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Author for correspondence:
Sorcha É. Mc Ginty
e-mail: sorcha.mcginty@ieu.uzh.ch

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The interplay between relatedness and horizontal gene transfer drives the evolution of plasmid-carried public goods

Sorcha É. Mc Ginty[1,2], Laurent Lehmann[3], Sam P. Brown[4,5] and Daniel J. Rankin[1,2]

[1]Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Building Y27, Winterthurerstrasse 190, 8057 Zürich, Switzerland
[2]Swiss Institute of Bioinformatics, Quartier Sorge Bâtiment Génopode, 1015 Lausanne, Switzerland
[3]Department of Ecology and Evolution, University of Lausanne, UNIL Sorge, Le Biophore, 1015 Lausanne, Switzerland
[4]Department of Zoology, The Tinbergen Building, South Parks Road, Oxford OX1 3PS, UK
[5]Centre for Immunity, Infection and Evolution, University of Edinburgh, West Mains Rd, Edinburgh EH9 3JT, UK

Plasmids carry a wide range of genes that are often involved in bacterial social behaviour. The question of why such genes are frequently mobile has received increasing attention. Here, we use an explicit population genetic approach to model the evolution of plasmid-borne bacterial public goods production. Our findings highlight the importance of both transmission and relatedness as factors driving the evolution of plasmid-borne public goods production. We partition the effects of plasmid transfer of social traits into those of infectivity and the effect of increased relatedness. Our results demonstrate that, owing to its effect on relatedness, plasmid mobility increases the invasion and stability of public goods, in a way not seen in individually beneficial traits. In addition, we show that plasmid transfer increases relatedness when public goods production is rare but this effect declines when production is common, with both scenarios leading to an increase in the frequency of plasmid-borne public goods. Plasmids remain important vectors for the spread of social genes involved in bacterial virulence thus an understanding of their dynamics is highly relevant from a public health perspective.

1. Introduction

Mobile genetic elements, such as plasmids, are ubiquitous in bacterial genomes [1–4]. Plasmids carry a wide range of different 'accessory' genes, and many factors are likely to influence which genes are carried on plasmids and why [5,6]. Plasmids are disproportionately likely to carry genes that code for secreted proteins [7]. As secreted proteins are costly to produce and may provide a benefit to other individuals (e.g. antibiotic resistance, [8]), they raise the question of why an individual should carry out a behaviour that is potentially costly to perform but benefits others. Theoretical explanations for public goods production (PG) have shown that such behaviour can evolve if there are either direct fitness benefits to the producer individual (i.e. mutual benefits for actor and recipient) or else indirect fitness benefits to the actor, so that kin selection is operating [9–11]. These social traits are of particular relevance in a public health sense as secreted factors are often known to be virulence determinants [7,12]. Plasmids are also known for their carriage of antibiotic resistance traits, and these traits can also, in some cases, be characterized as public goods due to the benefit they may confer on neighbouring cells, for example, β-lactamase exported from *Pseudomonas aeruginosa* in outer membrane vesicles [8].

Previous work has demonstrated that genes involved in bacterial PG and virulence are over-represented on mobile elements, or areas of the bacterial genome likely to have originated by horizontal transfer [7,13]. This association highlights the importance of gene mobility in bacterial social evolution [7,12]. It has been argued that horizontal gene transfer (HGT) of public goods

producing genes could act as a novel mechanism for the evolution of PG [12]. HGT of public good producing plasmids has the effect of converting previously non-producing cells into producers of a public good [12]. As such, the one-time 'cheater' cells are essentially forced to display a producer phenotype. However, if competing (incompatible) plasmids arise in a population that do not carry the gene for production of the public good then they can prevent the invasion of producer plasmids [14]. Thus, the benefits that a plasmid carrying a producer gene gains from infecting other cells are reduced in the presence of incompatible plasmids.

HGT via plasmids can potentially increase local relatedness by infecting previously unrelated neighbours. As relatedness is measured at the locus of interest [11] (which, in this case, is a gene on a plasmid), HGT thus has the potential to increase local relatedness [7]. More mobile genes may then create higher relatedness among their neighbours allowing kin selection to maintain costly public goods production. There are therefore two complementary ways in which public goods can be maintained via plasmids: infectious transfer (i.e. spread of plasmids into previously uninfected cells, directly increasing the number of plasmid carriers) and kin selection, where plasmids increase their within-host relatedness ensuring the production cost of public goods is going towards helping relatives.

While plasmid transfer may not be sufficient to maintain PG in every scenario [14], it is likely that both infectious transfer and kin selection may act as complimentary forces in the evolution of plasmid-borne PG [15]. Infectious transfer of the producer plasmid forces the receiving cell to adopt a producer phenotype, but, when the plasmid is rare, transmission also has the effect of increasing the relatedness between neighbouring cells (with respect to the producer gene). Kin selection then maintains PG between relatives. Both these processes could help to explain why so many social genes are transmitted horizontally [7]. There has been some debate as to whether kin selection truly plays a role in maintaining bacterial PG via HGT or, if instead, only the infectivity of the mobile genetic element has an effect (see [15,16]). It has recently been asserted that there is no definitive evidence that kin selection acts, in addition to the infectivity of mobile genetic elements, as a mechanism to maintain bacterial PG [16]. Here, we aim to examine whether relatedness can in fact be disentangled from infectivity as a process to support plasmid-borne PG. Previous work on plasmid evolution and persistence has drawn on epidemiological models of plasmid transfer [14,17–20], which assume large, well-mixed populations or has not explicitly partitioned the effects of infectivity and kin selection [12]. Here, we build an explicit population genetical model, incorporating horizontal transfer between local hosts. This allows us to examine the relative force of both infectivity and kin selection in the success of plasmid-borne genes. We can, in particular, explicitly calculate relatedness and examine how it is affected by horizontal gene transmission, a feature that has not been explored by previous models of plasmid-borne PG [14].

2. Model and results

(a) A model of plasmid-borne public goods production

(i) Life cycle

We use the neighbour-modulated fitness approach [9,21] to model a large population of host-associated bacteria. We assume a population of bacteria living in an infinite number of hosts (an infinite island model [22]), where there are N founder strains on each host. Hosts are referred to as 'patches' as they represent structure in the population. Patch generations are non-overlapping, and individuals are haploid. For purposes of mathematical tractability, we use a simplified bacterial life cycle where processes occur in separate phases, as described below. In reality, these processes may occur simultaneously, but separating them into discrete stages is a standard approach to make the analyses simpler [7,23–26]. Our model life cycle consists of five steps, as illustrated in the electronic supplementary material, figure S1:

(1) *Founding*. Each patch is colonized by N independent founder stains sampled from an infinite, panmictic pool of potential founder strains. Founder strains may be plasmid carriers or plasmid-free. With over 1700 complete plasmid genomes in GenBank [27], it is clear that plasmids are diverse in nature, although existing mechanisms may prevent infection with multiple types, such as plasmid incompatibility. For simplicity, and as plasmid competition in terms of social evolution has been modelled elsewhere [14], we assume only one plasmid type.

(2) *Reproduction*. All initial founder cells produce a large number of offspring such that by the end of the reproduction stage there are a very large number of individuals in each patch with a fraction $1/N$ of them descending from each founder strain. Plasmids are inherited vertically from parent to offspring. In nature, plasmids may be lost at this point, owing to a process known as segregation. However, as the rate of segregation is generally of the order of 10^{-6} or lower [28], we assume that segregation is negligible, and do not include it in our model. Parent cells die and selection on offspring does not occur at this stage.

(3) *Plasmid transmission*. Offspring interact randomly within the patch. Plasmid transmission rates have been found to vary widely [29] and transmission may be increased in areas of high bacterial density, such as biofilms [4] or during microbial blooms [30]. In addition, transfer can be influenced by additional environmental factors (as listed in [4]). We model plasmid transmission at the individual level. Conditional on contact between plasmid carriers and plasmid-free cells in a patch, transmission of the plasmid occurs with probability β. For simplicity, we assume that only uninfected cells can acquire a plasmid and that there is exactly one contact per cell pair during this stage. However, secondary infection may occur in nature and may be associated with decreased fitness [31].

(4) *Public goods production*. All plasmid-carrying offspring produce a public good which generates a benefit that is shared by all individuals within the same patch. The cost of producing the public good to the producer individual is represented by C (this includes the baseline cost of plasmid carriage, where we can write $C = C_C + C_G$, where C_G is the cost of public goods production and C_C is the cost of plasmid carriage [18]) and the benefit of producer behaviour (shared by all individuals within the same patch j) is represented by B. Public goods, for example, production of β-lactamase, which confers antibiotic resistance [8] or secreted virulence factors [32] are often horizontally transferred in bacteria. Offspring survival is determined by results of costs and benefits of public goods.

Table 1. List of model parameters.

parameter	definition
p_{ij}	an indicator variable taking the value one if founder i in patch j carries the plasmid, zero otherwise, this is a random variable whose value depends on the individual sampled.
p_{ij}^t	the value of p_{ij} when measured after transmission (denoted by the superscript t). Random interactions indicate this will be $p_{ij}^t = p_{ij} + (1-p_{ij})\sum_{k\neq i}(p_{kj}/N)\beta = p_{ij} + (1-p_{ij})\beta p_j$.
p	average frequency of carriers of the plasmid among individuals in the population such that $p = \mathrm{E}[p_{ij}] = \lim_{n\to\infty}[\sum_{ij} p_{ij}/(Nn)]$. A subscript t denotes when this is measured after transmission.
p_j^t	a random variable such that $p_j^t = \sum_i p_{ij}^t/N$ and denotes the average frequency of the plasmid in patch j. The value of p_j^t depends on the sampled individual (descending from founder i).
w_{ij}	a random variable indicating the fitness, after transmission, of individual i in patch j
w	average fitness, after transmission, across the population
β	per contact transmission probability of the plasmid
N	number of founder strains in a patch

(5) *Dispersal*. All cells disperse to form an infinite, panmictic pool of potential founders.

Our life cycle therefore consists of within patch plasmid transmission and public goods production, and global competition between all bacteria to found new patches. The effects of plasmid carriage and public goods production determine the success of the bacteria at this competitive stage.

(b) Model structure

We use a standard population genetical approach, and derive our model from the Price equation [33,34] in order to evaluate the change, Δp, in the average frequency, p, of the plasmid in the population;

$$\Delta p = \frac{1}{w}\mathrm{Cov}[w_{ij}, p_{ij}^t] + E[\Delta p_{ij}], \tag{2.1}$$

where w_{ij} represents the fitness of an individual, i, carrying the plasmid, in patch j; w refers to the mean fitness across the whole population; p_{ij}^t is an indicator variable taking the value one if descendant individual i in patch j carries the plasmid and zero otherwise (where the subscript t indicates it is measured after transmission stage); Δp_{ij} is the change in an individual's status (plasmid carrier or plasmid-free) within a generation; and the covariance and expectation is taken over all individuals in the population. The full derivation of this equation (2.1) is shown in electronic supplementary material, appendix A equations (A 1–A 2). A list of the parameters used in the model is found in table 1.

(c) Transmission ($E[\Delta p_{ij}]$)

The change in frequency due to transmission (also known as the transmission bias), $E[\Delta p_{ij}]$, is calculated using the life cycle described earlier. As $\mathrm{E}[\Delta p_{ij}] = \mathrm{E}[p_{ij}^t] - \mathrm{E}[p_{ij}]$ and $E[p_{ij}] = p$, we need only calculate $E[p_{ij}^t]$ which is given by

$$E[p_{ij}^t] = p^t = p + (1-p)\frac{N-1}{N}\beta p. \tag{2.2}$$

This equation is composed of the sum of the average population frequency of those individuals who originally carried plasmid (p) plus those non-carriers who were infected with the plasmid ($1-p$) at the transmission stage. Transmission is therefore frequency dependent (not density dependent as described in other studies (e.g. [20,35]). These plasmid-free individuals are infected with probability β by plasmid-carrying individuals that are descended from a different strain to their own $((N-1)/N)p$, where N is the number of founding strains in a patch. This gives the average frequency of the plasmid in the population after transmission (p^t).

Therefore, $\mathrm{E}[\Delta p_{ij}] = p + (1-p)((N-1)/N)\beta p - p$. Thus, we see that the change in frequency after one generation depends on the variance in the population, $p(1-p)$, and a transfer coefficient based on the number of strains and the probability of transfer per contact $((N-1)/N)\beta$, giving

$$E[\Delta p_{ij}] = p(1-p)\frac{N-1}{N}\beta. \tag{2.3}$$

(d) Selection ($(1/w)\ \mathrm{Cov}[w_{ij}, p_{ij}^t]$)

We consider a producer plasmid. The fitness of an individual, i, carrying the plasmid, in patch j, is, following our assumptions, given by:

$$w_{ij} = 1 - Cp_{ij}^t + Bp_j^t.$$

Therefore, mean fitness across the whole population, after transmission and fitness interaction, is

$$w = 1 + (B - C)p^t.$$

$\mathrm{Cov}[w_{ij}, p_{ij}^t]$ reduces to (see the electronic supplementary material, appendix B equation (B 1) for full details):

$$\mathrm{Cov}[w_{ij}, p_{ij}^t] = \mathrm{Var}[p^t](BR - C), \tag{2.4}$$

where $\mathrm{Var}[p^t]$ describes the variance in plasmid carriage across individuals and R refers to relatedness; this is a whole-group relatedness coefficient which measures the extent to which random recipients are more (or less) likely to carry the same plasmid as the actor than is an average cell in the patch [36]. Both $\mathrm{Var}[p^t]$ and R are measured after transmission and full derivations are found in electronic supplementary material, appendix B (equations B 2 and B 3 and B 6, respectively).

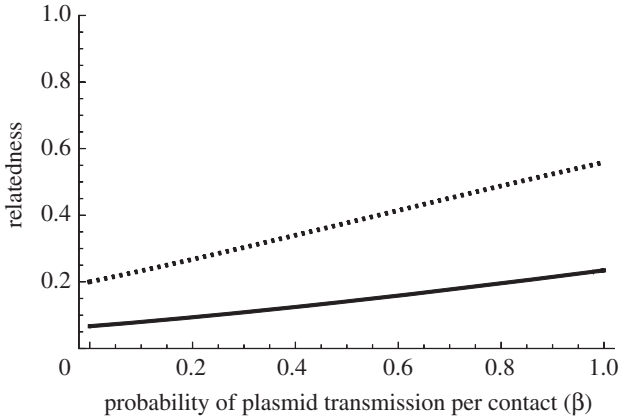

Figure 1. Increasing the probability of plasmid transmission per contact increases the relatedness within a population when the plasmid is rare. But an increased number of founders (N) leads to an overall decrease in relatedness and a decrease in the strength of the effect of increased plasmid transmission. Therefore, relatedness no longer increases to the same extent as plasmid transmission increases. $p = 0.001$. Solid line, $N = 15$; dotted line, $N = 5$.

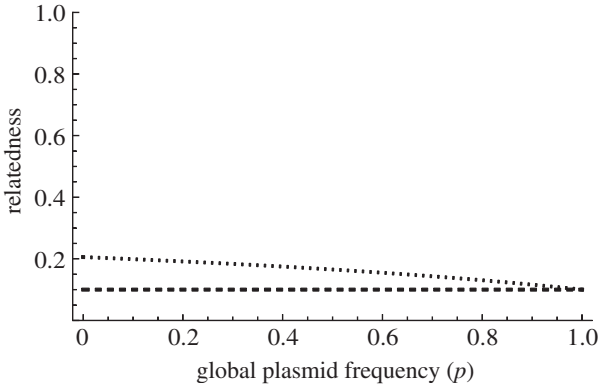

Figure 2. As the global frequency of the plasmid increases, whole-group relatedness (the extent to which random recipients are more (or less) likely to carry the same plasmid as the actor than is an average cell in the patch) decreases. $N = 10$, $\beta = 0.5$. The dotted line indicates relatedness where $\beta = 0.5$, the dashed line indicates relatedness in the absence of HGT i.e. $\beta = 0$. There is a decrease in relatedness as the global frequency of the plasmid increases (dotted line), whereas in the absence of horizontal gene transmission (dashed line) there is no concurrent decrease in relatedness as global plasmid frequency increases.

(e) Relatedness when the plasmid is rare

The full expression for R is complicated and thus we study special simple cases. First, we consider that the plasmid is rare (i.e. by setting $p \to 0$), in which case we obtain

$$R = \frac{1}{N}\frac{(N + (N-1)\beta)^2}{N^2 + (N-1)\beta^2}.$$

For an actor (i.e. a focal cell) in a patch containing N (number of founder strains in a patch) strains, one's own strain makes up $1/N$ of the total set of recipient strains (with whom one interacts), the second factor of relatedness is made up of the remaining strains and the effect of horizontal gene transmission. As such, as patch size (with larger N) becomes very large, the correlation among bacteria (relatedness) in the same patch tends to decrease. This is a generic feature of our models and is qualitatively equivalent to the decrease in relatedness as patch size increases under vertical transmission with limited dispersal [25,26]. This is illustrated in figure 1 where it is clear that relatedness increases with increased plasmid transmission but when the number of founders (N) is increased we see an overall decrease in relatedness and a decrease in the strength of the effect of increased plasmid transmission.

(f) Relatedness around small values of β

Allowing the frequency of the plasmid to vary freely, while assuming that the transmission parameter β is small, we can perform a first-order Taylor expansion of relatedness (equation (B3)) around $\beta \to 0$, which shows that

$$R = \frac{1}{N} + \frac{2((N-1)(1-p))\beta}{N^2}.$$

HGT will cease to increase relatedness when $p \to 1$ (figure 2), and we have $R = 1/N$ so that, when the plasmid is fixed, horizontal gene transmission no longer has an effect as all offspring will receive the plasmid from their respective parent cells.

This is a good approximation of relatedness for values of β up to around $\beta = 0.4$ (not shown). We find that irrespective of the probability of transmission per contact, as the number of founder strains (N) becomes very large, relatedness (R) goes to zero. Thus, the accumulation of relatedness depends on there being a finite number of founder strains for each patch. This is biologically likely as, for example, many pathogenic bacteria may infect a host starting from a relatively small number of founder cells [37], this number may be as low as 10 in many cases (placing a severe upper limit on strain numbers). A finite number of founder strains results in a significant variance in plasmid frequency between patches (i.e. hosts) at the founding stage.

(g) Feedback between relatedness and transmission

There appears to be a feedback between relatedness (equation (B 3)) and transmission. When p is rare in the population, i.e. close to zero, this implies that in many patches there are no plasmid-carrying lineages (descending from plasmid-carrying founders) and only a few patches which are infected by one or two plasmid-carrying lineages. This leads to an overall low global frequency of the plasmid. As a result, transmission of the plasmid within a patch will increase plasmid frequency in the patches where there are plasmid lineages but will have no effect on patches from which plasmid lineages are absent. This will lead to many plasmid-free patches (as before) and a few patches with high plasmid carriage. Therefore, the variance in plasmid frequency between patches increases and thus whole-group relatedness (R) increases concurrently. On the other hand, when p is close to 1 (close to fixation in the population), the variance between patches implies that there are many patches with N plasmid-carrying lineages and a few patches with N-1, N-2 plasmid-carrying lineages (i.e. which still have a few plasmid-free lineages). Thus, almost all bacteria across all patches already carry a plasmid before the transmission stage. Transmission will then increase plasmid frequency in the patches not fixed for plasmids (those which still have plasmid-free lineages) but will not affect the patches within which the plasmid is fixed. This will decrease the variance in plasmid frequency between

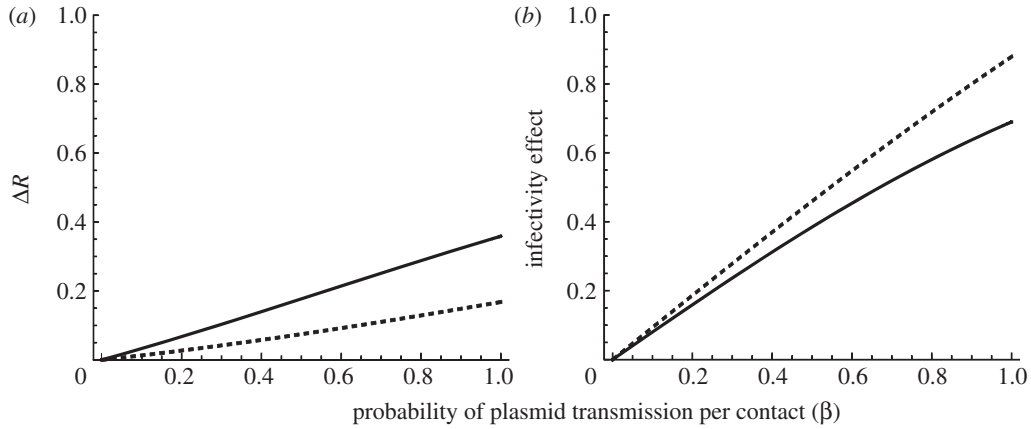

Figure 3. The probability of transmission per contact has a stronger effect on relatedness when founder numbers are low (as opposed to higher), whereas it has a stronger effect on infectivity when founder numbers are higher (as opposed to lower) when the plasmid is rare (i.e. $p \to 0$). (*a*) Indicates ΔR, the increase in relatedness owing to horizontal transfer. (*b*) Indicates the infectivity effect of the plasmid (the increase in local plasmid frequency caused by HGT of the plasmid). Low number of founders ($N = 5$) is denoted by solid lines and $N = 15$ denoted by dashed lines. The infectivity effect is increased with increasing transmission, and this is compounded by higher founder numbers (N), but relatedness decreases with increased founder numbers so that ΔR is decreased and increased transmission cannot compensate fully for this effect.

patches. Therefore, we see a decrease in whole-group relatedness (R) relative to its initial level when the plasmid was rare in the population as shown in figure 2.

(h) Plasmid spread is affected by relatedness and transmission

Substituting equations (2.2)–(2.4) into equation (2.1) gives the full expression for the change in the average frequency of the plasmid in the population

$$\Delta p = \frac{1}{w}(\mathrm{Var}[p^t](BR - C)) + p(1-p)\frac{N-1}{N}\beta. \tag{2.5}$$

Substituting equation (B 2), and equation (B 3), into this expression and performing an invasion analysis (of point $p \to 0$) by taking the partial differential of equation (2.5) with respect to p and setting p to zero, we find that the plasmid can spread from rare in the population (when $p \to 0$) provided

$$BR + \beta\frac{N(N-1)}{N^2 + \beta^2(N-1)} > C. \tag{2.6}$$

Here, R refers to relatedness after transmission and $\beta N(N-1)/(N^2 + \beta^2(N-1))$ accounts for the infectivity effect of the plasmid, that is, the increase in plasmid carriage caused by HGT of the plasmid.

Inequality (2.6) highlights the fact that different types of trait respond differently to being carried on plasmids. When there is a trait which is beneficial to the group (i.e. $B > 0$), both kin selection and infectivity influence its spread but for an individually beneficial trait (where $B = 0$ and $C < 0$), only infectivity plays a role. For traits involved in competition, i.e. those which impact negatively on the group (when $B < 0$) and which may be strictly selfish (i.e. $B < 0$ and $C < 0$), we see that the kin selection effect will hinder their spread. For traits which are parasitic (i.e. $C > 0$ and $B = 0$), infectivity alone plays a role and this must be greater than the parasite's cost in order for the trait to spread.

Using our expression for relatedness (equation (B3)), we can also calculate relatedness before transmission, which gives $1/N$; the probability of sampling two individuals from the focal strain. Thus, we see that when the plasmid is rare the change in relatedness over one generation (ΔR) is calculated by

$$\Delta R = \frac{N-1}{N}\beta\frac{N(2+\beta) - 2\beta}{N^2 + (N-1)\beta^2}.$$

HGT promotes the spread of the plasmid through both ΔR, the additional kin selection effect stemming from the extended identity-by-descent through horizontal spread of the plasmid, and the infectivity effect. Both of these effects are affected by the per contact transmission probability (β) but to different extents as can be seen from figure 3. It is clear from figure 3 that the probability of transmission has the greatest impact on the infectivity effect, supporting the assertion based on our Taylor expansion of R around $\beta \to 0$, that the increase in R due to HGT is at most of magnitude $1/N$ when the probability of transmission per contact is high ($\beta \to 1$) (assuming one contact per cell pair during the plasmid transmission phase of the life cycle).

We see that when taking the limit of inequality (2.6) when N approaches infinity that the kin selection effect drops out leaving the condition of invasion $\beta > C$. This illustrates that there remains an effect of transmission even after the effect of kin selection is removed suggesting that under some conditions infectivity may be the dominant component influencing the plasmid's spread. Our results clearly depend on our life cycle and, if transmission were to occur after the public goods interaction (i.e. if the order of stages 3 and 4 was reversed) then we would no longer see the kin selection effect but infectivity effect would remain.

Inequality (2.6) reveals that transmission is a powerful force in the model. Even costly plasmids or those which have no effect (i.e. $B = 0$), or even a negative effect, on the group (i.e. $B < 0$) can spread from rare provided transmission is high enough. This is a standard result from plasmid previous studies [20,38]. However, there are other factors, such as relatedness, in the model which are influenced by transmission. Transmission affects relatedness (figure 1) and we can explore the effects of relatedness on its own by looking

at what happens when transmission is rare. If transmission is absent ($\beta = 0$) then we find that equation (2.5) reduces to

$$\Delta p = p(1-p)\left(\frac{BR - C}{1 + Bp - Cp}\right),$$

where whole-group relatedness, in the absence of transmission, is given by $1/N$. In this case, the plasmid can spread from rare provided $BR > C$, i.e. if the producer lineage in patch receives a positive net fitness benefit, the public good will be selected for (a standard result from social evolution theory).

3. Discussion

Our model highlights the importance of both infectivity and kin selection as factors enabling PG traits on plasmids to spread through the population. While it has been argued that infection of uninfected cells alone is enough to drive bacterial public goods [16], it was shown that this does not apply when there is competition among plasmids in an unstructured environment [14], and it has been argued that HGT confers a strengthened inclusive fitness benefit to PG in a structured environment, as it increases relatedness on a local scale [7]. However, both infectivity and kin selection are complimentary [15] and our model helps to reconcile these two approaches. We find HGT favours plasmid-carried public goods through the dual effect of increasing local relatedness when the plasmid is rare and through the effects of transmission (increasing numbers of plasmid carriers).

It is well established that costly helping and thus PG can be maintained through interactions between relatives via kin selection [10,11]. It is important to consider that relatedness is always measured at the locus of interest [11]. In the case of our model, the locus of interest is always on a plasmid, and our model shows that, under local transmission, gene mobility can act to increase whole-group relatedness at the plasmid level (figure 1). Thus, in the case of plasmid-borne PG genetic relatedness between interacting bacterial cells after the infection period can be generated either through descent from the same founder (i.e. coalescence, which is independent of gene mobility) or through HGT (i.e. transmission of the plasmid) itself. In the absence of HGT relatedness between cells within the patch will be $1/N$.

It is clear that HGT will increase the number of local cells that carry the plasmid, and thus increase the probability of identity in plasmid carriage, relative to the rest of the population. In the case of plasmid-carried PG, the results of our model show that there is an interesting feedback between transmission and relatedness: if individuals are less related in a patch there will be a higher number of cells for the plasmid to infect which will increase overall transmission, whereas if patches are homogeneous a plasmid will never find itself in a patch with uninfected cells. In other words, low relatedness at the plasmid level facilitates plasmid transmission (as there are available cells to infect) and thus an increase in relatedness (figure 1) when the plasmid is rare. However, high initial plasmid frequency results in less available cells for the plasmid to infect (i.e. decreased transmission). $E[p_j^t p_j^t]$, the probability that two individuals sampled randomly from patch j bear the focal allele, will continue to increase until the plasmid reaches fixation even when the plasmid is at high initial frequencies. As the plasmid becomes fixed in many patches, transmission will subsequently only increase plasmid frequency in those patches not fixed for plasmids. This leads to a decrease in the variance in plasmid frequency between patches. Therefore, we see a decrease in whole-group relatedness (R) as the global frequency of p increases (figure 2) but we will continue to see an increase in $E[p_j^t p_j^t]$ under the same conditions. This aspect of our model is novel because it exhibits a subtle feature of horizontal gene transmission across the population. We expect the increase in the local frequency of a producer plasmid ($E[p_j^t p_j^t]$,) within a patch to favour public goods production. While one may expect to observe such production of public goods associated with the highest relatedness value, at least on a local scale, we demonstrate that a low relatedness value may be associated with success of PG under certain conditions (i.e. that this decreased relatedness is associated with high global frequencies of p).

Transmission therefore has two main impacts in plasmid dynamics; firstly via direct transmission gains, and secondly, via changes to population structure R, which modify selection on social traits. The direct gain via transmission [16] can potentially work in a well-mixed population under certain conditions. However, this model has an important limitation, namely that plasmids of the same incompatibility type may exist in the population which do not carry the producer gene [39]. If two plasmids are incompatible it means that cells cannot carry both plasmids. In this case, the advantage of transmission will break down (assuming both plasmids transmit with the same probability). This means that, in a well-mixed population the non-producer plasmid will always invade as it gets a benefit from the producer plasmid (B) without paying the cost (C). Under such a scenario and with no infectious transmission, population structure, which allows for interactions between relatives, is needed for producer plasmids to persist when in competition with non-coding plasmids.

The importance of interactions between related individuals is further highlighted by the impact that changing the number of founders (N) has in our model. This number has two effects in our model. A higher number of founder strains means that producers are more likely to interact with more cheaters and the public good must be shared among more individuals, while a lower number of founders favours the public goods trait, as the offspring of the founders will be more related to each other, within a patch. Increasing relatedness via transmission of the plasmid feeds into this mechanism. However, a second effect of the number of founders is that, when the plasmid is rare, it increases the chance that there are uninfected cells with a plasmid, and thus favours 'infectivity' as a mechanism to promote plasmid-borne public goods production. Thus, infectivity and relatedness combine to promote public goods. The importance of the transmission term can be seen by the result that a sufficiently high transfer probability can be used to spread a purely costly plasmid, which has a negative impact on other individuals ($-B$) or which has no impact on other cells ($B = 0$) and only incurs a cost to the carrier. In this case, a sufficiently high transmission probability allows the otherwise costly the producer plasmid to persist. However, the selection term would disfavour a highly selfish and highly infectious plasmid, as the indirect fitness costs of damaging related neighbours would be increasingly severe with increasing transmission.

We assume that our public good is continuously produced by the producer cells and not recycled or regulated by its concentration in the environment. It has recently been

demonstrated that such regulation of a durable public good greatly reduces the selection for cheaters in that environment [40]. However, as the authors point out, upon invasion of a new patch, the cost of production must be paid in full, at least in this initial period. Thus, in this scenario, a high number of founders remains a threat to public goods production which can be dealt with via an increased transmission probability suggesting the advantage of durable public goods that are facultatively regulated may be maximized when carried on plasmids with relatively high transfer probabilities.

As the size of the founding inoculum is of clinical relevance and can vary for different pathogens [41], this model is useful as it demonstrates the effects of founder size (which can be no greater than inoculum size). When N is high, that is, when there is a large and diverse founding inoculum, we see a decrease in the effects of transmission on relatedness (figure 1) as well as an increase in the transmission bias term. Thus, for a high inoculum threshold direct transmission gains, i.e. transmission from carrier cells to plasmid-free cells is more important than the effect of transmission on relatedness (and consequently kin selection). However, for a low inoculum threshold the effect of transmission on relatedness is of greater importance and kin selection plays a greater role. We can therefore predict that when inoculum thresholds are low, the plasmids present are more likely to be those coding for public goods than when inoculum thresholds are high (as at low inoculum sizes such plasmids retain the advantage of kin selection).

Plasmids, among the key vectors of HGT, are present in all branches of the bacterial 'tree of life' and have been found in all bacterial communities studied to date [4]. They may act as vehicles for the horizontal transfer of genes between distantly related bacterial species, contributing to bacterial speciation and adaptation [42]. This ability to spread infectiously and reprogram the functionality of host cells may also have potential for use in new medical intervention 'Trojan horse' strategies [43]. More generally, an understanding of plasmids is essential to an understanding of evolution of bacterial traits, such as virulence and antibiotic resistance, which have an impact on human health. Our model focuses on plasmid transmission dynamics without incorporating complex epidemiology. In order to use this model to examine bacterial infections and the spread of antibiotic resistance via plasmids, it would be necessary to incorporate it into a nested model, allowing variable between-host dynamics and population sizes [44], to more accurately reflect the dynamics of an infectious pathogen.

In summary, we can conclude that the interaction between relatedness and infectivity is central to a complete understanding of plasmid-borne public goods production and the potential importance of HGT in the spread of producer traits.

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