

# Multiple plasmid interference – Pledging allegiance to my enemy's enemy

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## ABSTRACT

As shown in the previous article, two distinct conjugative plasmids sometimes interact within bacterial cells, implicating changes of transfer rates. In most cases of interactions within bacteria, the transfer of one of the plasmids decreases. Less frequently, the transfer rate of one of the plasmids increases. Here we analyse what happens if three distinct conjugative plasmids colonize the same bacterial cell. Our aim is to understand how interactions between two plasmids affect the transfer rate of the third plasmid. After showing that plasmids interact in 59 out of 84 possible interactions we show that, with some exceptions, if the transfer rate of a plasmid decreases in the presence of a second plasmid, a decrease is also observed in the presence of a third plasmid. Moreover, if the conjugation rate of a plasmid increases in the presence of another, an increase is also observed if there is a third plasmid in the cell. Both types of interactions are mostly independent of the third plasmid's identity, even if sometimes the third plasmid quantitatively distorts the interaction of the other two plasmids. There is a bias towards negative intensifying interactions, which provide good news concerning the spread conjugative plasmids encoding antibiotic-resistance genes and virulence factors.

## 1. Introduction

Interactions between three or more different biological entities often lead to the emergence of new phenomena. Focusing only in the bacterial world, one finds examples in very different contexts such as the ecological effect of colicinogenic bacteria (Kerr et al., 2002), biofilm formation (Mitri et al., 2011; Momeni et al., 2013), pathogenicity in *Salmonella* (Diard et al., 2013) or quorum sensing in *Bacillus subtilis* (Pollak et al., 2016).

Bacterial cells can harbour several plasmids, and they can influence each other employing diverse systems. Exclusion systems prevent host cells from receiving a related plasmid (reviewed in Garcillan-Barcia and de la Cruz, 2008). Incompatibility, due to replication and partition systems, precludes two related plasmids from persisting in the same host cell (reviewed in Novick, 1987). Different plasmids, however, tend to be compatible. Toxin-Antitoxin loci, also known as post-segregational killing (psk) systems consist of a stable toxin and an unstable antitoxin. Host cells die if they lose the plasmid encoding such systems because they require continuous production of the antitoxin to counteract the effect of the stable toxin. During intracellular plasmid competition, psk<sup>+</sup> plasmids displace psk<sup>-</sup> plasmids, otherwise the host cell

dies (Cooper and Heinemann, 2000). Plasmids may also encode fertility inhibition mechanisms, responsible for repressing their own horizontal transfer. Paradoxically, by inhibiting their own transfer, such plasmids prevail in bacterial populations, while plasmids not repressing their own transfer become too costly to their hosts, which leads to their counter-selection (Haft et al., 2009).

Furthermore, plasmids can employ strategies to affect the horizontal transfer of competitor plasmids ((Cascales et al., 2005; Chen and Kado, 1994; Datta et al., 1971; Fong and Stanisich, 1989; Gasson and Willetts, 1975; Gasson and Willetts, 1977; Goncharoff et al., 1991; Hochmannova et al., 1985; Hochmannova et al., 1982; Juhas et al., 2007; Maindola et al., 2014; Miller et al., 1985; Olsen and Shipley, 1975; Pinney and Smith, 1974; Sagai et al., 1977; Santini and Stanisich, 1998; Tanimoto and Iino, 1983; Ward et al., 1991; Willetts and Skurray, 1980; Winans and Walker, 1985; Yusoff and Stanisich, 1984) and companion article (Gama et al., 2017)).

These previous works, however, are insufficient to predict how two plasmids interact with a third one (in this work we define “interaction” as any influence on the transfer rate of a plasmid). For example, considering three plasmids “A”, “B” and “C” where “A” increases the transfer of plasmid “C” and “B” decreases the transfer of “A”, what will

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happen when “C” is simultaneously in the presence of those two plasmids? Will the influence of plasmid “B” towards “A” result in an attenuation of the effect of “A” on “C”? Or will “B” be unaffected because “B” targets a function specific for plasmid “A”? As a second example, suppose that both plasmids “A” and “B” decrease the transfer of plasmid “C”. When the three plasmids occupy the same cell simultaneously, will the effect of “A” and “B” complement each other intensifying the inhibition of the transfer of plasmid “C”?

To understand how two plasmids influence the transfer rate of a third one, we compared the conjugation rates of a given plasmid when alone, in the presence of two plasmids and in the presence of either one of them. With these experiments, we will be able to detect distorting interactions in which the values of the conjugation rate of a given plasmid in the presence of a third plasmid differs from those when only a second plasmid is present. Specifically, among negative and positive interactions, we expected two main types of interactions: attenuating distortions, in which the effect of a plasmid on another is alleviated due to the presence of a third plasmid in the cell, or intensifying distortions, in which the effect of a plasmid on another one is heightened when a third plasmid is present in the cell.

## 2. Materials and methods

### 2.1. Bacterial strains and plasmids

We used the following bacterial strains: *E. coli* K12 MG1655 and *E. coli* K12 MG1655  $\Delta ara$  (unable to metabolize arabinose). We used 11 natural conjugative plasmids, whose properties are summarized in Supp. Table S1.

### 2.2. Generation of plasmid harbouring-strains

We produced a total of 28 strains of *E. coli* K12 MG1655  $\Delta ara$  carrying all possible combinations of three plasmids (not all combinations were possible due to incompatibility and selective markers). These strains resulted from overnight matings between two strains of *E. coli* K12 MG1655  $\Delta ara$  (produced in the accompanying article (Gama et al., 2017)), one carrying a single plasmid and the other carrying two plasmids. Transconjugants were selected in Lysogeny Broth (LB) supplemented with agar (1,5%) and the required antibiotics.

### 2.3. Conjugation assays

After overnight growth at 37 °C with agitation, donor (*E. coli* K12  $\Delta ara$ ) and recipient (*E. coli* K12  $ara^+$ ) strains were inoculated ( $10^8$  total bacteria) in 15 mL tubes containing 5 mL of LB in a ratio of 1:1. Conjugation assays were performed at 37 °C for 90 min without agitation. To quantify donor and recipient bacteria, we plated suitable culture dilutions (in  $MgSO_4$  0.01 M) in Tetrazolium Arabinose (TA) medium, where, due to differences in arabinose metabolism, the donor strain forms red colonies and the recipient strain forms white colonies. To quantify transconjugants, we plated suitable culture dilutions in M9 minimal solid medium supplemented with arabinose (0.4%) and adequate antibiotics. Logarithm of conjugation rates ( $\gamma$ ) was calculated as:  $\gamma = \log_{10} \left( \frac{T}{\sqrt{D \cdot R}} \right)$ , considering D, R and T respectively as the number of donors, recipients and transconjugants per millilitre.

### 2.4. Determination of distorting interactions

Classification of plasmid interactions in triplets (strains carrying three plasmids) follows Supp. Fig. S1 (explained in supplementary information). We use the following definitions:

No-interaction: we considered that the co-resident plasmids did not interact with the analysed plasmid if its conjugation rate was not affected in either the double (strain carrying two plasmids) or in the

triplet.

Non-distorting interactions: these interactions occurred if the effect observed in the triplet was identical to the strongest effect observed in the doubles.

Distorting interactions: an interaction is distorting if the effect observed in the triplet differed from the strongest effect observed in the doubles.

The type of interaction could not be determined if the effect observed in the triplet was simultaneously indistinguishable from the strongest effect observed in doubles and from when the analysed plasmid was alone.

### 2.5. Statistics

Statistical tests were performed in R version 3.2.0, available at <http://www.rstudio.com/> (R Core Team, 2015).

## 3. Results and discussion

### 3.1. To interact or not to interact, that is the question

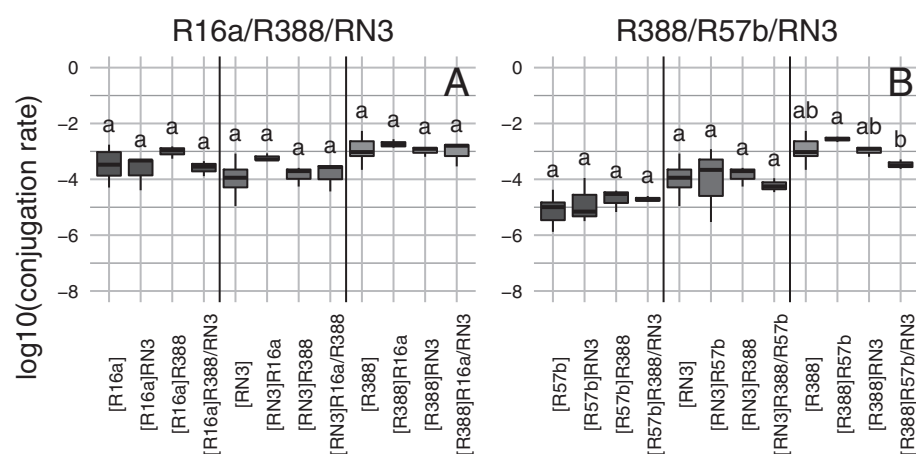
We measured the conjugation rates of each plasmid present in bacteria harbouring three plasmids simultaneously, using a sample of eleven different naturally-occurring plasmids. For each combination of three plasmids, we studied the transfer rate of each plasmid – this allowed us to check for three putative interactions. Indeed, assuming that a combination comprises plasmids “A”, “B” and “C”, we have three possible interactions to consider and three questions. First, how does the interaction between “B” and “C” affect the transfer rate of plasmid “A”? Second, how does the interaction between “A” and “C” affects the transfer rate of plasmid “B”? Third, how does the interaction between “A” and “B” affects the transfer rate of plasmid “C”? Since we analysed 28 combinations of three plasmids, we studied a total of 84 possible interactions. We detected plasmid interactions in 59 of the 84 cases (70,2%). Except for combinations R16a/R388/RN3 and R388/R57b/RN3 (Fig. 1), there are interactions in all the other 26 combinations (Supp. Fig. S2).

Through the analysis of the transfer rates in each combination, one can see that, if a co-resident plasmid was inhibitory towards a given plasmid (negative interaction), it remained inhibitory in the presence of a third plasmid. For instance, in the accompanying article (Gama et al., 2017), we observed inhibition of plasmid RP4 in combinations with another plasmid; now we still observe its inhibition when in the presence of two co-resident plasmids (Supp. Fig. S2I). The reverse is also true: a plasmid increased its conjugation rate in the presence of another (positive interaction), independently of the third plasmid's identity, which is illustrated by R16a in any combination involving either plasmid F or R124 (Supp. Fig. S2E). Despite this general trend, there are some exceptions.

We have seen that, in most cases, the presence of a third plasmid did not alter the qualitative effect of the second plasmid, that is, negative interactions continue to be negative even in the presence of a third plasmid, and positive interactions continue to be positive even in the presence of a third plasmid. Quantitatively, however, there may be some distortion, that is, the values of the conjugation rates in the presence of a third plasmid could differ from those when only a second plasmid was present. To test for distortion events, we compared the conjugation rates of a given plasmid when alone, in the presence of both co-resident plasmids and in the presence of each plasmid. Then, we classified the interactions as intensifying or attenuating, based on the groups resulting from the Tukey multiple-comparison test, as outlined in supplementary information (Supp. Fig. S1).

### 3.2. Distorting interactions: how frequent?

Overall, we observed 59 interactions among the possible 84



**Fig. 1.** Plasmid combinations without interactions. Titles indicate the combination of three plasmids. In these combinations, plasmids do not seem to interact. Analysis for each plasmid are separated by vertical lines. The horizontal axis indicates the combinations of one, two or three plasmids; the analysed plasmid is indicated in brackets. Conjugation rates for combinations of two or three plasmids were measured in triplicate. ND – not detected (no transconjugant colonies in any replicates). PD – partially detected (no transconjugant colonies in some replicates). For the sample sizes of individual plasmids, see accompanying article (Gama et al., 2017). Annotations above the boxes represent the results of Tukey's multiple comparison test; plasmids with different letters are significantly different (p-value < 0.05).

interactions: 20 distorting interactions and 39 non-distorting interactions. Among the 59 observed interactions, we discerned 33.9% of distorting interactions and about twice as much of non-distorting interactions (66.1%). We focused on the prevalence of each type of distorting interaction: positive versus negative and attenuating versus intensifying. Among the 20 distorting interactions, we observed seven attenuating interactions, two of which concerning positive interactions and five concerning negative interactions. The remaining 13 interactions were all negative intensifying interactions. We analysed if the frequencies of these interactions deviated from what would be expected and we found that they did (Barnard's test, two-sided, p-value = 0.04). Thus, this suggests a bias towards negative intensifying interactions. We will now discuss the various observed scenarios: cases of positive and of negative distorting interactions.

### 3.3. Distorting interactions: are they positive?

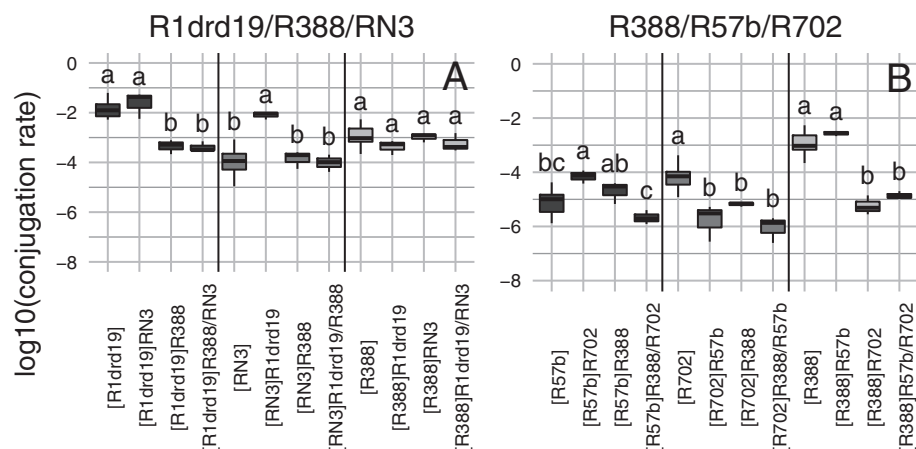
Among the observed distorting interactions, two were positive, which means that the analysed plasmid increased its conjugation rate in the presence of co-resident plasmids. These interactions occurred in the combinations R1drd19/R388/RN3 and R388/R57b/R702 (Fig. 2). For example, in the first combination (R1drd19/R388/RN3), we observed a positive interaction between plasmids R1drd19 and RN3 (Fig. 2A). That is, the conjugation rate of plasmid RN3 was higher when plasmid R1drd19 was the only co-resident plasmid in the host cell. By contrast, the presence of R388 as a third plasmid suppressed this positive interaction, as the conjugation rate of RN3 was no longer different from that observed when RN3 occupied the host cell alone. When only R388 and RN3 inhabited the same host cell, they did not interact; however,

R388 decreased the conjugation rate of plasmid R1drd19 when these two plasmids occupied the host cell simultaneously. Therefore, the suppressive action of R388 on the conjugation rate of RN3 was an indirect effect resulting from the inhibition of R1drd19 by R388, which in turn prevented the positive effect of R1drd19 on RN3. The same reasoning applies to the other plasmid combination.

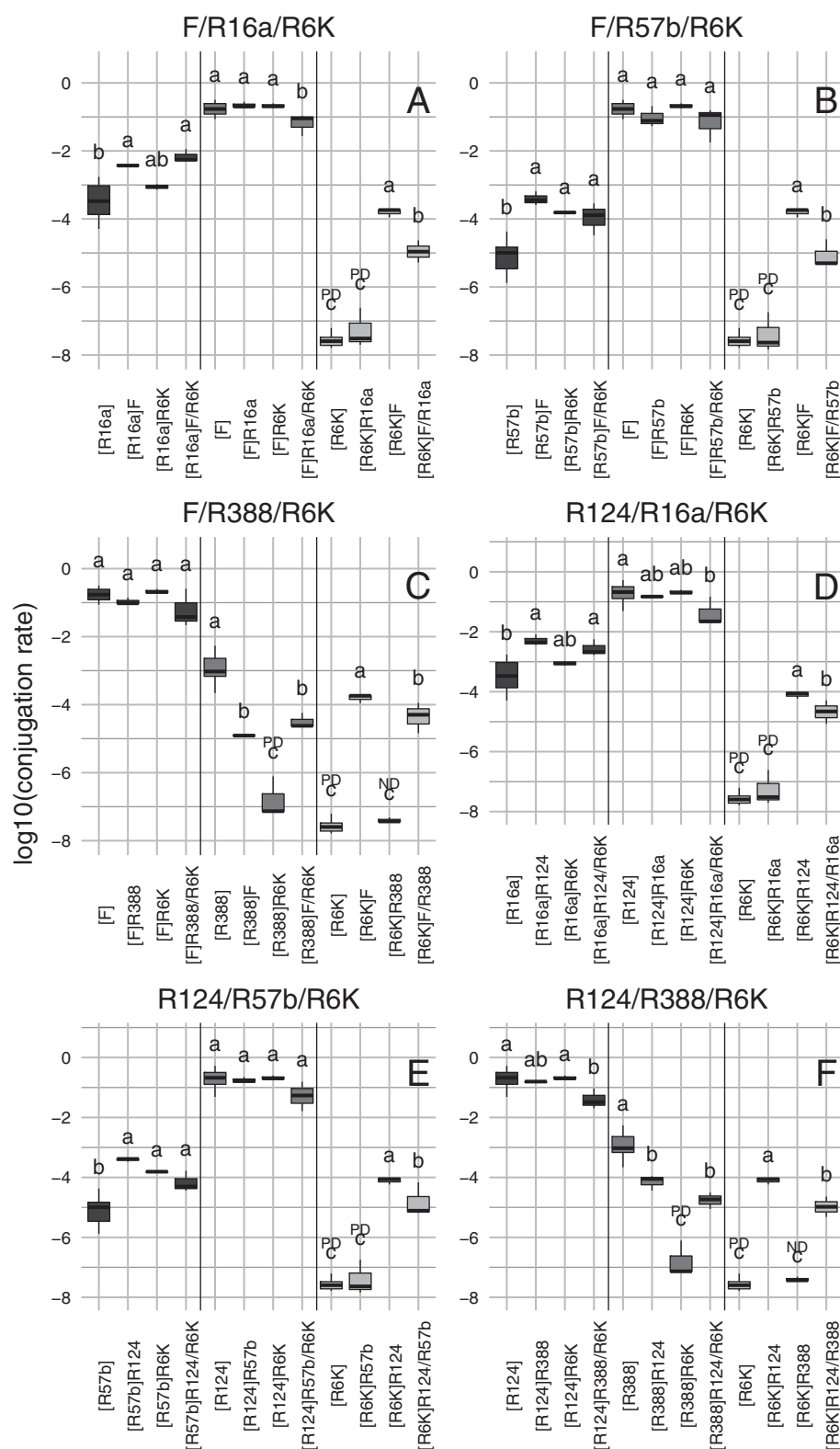
We only considered two cases of positive attenuating interactions, while the six cases involving plasmid R6K (Fig. 3) were instead regarded as non-distorting negative interactions. The conjugation rate of plasmid R6K, when occupying the host cell alone, is near the experimental limit of detection, which made it impossible to recognize any negative interactions targeting R6K. We argue that the lower conjugation rate of plasmid R6K observed in Fig. 3, when co-residing the bacterial cell with another two plasmids, was due to negative interactions that we could not detect previously. Furthermore, we argue that negative interactions are dominant over positive interactions. In the accompanying article (Gama et al., 2017), we showed that plasmids R388 and R1drd19 simultaneously had positive and negative effect on plasmids R1 and RP4, respectively, and that the negative effects prevailed when cells harboured two plasmids. Therefore, we do not consider these six observations involving plasmid R6K as distorting interactions.

### 3.4. Distorting interactions: how low can plasmids go?

We will now focus on negative interactions, that is, inhibitory interactions that result in decreased conjugation rates. We observed 18 negative distorting interactions (shown in Figs. 3, 4 and 5). We can see in Fig. 3C, F and Fig. 4 that plasmid R388 is the target of distorting



**Fig. 2.** Plasmid combinations exhibiting distorting positive interactions. Positive interactions are those in which the conjugation rate of the analysed plasmid increases in the presence of at least one of the co-resident plasmids. Attenuating positive interactions are those in which a conjugation rate increases in the presence of a co-resident plasmid but not of both co-resident plasmids. Explanation of this figure as in Fig. 1.



**Fig. 3.** Distorting interactions regarded as non-distorting negative interactions. These are the interactions in which the positive effect directed to the analysed plasmid decreased when this plasmid shared the cell with two other plasmids. Alternatively, these interactions can be regarded as non-distorting negative interactions because they could represent inhibitory effects, which we did not detect in the accompanying article (Gama et al., 2017) due to experimental limitations. Explanation of this figure as in Fig. 1.

negative interactions in all these five combinations.

In the combinations F/R388/R6K and R124/R388/R6K, there is inhibition of plasmid R388 by both the other two plasmids of the combination (Fig. 3C, F). When plasmid R388 co-resided with plasmid R6K, the latter provided a strong inhibition over R388. If either F or R124 was also present in the cell, the inhibitory effect of R6K was not so strong. These are interesting cases of negative interactions because the

overall inhibition of plasmid R388 when either F or R124 co-resided with R6K was similar to the inhibitory power of plasmids when either F or R124 were alone with R388. Therefore, the inhibition by plasmid R6K became negligible. However, one cannot infer that the inhibition by plasmid R6K was totally suppressed since it could only be reduced to a level equal or less than the level of the inhibition provided by the other co-resident plasmid.

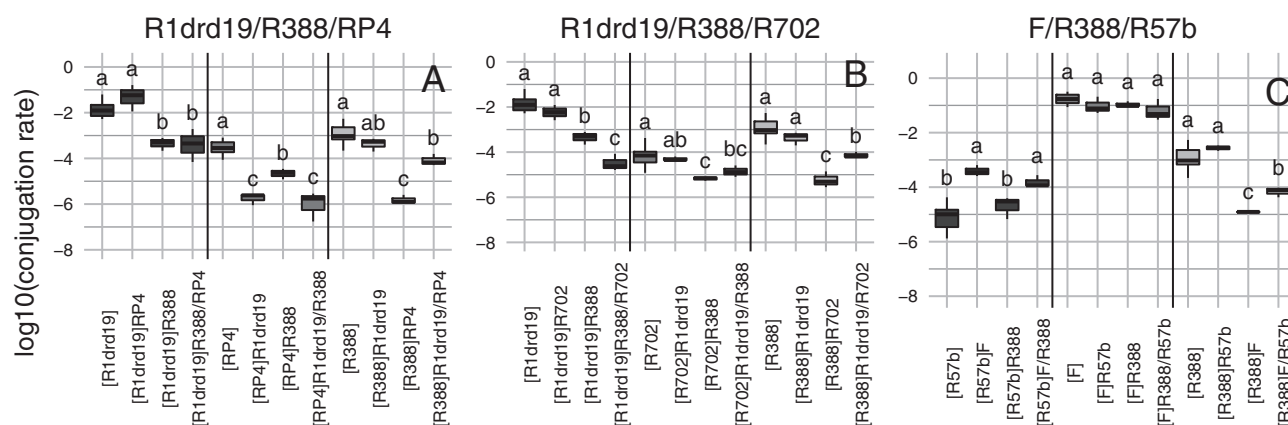


Fig. 4. Plasmid combinations exhibiting distorting attenuating negative interactions. Negative interactions are those in which the conjugation rate of the analysed plasmid decreases in the presence of at least one of the co-resident plasmids. These interactions are classified as attenuated when the inhibitory effect (on the analysed plasmid) exerted by only one co-resident plasmid is stronger than the overall effect exerted by the two co-resident plasmids. Explanation of this figure is as in Fig. 1.

On the three combinations shown in Fig. 4, only one of the co-resident plasmids inhibited plasmid R388. However, there was attenuation of the inhibition of R388 when all three plasmids were present simultaneously in the same host cell. This suggests that the third plasmid partially blocked the inhibitory effect inflicted on R388.

The five combinations mentioned above, as the previous three combinations concerning positive interactions, illustrate attenuating interactions, which means that there is alleviation of the effect of a plasmid on another. Conversely, other combinations (Figs. 3A, D, E, 4B and 5), involve intensifying interactions, that is, the overall effect is strengthened. For instance, one can observe that in both combinations R1/R388/R702 and R1drd19/R388/R702 the interaction between plasmids R702 and R388 magnifies the inhibitory effect of R388 towards either R1 and R1drd19, although plasmid R702, per se, did not inhibit either of the two plasmids in the absence of R388 (Figs. 5F and 4B).

One can also observe complementation of inhibitory mechanisms, which results in intensifying negative interactions. That is, when both co-resident plasmids inhibit the analysed plasmid, the overall inhibitory effect increases. This differs from the previous cases where only one of the plasmids inhibited the analysed plasmid, although the effect could be somewhat intensified. Focusing on the intensified inhibition of plasmid RP4, we can observe complementation between the mechanisms of inhibition encoded by plasmids R57b, R6K and R388, for the overall inhibition caused by two co-resident plasmids was stronger than the individual inhibitory effects (Fig. 5A–C).

### 3.5. Noisy interactions

As discussed above, for several combinations of three plasmids, one can find a reasonable explanation for the observed distorting interactions. Some cases, however, seem to be more complex, such as the intensifying inhibition of plasmids F and R124 (Fig. 3A, D, and F). Results (Figs. 3 and 4C, Supp. Fig. S3 A–C) show that neither F nor R124 were inhibited in cells harbouring only two plasmids. By comparing the conjugation rates when these plasmids inhabited the host cell with two co-resident plasmids with those when no co-resident plasmid existed (Supp. Fig. S2), we detected no inhibitory interactions. However, according to Fig. 3A, D and F, there is inhibition of plasmids F and R124. Therefore, the inhibitory effect on these plasmids was very small, since its detection was not consistent. Since observations of such interactions are not consistent, they may result from stochastic experimental fluctuations. These facts prompt us to speculate that a destabilizing phenomenon arises as the number of different plasmids present in the host cell increases. Such unspecific interactions between the plasmids would provide the stochastic noise observed. Furthermore, considering the

near-identical plasmids R1 and R1drd19, one should expect plasmid R1drd19 to be less affected by these fluctuating interactions. The reason is that R1drd19 has a higher level of expression of conjugation genes, eventually buffering the stochastic noise (Koraimann et al., 1991). Differences observed between the combinations R1/R388/RN3 and R1drd19/R388/RN3 could reflect such hypothesis (Figs. 2A and 5G).

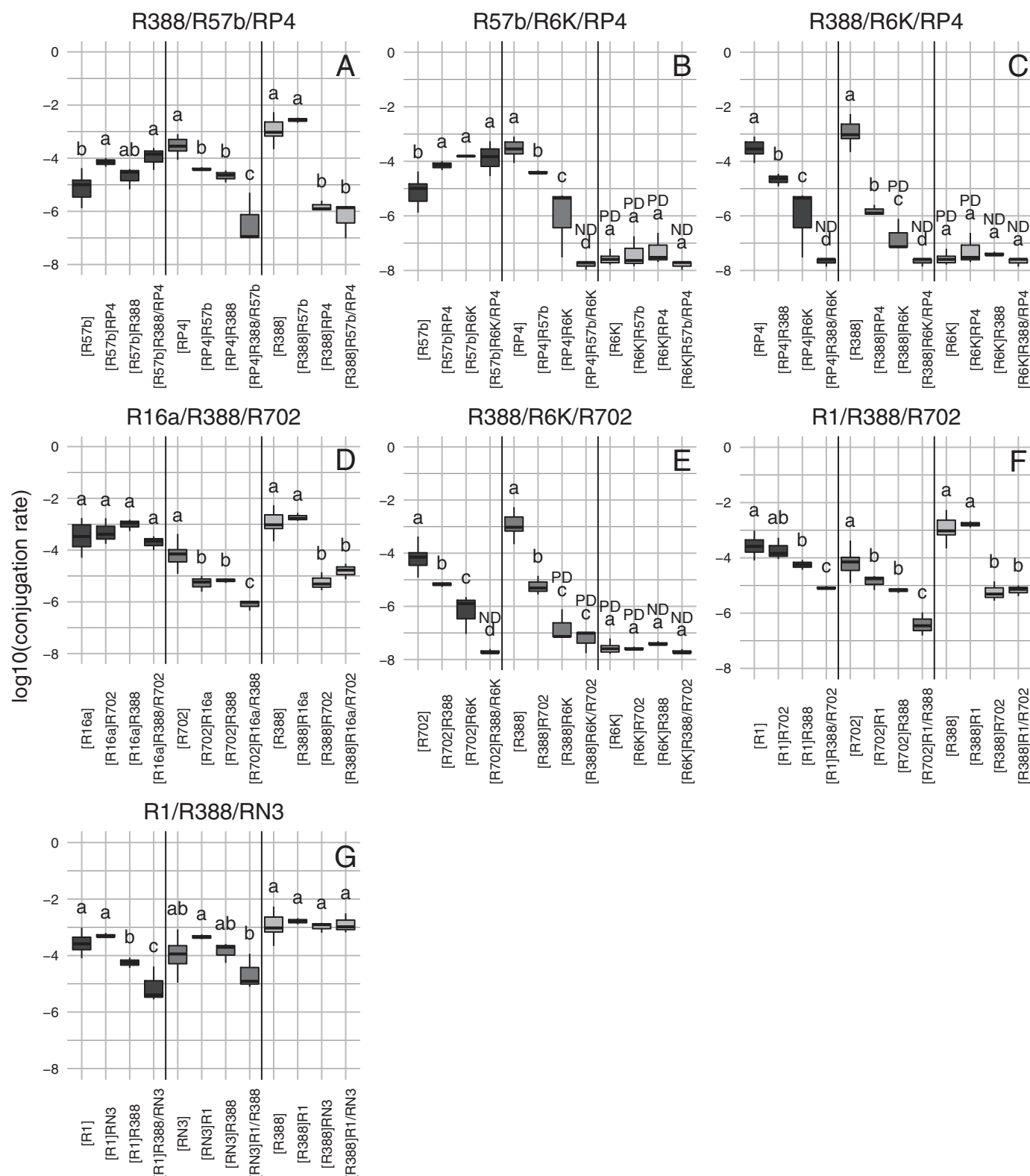
## 4. Conclusions

In this work, we show that plasmids interact in most of the combinations of three plasmids. Among 84 possible interactions, we detected interactions in 59 of them. We analysed if the interactions between two plasmids would influence the transfer rate of a third co-infecting plasmid. We termed such kind of interactions as distorting interactions and in a total of 59 interactions we detected 20 cases, approximately 1/3 of the interactions. We further categorized distorting interactions as intensifying or attenuating. Most (13 cases) of the observed distorting interactions were intensifying, representing about 2/3 of all distorting interactions. Furthermore, all intensifying interactions exerted an inhibitory effect on a target plasmid, showing that inhibitory mechanisms complement each other. Given the high proportion of intensifying inhibitory interactions it seems that mechanisms were selected not only to decrease a rival plasmid's transmission but also to have the ability to enhance inhibitory mechanisms encoded by other plasmids. This would be a good strategy if plasmids were selected to decrease the transmission of other plasmids present in the same cell. Another good strategy for a plasmid would be to attenuate the positive effect of other plasmids, which we observe in the two cases of attenuating positive interactions. These two cases and the above 13 cases illustrate situations resulting in reduced plasmid transmission. In the context of public health, these are good news suggesting the potential to decrease of the dissemination of (multiple) antibiotic-resistance determinants and virulence factors encoded in conjugative plasmids.

However, among the cases of distortions, five do not follow strategies to enhance the inhibition nor to attenuate positive effects of co-inhabiting plasmids. These are the cases of attenuating negative interactions. In such cases plasmids inhibit the transfer rate of a target plasmid, but in the presence of a third plasmid, this inhibitory effect is not so strong, thus alleviating the effect on the target plasmid. This would be a worrisome scenario for the antibiotic-resistance issue if attenuation was 100% effective. However, in all these five cases, attenuation is not that strong which means that the target plasmid is still inhibited. Thus, these bad news are not as bad as they could be.

In this work, we show that the outcome of the interactions between three co-inhabiting plasmids is non-trivial, meaning that it is not





**Fig. 5.** Plasmid combinations exhibiting distorting intensifying negative interactions. Negative interactions are those in which the conjugation rate of the analysed plasmid decreases in the presence of at least one of the co-resident plasmids. These interactions are classified as intensifying when the overall inhibitory effect (on the analysed plasmid) exerted by the two co-resident plasmids is stronger than the individual effect of any of the co-resident plasmids. Explanation of this figure as in Fig. 1.

predictable. For instance, when two plasmids inhibit a third plasmid, we observed three different possible outcomes: attenuated inhibition, intensified inhibition and non-distorted inhibition.

It is yet important to note that the number of interactions might have been underestimated because some conjugation rates fell under

the experimental limit of detection, making it impossible to detect negative interactions. Nonetheless, we can conclude that intensifying distorting interactions tend to be negative. Such a bias, lead us to conclude that these inhibitory mechanisms may complement each other, resulting in stronger inhibition. This points towards interference

competition in triple plasmid carriage.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.plasmid.2017.08.002>.

## References

- Cascales, E., et al., 2005. *Agrobacterium tumefaciens* oncogenic suppressors inhibit T-DNA and VirE2 protein substrate binding to the VirD4 coupling protein. *Mol. Microbiol.* 58, 565–579.
- Chen, C.Y., Kado, C.I., 1994. Inhibition of *Agrobacterium tumefaciens* oncogenicity by the *osa* gene of pSa. *J. Bacteriol.* 176, 5697–5703.
- Cooper, T.F., Heinemann, J.A., 2000. Postsegregational killing does not increase plasmid stability but acts to mediate the exclusion of competing plasmids. *Proc. Natl. Acad. Sci. U. S. A.* 97, 12643–12648.
- Core Team, R., 2015. R: A Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Datta, N., et al., 1971. Properties of an R-factor from *Pseudomonas aeruginosa*. *J. Bacteriol.* 108, 1244–1249.
- Diard, M., et al., 2013. Stabilization of cooperative virulence by the expression of an avirulent phenotype. *Nature* 494, 353–356.
- Fong, S.T., Stanisich, V.A., 1989. Location and characterization of two functions on RP1 that inhibit the fertility of the IncW plasmid R388. *J. Gen. Microbiol.* 135, 499–502.
- Gama, J.A., 2017. Conjugation efficiency depends on intra and intercellular interactions between distinct plasmids: plasmids promote the immigration of other plasmids but repress co-colonizing plasmids. *Plasmid* 135 <http://dx.doi.org/10.1016/j.plasmid.2017.08.003>. (in press).
- Garcillan-Barcia, M.P., de la Cruz, F., 2008. Why is entry exclusion an essential feature of conjugative plasmids? *Plasmid* 60, 1–18.
- Gasson, M.J., Willetts, N.S., 1975. Five control-systems preventing transfer of *Escherichia coli* K-12 sex factor F. *J. Bacteriol.* 122, 518–525.
- Gasson, M.J., Willetts, N.S., 1977. Further characterization of F fertility inhibition systems of unusual Fin<sup>+</sup> plasmids. *J. Bacteriol.* 131, 413–420.
- Goncharoff, P., et al., 1991. Structural, molecular, and genetic-analysis of the *kilA* operon of broad-host-range plasmid RK2. *J. Bacteriol.* 173, 3463–3477.
- Haft, R.J.F., et al., 2009. Competition favours reduced cost of plasmids to host bacteria. *ISME J.* 3, 761–769.
- Hochmannova, J., et al., 1982. Molecular and genetic properties of plasmid R 485 conferring resistance to sulfonamides. *J. Gen. Microbiol.* 128, 529–537.
- Hochmannova, J., et al., 1985. New replication mutant pNH602 and its relationship to plasmid pAs3, another deletion derivative of plasmid R6K. *Folia Microbiol.* 30, 407.
- Juhas, M., et al., 2007. Sequence and functional analyses of *Haemophilus spp.* genomic islands. *Genome Biol.* 8 (R237).
- Kerr, B., et al., 2002. Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. *Nature* 418, 171–174.
- Koraimann, G., et al., 1991. Repression and derepression of conjugation of plasmid R1 by wild-type and mutated *finP* antisense RNA. *Mol. Microbiol.* 5, 77–87.
- Maindola, P., et al., 2014. Multiple enzymatic activities of ParB/Srx superfamily mediate sexual conflict among conjugative plasmids. *Nat. Commun.* 5, 5322.
- Miller, J.F., et al., 1985. F-factor inhibition of conjugal transfer of broad-host-range plasmid RP4 - requirement for the protein product of *pif* operon regulatory gene *pifC*. *J. Bacteriol.* 163, 1067–1073.
- Mitri, S., et al., 2011. Social evolution in multispecies biofilms. *Proc. Natl. Acad. Sci. U. S. A.* 108 (Suppl. 2), 10839–10846.
- Momeni, B., et al., 2013. Strong inter-population cooperation leads to partner intermixing in microbial communities. *elife* 2, e00230.
- Novick, R.P., 1987. Plasmid incompatibility. *Microbiol. Rev.* 51, 381–395.
- Olsen, R.H., Shipley, P.L., 1975. RP1 properties and fertility inhibition among P-incompatibility, N-incompatibility, W-incompatibility, and X-incompatibility group plasmids. *J. Bacteriol.* 123, 28–35.
- Pinney, R.J., Smith, J.T., 1974. Fertility inhibition of an N group R factor by a group X R-factor, R6K. *J. Gen. Microbiol.* 82, 415–418.
- Pollak, S., et al., 2016. Facultative cheating supports the coexistence of diverse quorum-sensing alleles. *Proc. Natl. Acad. Sci. U. S. A.* 113, 2152–2157.
- Sagai, H., et al., 1977. Inhibition and facilitation of transfer among *Pseudomonas aeruginosa* R plasmids. *J. Bacteriol.* 131, 765–769.
- Santini, J.M., Stanisich, V.A., 1998. Both the *fipA* gene of pKM101 and the *pifC* gene of F inhibit conjugal transfer of RP1 by an effect on *traG*. *J. Bacteriol.* 180, 4093–4101.
- Tanimoto, K., Iino, T., 1983. Transfer inhibition of RP4 by F-factor. *Mol. Gen. Genet.* 192, 104–109.
- Ward, J.E., et al., 1991. Activity of the *Agrobacterium* T-DNA transfer machinery is affected by *virB* gene-products. *Proc. Natl. Acad. Sci. U. S. A.* 88, 9350–9354.
- Willetts, N., Skurray, R., 1980. The conjugation system of F-like plasmids. *Annu. Rev. Genet.* 14, 41–76.
- Winans, S.C., Walker, G.C., 1985. Fertility inhibition of RP1 by IncN plasmid pKM101. *J. Bacteriol.* 161, 425–427.
- Yusoff, K., Stanisich, V.A., 1984. Location of a function on RP1 that fertility inhibits IncW plasmids. *Plasmid* 11, 178–181.