators has the chance to spread in the population.

## 2. Results

In the following, we analyse both mechanisms in detail, starting with the group-fixation mechanism. For long separate evolution of groups, $T \gg 1$, all groups reach a stationary state: they consist of either cooperators or free-riders only with a group sizes of $(1 + p)(1 - c)K$ and $K$, respectively. The global fraction of cooperators is then

$$x' = \frac{(1+p)(1-c)P_C}{(1+p)(1-c)P_C + (1-P_C)}. \tag{2.1}$$

$P_C$ denotes the probability for a group to consist of only cooperators after assortment. In first order, only initially purely cooperative groups contribute to $P_C$, whereas all mixed groups are taken over by free-riders, such that

$$P_C = \frac{1}{e^{n_0} - 1} \sum_{v_i=1}^{\infty} \frac{n_0^{v_i}}{v_i!} x_0^{v_i} + \mathcal{O}\left(\frac{1}{K}\right) = \frac{e^{n_0 x_0} - 1}{e^{n_0} - 1} + \mathcal{O}\left(\frac{1}{K}\right).$$

If $x'$ exceeds the initial fraction of cooperators, $x_0$, the group-fixation mechanism is strong enough to overcome the advantage of free-riders. As $P_C$ increases with the initial fraction of cooperators, there is an unstable fixed point $x_u^*$, implicitly defined by $x_u^* = x' = x_0$ in equation (2.1): for initial fractions, $x_0$, above $x_u^*$, a purely cooperative population is reached after several regrouping events. In contrast, when starting below, $x_0 < x_u^*$, cooperators become extinct in the population. This bistable behaviour is illustrated in figure 2 where depending on the initial value $x_0$ the global fraction of cooperators is shown after a large regrouping time $T = 20$.

In contrast to the group-fixation mechanism, the group-growth mechanism acts for small times, where groups strongly grow. As cooperation enhances the growth speed of colonies, more cooperative groups have a larger weight in the average (1.2) even though $\dot{\xi}_i \leq 0$ holds in each group. Depending on the parameters, this positive effect is able to compensate for the selection disadvantage of cooperators. This can be quantified performing a van Kampen expansion of the master equation (see the electronic supplementary material). For binomial distributed groups, the change in the fraction of cooperators at time $t = 0$ is given by

$$\frac{\mathrm{d}}{\mathrm{d}t} x \propto \left[ -c(1 + px) + \frac{p}{n_0} \right] x(1 - x). \tag{2.2}$$
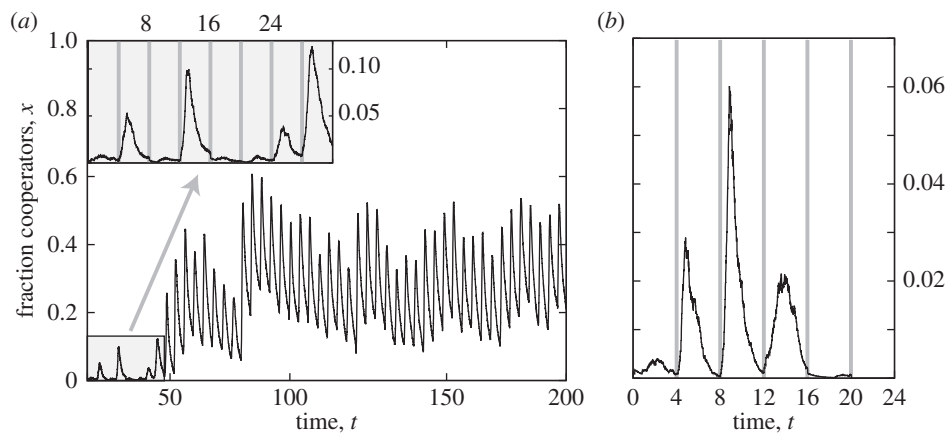
**Figure 3.** Two exemplary realizations for the evolution of cooperation from a single mutant. The parameters $T = 4$ and $n_0 = 5$ correspond to a point in the coexistence regime indicated by the black square in figure 2. In (a), a coexistence trajectory is shown, whereas (b) shows extinction of cooperators. For small times (zoom in inset a and b) cooperators have to survive a highly stochastic process: cooperators start with only one mutant, the population is subject to exponential growth which amplifies fluctuations, and regrouping events add an additional level of stochasticity. In addition, also for larger times the trajectory oscillates around the fixed point (for a detailed explanation, see main text) and is therefore prone to extinction.

The first term accounts for the selection advantage of free-riders for the growth advantage of more cooperative groups, whereas the second one reflects the growth advantage of more cooperative groups. The initial change, $(\mathrm{d}/\mathrm{d}t)x$, is larger if $x_0$ is small meaning that higher selection disadvantages can be overcome. Under regrouping after time $T$, the group-growth mechanism results in a stable fixed point $x_S^*$. As the group-growth mechanism relies only on variance in group composition, but not on the existence of purely cooperative groups, it acts for much stronger population bottlenecks, $n_0$, than the group-fixation mechanism does. However, as it is caused by population growth, it can act only for short regrouping times $T$.

Repeated regrouping corresponds to an iterative map. The underlying dynamics (group formation and group evolution) result in an effective drift: $\Delta x = x(T) - x_0$. Depending on the strength of both mechanisms, and thus $n_0$ and $T$, five distinct fixed point scenarios can be distinguished; examples for the corresponding stability plots ($\Delta x$) are shown in figure 2. Besides the discussed *bistable* (only group-fixation) and *stable coexistence scenario* (only group-growth), there can be a *bistable coexistence scenario* (group-growth and fixation mechanism). In addition, there are the *scenarios of only cooperation* and *only defection*, where the sole stable fixed points are $x^* = 1$ or $x^* = 0$, respectively.

To this point, we have seen that the internal dynamics on the intragroup level result in an effective drift for the iterative map, which can support cooperation. We now want to examine whether this drift allows for the evolution of cooperation. In other words, we investigate if a single cooperative mutant can survive and spread in a free-riding population. In figure 3, we show two exemplary trajectories for the time evolution of the fraction of cooperators. The parameters $T = 4$ and $n_0 = 5$ correspond to the coexistence regime in figure 2 (black square). The simulations are performed by placing a single cooperative mutant in one of the $M = 200$ groups; this corresponds to an average initial fraction of cooperators of $x_0 = 1/(n_0 M) = 0.001$. In figure 3a, a coexistence trajectory is shown, whereas in figure 3b, cooperators go extinct at the fifth regrouping event ($t = 20$). There are strong differences between these trajectories and the ones known from nonlinear dynamics models without an iterative map which are

commonly used to analyse coexistence scenarios: owing to the dynamics during group evolution, the fraction of cooperators does not only fluctuate slightly at the fixed point, but oscillates around it. The reason is the interplay of the group-growth mechanism with the ensuing decline in the fraction of cooperators owing to the selection disadvantage of cooperators in each group: after each regrouping event, the group growth mechanism causes an increase in the level of cooperators. Later on, when population growth slows down as groups are already close to their carrying capacity, the group-growth mechanism is not effective anymore. Thus, the fraction of cooperators declines owing to their growth disadvantage within each group. This dynamic pattern is then repeated in each life cycle causing the oscillatory behaviour. But, not only oscillations increase the variability, also demographic fluctuations are particularly strong. The reason is that small variations in the fraction of cooperators are exponentially amplified during group growth. Therefore, for large times ($t > 100$ in figure 3a) when the steady state is already reached the exact position of the maxima and minima in each life cycle substantially varies between different regrouping events. In addition, the regrouping mechanism increases the randomness, i.e. owing to the stochastic reformation of groups the fraction of cooperators can change during this process. In the inset of figure 3a, the first eight regrouping steps are magnified. For instance, during the second ($t = 8$) and fourth ($t = 16$) regrouping events, a substantial drop in the fraction of cooperators is present, whereas for other regrouping events it is hardly changed.

This analysis already shows that the question whether a single mutant can survive is much less trivial than suggested by the phase diagram (figure 2): mutants have to overcome two hampering factors which diminish the positive drift of the iterative map. First, in each group, free-riders are favoured over cooperators owing to the costs to produce the public goods $c$: during group evolution, the fraction of cooperators declines in each initially mixed group. Thus, if group evolution was not interrupted by the formation of new groups, cooperators would die out in those mixed groups. Therefore, in all parameter regimes (including the ones with a stable cooperation fixed point), the fixation probability of a free-rider is higher than that of a cooperator
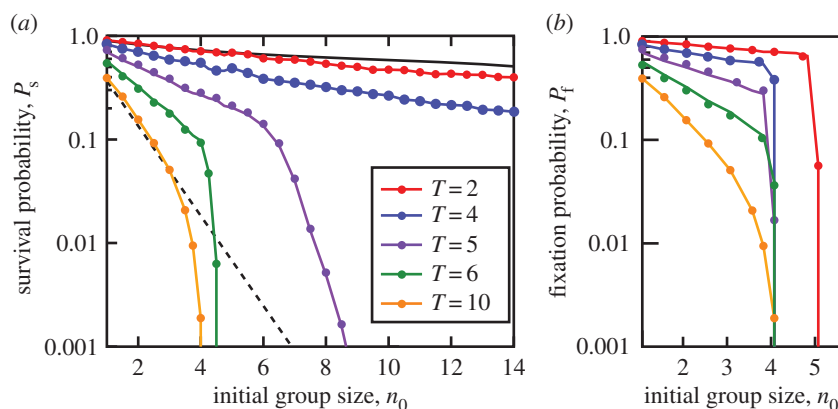
**Figure 4.** Survival probability (*a*) and fixation probability (*b*) of cooperation. When starting with a single mutant, the survival probability of cooperators, $P_s$, and their fixation probability, $P_f$, are shown after 500 regrouping events. Interestingly, both probabilities are fairly large over a large parameter regime. Different colours correspond to different regrouping times: $T = \{2$ (red), 4 (blue), 5 (purple), 6 (green), 10 (orange)$\}$, $M = 200$, number of realizations $R \geq 10^5$. Other parameters: $p = 10$, $K = 100$, $c = 0.1$. Black lines are calculated with our analytical approximation, equation (2.3), for $T = 2$ (solid) and $T \to \infty$ (dashed). (Online version in colour.)

during group evolution. Second, even if cooperators have survived group evolution, it is not assured that they survive regrouping as this process follows a random distribution. Especially for small fractions of cooperators, cooperators are more likely to die out than free-riders during regrouping. Taken together, it is not obvious whether the advantages of cooperators are sufficient to enable the evolution of cooperation from a single mutant.

In the following, we study this issue by analysing the *fixation probability* $P_f$, and the *survival probability* $P_s$. $P_f \equiv P_{\text{fix},C}$ denotes the probability that a single cooperative mutant will take over the entire population, whereas $P_s \equiv 1 - P_{\text{fix},F}$ is the probability that free-riders have not fixated yet in the population. Both probabilities are time-dependent, but reach quasi-stationary values under repeated regrouping (see the electronic supplementary material). Again starting with one cooperator, we performed stochastic simulations of the population dynamics, each realization with 200 regrouping events. In figure 4, the probabilities $P_s$ (figure 4*a*) and $P_f$ (figure 4*b*) depending on $n_0$ are shown for different regrouping times $T$. Owing to the life cycle dynamics, the fixation behaviour here is more intricate than for standard evolutionary dynamics. In those standard models, it is the population size only that determines the weight of fluctuations with respect to the deterministic drift [41]. In the limit of large population sizes fluctuations become irrelevant, and a stable fixed point is reached with absolute certainty. In contrast, for our life cycle model, the stability diagram and the deterministic drift themselves depend on the initial population size $n_0$: cooperators are less favoured for higher values of $n_0$. For small $n_0$, the regime of pure cooperation with a stable fixed point at $x^* = 1$ is present, cf. figure 2. In accordance with that both fixation and survival probability are equal and fairly large. With increasing initial population sizes $n_0$, the strength of the group-growth mechanism is reduced, and we observe a decline in the survival and fixation probabilities. This decline is faster for larger regrouping times, because cooperators stay longer in the evolution step and the thereby created selection disadvantage, $s$, accumulates during the time interval $[0, T]$. Further increasing initial group sizes causes a steep drop in the fixation probability. The reason is that upon increasing $n_0$ one is leaving the regime of pure cooperation, cf. figure 2 red line. Hence, the fixation probability of cooperators dramatically declines.

While the so far discussed fixation probabilities only depend quantitatively on the regrouping time $T$, the survival probabilities are also qualitatively influenced by this parameter. For small $T$, the system is in the coexistence regime which implies that cooperators neither fully take over the population nor die out easily. Thus, the survival probability shows only a slow decay with $n_0$ in this regime. In contrast, for larger regrouping times, the dynamics becomes unstable. Because the dynamics start with a single cooperative mutant, the group-fixation mechanism is not efficient as it is unlikely that the threshold above which cooperators are favoured is reached. Therefore, the survival probability declines rapidly with increasing $n_0$.

Let us finally discuss how the survival probabilities can be understood, based on analytic arguments. To this end, we approximate the dynamics by considering the first life cycle only, i.e. the first group-evolution steps and the ensuing formation of new colonies. As most extinction events of cooperators happen at the beginning, this approximation captures the extinction dynamics qualitatively correctly (see electronic supplementary material). The success of a cooperator crucially depends on the size of the group it is living in. For larger groups, it has to compete with more free-riders and its survival chances are diminished. As the group sizes are Poisson-distributed, the probability that a cooperator emerges in a group of size $k$ is given by $n_0^{k-1}/(k-1)! e^{-n_0}$. The probability that cooperators survive the first regrouping step is the probability that not all $Mn_0$ newly formed groups are purely free-riding. For a realization with a fraction of $x$ cooperators before regrouping, this probability is given by $1 - e^{-xn_0 M}$. Taken together, this leads to the overall survival probability

$$P_S = \frac{e^{-n_0} \sum_{k=0}^{\infty} n_0^{k-1}}{(k-1)! \langle 1 - e^{-x(k,T)n_0 M} \rangle}, \qquad (2.3)$$

where $\langle .. \rangle$ denotes an average over all possible realizations. For large regrouping times, cooperators die out in all initially mixed groups, i.e. $x = 0$ for $k > 1$. Thus, the survival probability simplifies to $e^{-n_0}$, which is in perfect agreement with our simulation results (figure 4 dashed black line). For smaller $T$, the group-growth mechanism is dominant, and cooperators also survive in initially mixed groups leading to even higher values of the survival probabilities. For $T = 2$, where the group-growth mechanism results in a
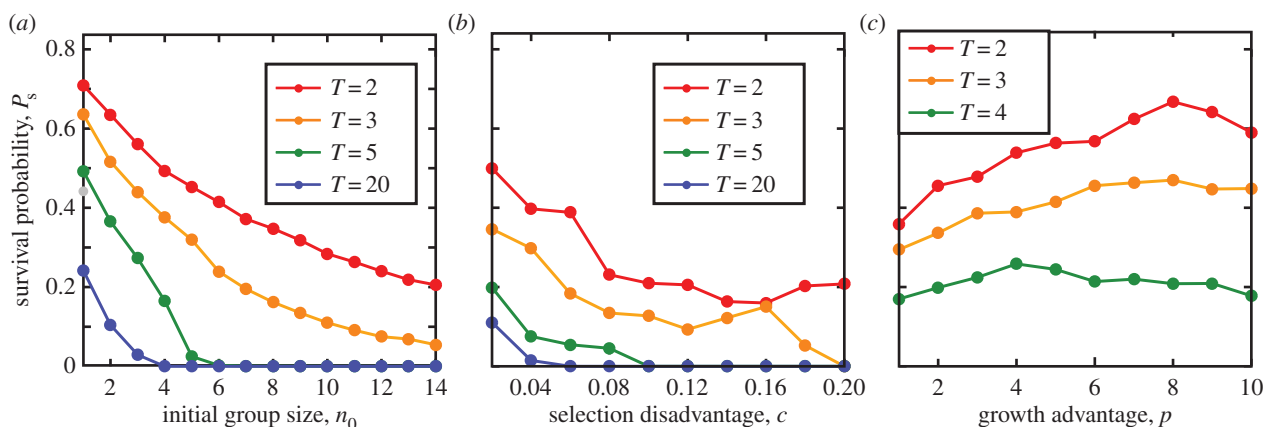
**Figure 5.** Robustness of the survival probabilities for different model parameters. (*a*) The impact of exponentially distributed regrouping times depending on $n_0$. (*b*) The impact of varying selection disadvantages is investigated for $p = 10$ and $n_0 = 5$. (*c*) The survival probability for different values of $p$ scaling the growth advantage mediated by the public good is shown. Even for small values of $p$ the group-growth mechanism is effective and supports the onset of cooperation. If not varied, $n_0 = 5$ and $c = 0.1$. Other parameters are as in figure 4. (Online version in colour.)

stable fixed point, we compare the approximation with the simulated survival probabilities and find good agreement (cf. Figure 4 solid black line).

To further support the idea that ecological factors can explain the onset of cooperation, we study the robustness of our findings against changes of the model parameters. In particular, we vary $p$ scaling the benefit of the public good, and $c$ measuring the metabolic costs owing to the production of a public good. In figure 5, we show the survival probabilities for different regrouping times and $n_0 = 5$ depending on $c$ (figure 5*b*) and $p$ (figure 5*c*). Interestingly, already for comparably small values of $p$ the positive impact of a public good on the population size notably supports cooperation and thereby increases the survival probability. In contrast increasing costs $c$ decrease the survival chances, but the benefits of cooperation are strong enough to compensate for selection disadvantages of up to 20%. In addition, we also demonstrate that our results do not depend on the assumption that the regrouping time is fixed. In figure 5*a*, survival probabilities are shown for regrouping times exponentially distributed with $\bar{T}$ according to $p(T) = (1/\bar{T}) \exp [-T/\bar{T}]$. Again, the survival probabilities are fairly large and our conclusion that a simple life cycle supports cooperators strong enough to overcome their selection disadvantages is not changed. All in all, we therefore conclude that our results are robust against changes in the details of the parameters and model assumptions and thereby constitute possible mechanisms enabling the onset of cooperation.

## 3. Conclusion

In this article, we studied the impact of ecological factors such as population growth and population bottlenecks on the evolutionary dynamics of cooperating individuals. Our main findings can be subdivided into two blocks: first, we analysed the evolutionary dynamics acting on cooperators already abundant in a population; second, we studied the survival chances of single mutants emerging in a purely non-cooperating environment.

In the first part, we were employing our recently introduced model [39] to study how a restructuring mechanism combined with typical growth conditions influences the

evolutionary dynamics of public good producing bacteria. Depending on the inoculation size $n_0$ and the regrouping time $T$, regimes of stable cooperation, coexistence between cooperators and free-riders and bistability emerge. Those regimes arise over a broad parameter regime even though the worst-case scenarios for cooperators are assumed whenever model assumptions have to be made. Therefore, we believe that the mechanisms still apply in more realistic evolutionary scenarios where for example reassortment is not completely random or public goods are not equally distributed between all individuals [8]. Other studies focusing on different aspects of the interplay between evolution and ecological factors support our findings: for instance, the impact of exponential growth following bottlenecks in infinite populations was shown to support cooperators [19]. In [20], the competition between groups sharing a bounded global population's size and thereby competing for resources was investigated, whereas in [27], the impact of mutations on the beneficial effects of population growth for groups starting with only one individual was studied. In addition, the frequency of ecological disturbance and resource supply plays a crucial role for the resulting level of cooperation [54,55]. Furthermore, beneficial effects for cooperators were also found when reassortment is not random but environmentally driven [56–58]. All studies emphasize the important role of ecological factors for understanding cooperation. In contrast to the mentioned studies, we focused on a description of bacterial growth, starting with an exponential growth phase reaching a carrying capacity later on. The different growth regimes (exponential growth and stationary state) influence the evolutionary dynamics differently: both related mechanisms (group-growth and group-fixation) favour cooperators, but as confirmed by analytical arguments presented above, the strength of the group-growth mechanism increases with small $x_0$, whereas the strength of group-fixation mechanism decreases. Therefore, both mechanisms can be associated with two different fixed point scenarios (stable and unstable). We present a full parameter study of the ensuing regimes of cooperation (fully cooperative, coexistence, bistability, bistable coexistence and purely free-riding) for both key parameters, $n_0$ and $T$.

In the second part of our paper, we focused on the question whether those beneficial mechanisms can also explain

the onset of cooperation from a single mutant. Similar questions were extensively studied for evolutionary dynamics without regrouping where the factors influencing fixation probabilities for neutral, beneficial and deleterious mutations were investigated carefully [59–61]. However, the non-iterative map caused by regrouping makes a new study essential as it alters many aspects of the evolutionary dynamics. Here, we demonstrate that ecological factors increase the survival and fixation probabilities of cooperators substantially. In particular, the group-growth mechanism allows for the robust establishment of cooperation as it does not rely on a threshold fraction of cooperators to act effectively. Remarkably, the probability for a single mutant to succeed decreases only slowly with increasing $n_0$, and growth thus allows the onset of cooperation without the requirement of extremely narrow population bottlenecks. Owing to the robustness against parameter changes and the worst-case assumptions employed,

our model provides a proof of principle that ecological factors might explain the onset of cooperative behaviour. Once cooperation is established in a population, more advanced mechanisms, which rely on cooperators already present in a population, like kin discrimination or other active forms of positive assortment, may evolve to further stabilize cooperative behaviour [1,8,62–66].

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