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Co-resident plasmids travel together

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ABSTRACT

Conjugative plasmids encode genes that enable them to transfer, by conjugation, from a given host cell to another cell. Conjugative transfer, despite being an important feature of conjugative plasmids, is not constitutive for most plasmids, the reason being that genes involved in horizontal transfer are mostly repressed. Only upon their transient de-repression are plasmids able to transfer horizontally. If host cells harbour multiple plasmids, their simultaneous transfer depends on simultaneous transient de-repression of all plasmids. If de-repression of different plasmids was random and independent events, simultaneous de-repression should be a rare event because the probability of simultaneous de-repression would be the product of the probabilities of de-repression of each plasmid. Some previous observations support this hypothesis, while others show that co-transfer of plasmids is more frequent than this reasoning indicates. Here, we show that co-transfer of multiple plasmids mainly results from non-independent events: the probability that all plasmids within a cell become de-repressed is much higher than if de-repression of plasmids genes were independent. We found a simple model for the probability of co-transfer: the plasmid having the lowest conjugation rates is the one who limits co-transfer. In this sense, cells receiving the plasmid with the lower transfer rate also receive the other plasmid. If de-repression happens simultaneously on co-resident plasmids, common cues may stimulate de-repression of distinct plasmids.

1. Introduction

Conjugative plasmids are well known genetic elements able to transfer horizontally between hosts but it is currently unknown how multiple plasmids transfer from the same host cell. Here we study co-transfer of plasmids inhabiting the same cell. In general plasmids do not express the conjugative machinery constitutively (for a review of the conditions affecting such expression, see Frost and Koraimann, 2010). Fertility inhibition systems (FIN), encoded by the plasmid, prevent the expression of its own conjugative machinery. However, de-repression occurs transiently allowing the horizontal transfer of the plasmid briefly. Paradoxically, although this feature reduces their frequency of horizontal transfer, it may enhance their ability to persist in nature (Dionisio, 2005; Dionisio et al., 2002; Lundquist and Levin, 1986). Moreover, these systems may affect other plasmids present in same the cell.

The paradox can be solved by understanding that conjugation may impose a cost on bacterial cells, which can be detrimental to plasmids. One of the sources of this cost is the expression of the conjugative machinery which reduces the growth rate of the plasmid's host cells. Consequently, such cells would be displaced by plasmid-free bacteria not incurring a fitness cost (Dahlberg and Chao, 2003; Turner, 2004; Turner et al., 1998). Indeed, it was shown in populations of bacteria harbouring two types of plasmids, FIN^+ and FIN^-, that hosts harbouring the FIN^+ variant grew faster than hosts harbouring the FIN^-, therefore outcompeting them (Haft et al., 2009).

However, there are other causes for costs. Male-specific phages are viruses that specifically infect bacteria harbouring conjugative elements, and target them via surface exposed conjugative pili. Therefore, in the presence of these types of viruses, expression of the conjugative machinery tends to be counter-selected. This outcome may result in either bacteria losing the plasmid or in plasmids unable to express their conjugative machinery (Jalasvuori et al., 2011), which in turn greatly reduce the plasmid's frequency in bacterial populations (Ojala et al., 2013). Fertility inhibition plays an important role in plasmid maintenance (Dionisio, 2005), since male-specific phages rarely target their host cells. This is because plasmid de-repression is only transitory.

Another consequence of the expression of the conjugative machinery is lethal zygosis (Alfoldi et al., 1957). This form of lethality occurs due to the cellular contact mediated by *pili* during the conjugative process. Such contacts lead to excessive permeability of the membrane of the recipient cells (Gross, 1963; Skurray and Reeves,

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1973).

Although fertility inhibition proves beneficial, it is still unclear when plasmids should express the conjugative machinery. Some signals that trigger the expression of such machinery have already been identified and are reviewed (Frost and Koraimann, 2010; Koraimann and Wagner, 2014). One of such signals is the presence of recipient cells in the bacterial population, but others exist, such as environmental, nutritional and stressful stimuli. Upon such signals, expression of the conjugative machinery is turned on and plasmid transfer occurs.

Bacteria frequently harbour distinct plasmids (Caugant et al., 1981; Sherley et al., 2003). The question here is: how frequently do these distinct plasmids transfer together? To our knowledge, few studies have addressed this question.

Due to transient de-repression, only a small proportion of plasmid-harbouring bacteria engages in conjugation (for example, plasmid R1 only expresses the conjugative machinery in 1/1000 cells it occupies (Frost and Koraimann, 2010)). If the expression of the conjugative machineries of the two plasmids was activated independently of each other's regulation mechanisms, the probability of simultaneous transmission from the same cell should be the product of the probabilities of de-repression. Considering that only 1/10 to 1/2000 cells engage in conjugation (Frost and Koraimann, 2010), let us consider a cell harbouring two hypothetical plasmids, “A” and “B”. For example, the de-repressed portion of cells carrying plasmids “A” and “B” is respectively 1/100 and 1/1000. Thus, if plasmid transfer is independent, only $1/10^2 \times 1/10^3 = 1/10^5$ of the cells carrying both plasmids would transfer both plasmids simultaneously. This hypothesis led previous authors to speculate that simultaneous transfer of two plasmids from the same donor cell to be a rare event (Romero and Meynell, 1969). Some of their observations agreed with this hypothesis: using strains harbouring two natural conjugative plasmids, they observed that co-transfer rates of the plasmids were identical to the product of individual transfer rates (two out of three cases). They further studied de-repressed plasmid variants and found that, if cells harboured a pair of such plasmids, co-transfer was still independent. They reached the same conclusion when cells harboured simultaneously a repressed and a de-repressed plasmid. For another pair of plasmids, however, the co-transfer rate was low, but not as much as the product of individual transfer rates (one out of three cases). Another study focused on a strain harbouring simultaneously three conjugative plasmids (Bouanchaud and Chabbert, 1969) and showed simultaneous co-transfer of either two or three plasmids to occur more frequently than expected if plasmid transfer events were independent.

These previous studies (Bouanchaud and Chabbert, 1969; Romero and Meynell, 1969), not only differ in their conclusions, but also do not fit with today's knowledge that some specific signals, as mentioned before, are indeed responsible for triggering the expression of the conjugative machinery (Frost and Koraimann, 2010; Koraimann and Wagner, 2014). Such signals could affect both plasmids, causing their simultaneous de-repression. In such scenario, the expression of both conjugative machineries is not independent, thus one may expect the simultaneous transfer of both plasmids to be limited by the plasmid exhibiting the lowest transfer rate. That is, the limiting factor is the plasmid that transfers less.

We aim to understand the rules of plasmid conjugation, and solve the conundrum concerning plasmid co-transmission. Here, we study co-transfer of plasmids using a larger experimental sample, spanning several incompatibility groups. Ultimately, we compare the observed data with the predictions of the above two models.

2. Materials and methods

2.1. Bacterial strains and plasmids

We used *E. coli* K12 MG1655 and *E. coli* K12 MG1655 Δ*ara* (unable to metabolize arabinose), respectively as recipient and donor strains. We used 11 natural conjugative plasmids, with properties summarized in Supp. Table S1. Donor strains harbouring combinations of two and three plasmids were produced in accompanying articles (Gama et al., 2017a,b).

2.2. Co-transfer assays

We inoculated 10^8 total bacteria, in a 1:1 donor: recipient ratio, into a 15 mL tube containing 5 mL of Lysogeny Broth. Tubes were incubated at 37 °C without agitation for 90 min. We diluted the cultures in $MgSO_4$ 0.01 M and plated them in Tetrazolium Arabinose medium to quantify donor and recipient bacteria (respectively red and white colonies due to their differential arabinose metabolism); and in M9 minimal solid medium supplemented with arabinose (0.4%) and suitable antibiotics to quantify transconjugants for two and three plasmids. We calculated the logarithm of conjugation rates (γ) as: $\gamma = log_{10}\left(\frac{T}{\sqrt{D^*R}}\right)$, where D, R and T represent, respectively, the number of donors, recipients and transconjugants per millilitre.

2.3. Models of plasmid co-transfer

We measured the conjugation rates of each plasmid in all plasmid combinations, as well as the co-transfer rate of plasmid pairs and triplets. It should be noted that the conjugation rate of a given plasmid may vary, depending on co-resident plasmid(s), thus differing from its conjugation rate when alone in the host cell (Gama et al., 2017a,b). We calculated the expected values of plasmid co-transfer according to different models. We consider γ1, γ2, γ3 as the respective conjugation rates of plasmids 1, 2 and 3, and γ1:2, γ1:3 and γ2:3 as the co-transfer rates of pairs of plasmids.

2.4. Independent transfer model (ε)

As explained in the introduction, there are some observations in which co-resident plasmids transfer independently of each other. If the expression of the conjugative machinery of co-inhabiting plasmids is activated independently of each other's regulation mechanisms, the rate of co-transfer should be given by the product of the conjugation rates of each plasmid. Since we consider log-transformed conjugation rates, the expected co-transfer rate is calculated as the sum of the logarithm of the conjugation rates of the plasmids:

$\varepsilon = \gamma1 + \gamma2$ or $\varepsilon = \gamma1 + \gamma2 + \gamma3$.

2.5. Least conjugative plasmid model (τ)

Due to some observations of co-transfer rates higher than those predicted if plasmid co-transfer was independent, we propose another model. We propose that the number of recipients receiving both co-resident plasmids is limited by the number of recipients receiving the plasmid exhibiting the lowest conjugation rate. This implies that the expected rate of co-transfer should be equal to the conjugation rate of the lowest conjugative plasmid.

$\tau = \min(\gamma1, \gamma2)$ or $\tau = \min(\gamma1, \gamma2, \gamma3)$.

2.6. Least conjugative pair model (π)

We will see in the results section that when analysing co-transfer of three plasmids, neither model ε nor τ explain the values observed. Since co-transfer of three plasmids logically depends on the co-transfer of two plasmids, co-transfer of three plasmids should be, at most, identical to the rate of co-transfer of the least conjugative pair of plasmids of the combination (in this article we show that the transfer rate of the least conjugative pair is not identical to the transfer rate of the plasmid exhibiting the lowest conjugation rate). Therefore, we propose another model in which the number of recipients receiving the three plasmids is