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Plasmid Persistence: Costs, Benefits and the Plasmid Paradox

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Abstract

Plasmids are extrachromosomal DNA elements that can be found throughout bacteria, as well as in other domains of life. Nonetheless, the evolutionary processes underlying the persistence of plasmids are incompletely understood. Bacterial plasmids may encode genes for traits which are sometimes beneficial to their hosts, such as antimicrobial resistance, virulence, heavy metal tolerance, and the catabolism of unique nutrient sources. In the absence of selection for these traits, however, plasmids generally impose a fitness cost on their hosts. As such, plasmid persistence presents a conundrum: models predict that costly plasmids will be lost over time, or that beneficial plasmid genes will be integrated into the host genome. However, laboratory and comparative studies have shown that plasmids can persist for long periods, even in the absence of positive selection. Several hypotheses have been proposed to explain plasmid persistence, including host-plasmid co-adaptation, a hitchhiking, cross-ecotype transfer, and high plasmid transfer rates, but there is no clear evidence that any one model adequately resolves the plasmid paradox.

Keywords: Plasmid, evolution, antibiotic resistance, compensatory evolution, epistasis

38 **Introduction:**

39 Bacterial plasmids are generally small (average 80 kb) circular extrachromosomal DNA
40 elements that are capable of semi-autonomous replication through the recruitment of host cell
41 machinery (Sherratt 1974; Kado 2014; Shintani et al. 2015). Plasmids do not typically encode
42 essential genes required by the host, but instead carry genes which may help the host adapt to
43 novel environments, such as in the case of antibiotic exposure or alternative nutrient sources (Lili
44 et al. 2007; Slater et al. 2008; Rankin et al. 2011). In addition, plasmids may carry genes
45 encoding plasmid-specific functions, such as self-replication, partitioning, and conjugative
46 transfer (Bennett 2008). Despite the obvious benefit of plasmid-borne genes and traits under
47 some circumstances, the reasons underlying continued plasmid persistence – as opposed to loss
48 or integration – are incompletely understood; the puzzle posed by plasmid persistence has been
49 coined ‘the plasmid paradox’ by Harrison and Brockhurst (2012). Plasmid loss due to purifying
50 selection would be expected when the costs of plasmid carriage outweigh the benefits. By
51 contrast, integration would be expected for beneficial plasmid genes, minimizing random loss
52 during cell division. In this review, we provide an overview of the benefits and costs of plasmid
53 carriage, and discuss the possible reasons for the continued persistence of plasmids.

54

55 **Benefits of Plasmid Carriage**

56 The range of possible explanations for continued plasmid persistence has two extremes.
57 At one end, plasmids exist entirely as selfish entities, only concerned in their continued survival
58 (Bergstrom et al. 2000; Slater et al. 2008). At the other, plasmids can confer some unconditional
59 benefit on their bacterial hosts. For the most part, neither extreme is likely to hold true - many

plasmids are not unconditionally beneficial, insofar as they often confer costs on their hosts (see below). Similarly, transfer rates of most conjugative plasmids are likely not high enough for persistence as purely selfish elements, and of course non-conjugative plasmids are not readily transferred on their own (Bergstrom et al. 2000). Thus, we expect that most plasmids must be beneficial to their hosts – at least some of the time – to be maintained in populations.

Plasmid-encoded benefits are typically related to survival in novel environments and conditions, with many plasmids encoding genes for antibiotic resistance, virulence, tolerance to heavy metals, or for the metabolism of unique carbon sources, as well as nitrogen fixation, plant gall formation, and root nodulation (Masterson et al. 1982; Eberhard 1989; Nies 1999; Beceiro et al. 2013; Ramirez et al. 2014; Pal et al. 2015). Not only can plasmid-encoded genes provide a potential benefit to their host, they may influence both the environment and neighbouring cells, for example through production of helpful enzymes or nutrients, or through production of harmful chemicals (Rankin et al. 2011). Plasmids can thus be a source of genes that help the host adapt to novel environments (Eberhard 1989).

Resistance to antibiotics is one of the better studied benefits conferred by plasmids, and one that is increasingly relevant in clinical settings. Plasmids carry resistance genes to most major classes of antibiotics, with genes for protection against aminoglycosides, beta-lactams, and tetracyclines most commonly found (Bennett 2008; Pal et al. 2015). Plasmid-encoded resistance occurs via the same mechanisms as resistance caused by chromosomal mutations: by altering the permeability of the cell membrane, deactivation or modification of the antibiotic, and the modification of drug targets (Bennett 2008; Blair et al. 2015). Carbapenemases, for example, which include β -lactamases from classes A, B, and D, are now commonly found on plasmids, and these enzymes work by hydrolyzing, and thus inactivating, carbapenem antibiotics (Blair et

al. 2015). Over and above issues with single resistance genes, multidrug-resistant (MDR) plasmids are increasingly common. For example, it was found that 40% of plasmids containing quinolone resistance genes that were isolated from strains obtained from dogs and cats with UTIs confer MDR, with additional resistances to tetracycline, sulphonamides or beta-lactams frequently observed (Liu et al. 2012; Poirel et al. 2012). The rapid dissemination of MDR plasmids by horizontal gene transfer complicates both the treatment of infections and public health measures to control resistance.

Deleterious Effects of Plasmid Carriage

While under particular conditions many plasmid-borne traits confer a benefit to their hosts, the wider fitness effects of plasmids are not always clear. For example, a plasmid may be beneficial when its bacterial host is exposed to a novel stressor, but detrimental once the stressor is removed (Vogwill and MacLean 2015). Under these conditions, it is expected that bacterial strains lacking the costly plasmid outcompete the plasmid-bearing strain due to the fitness cost of plasmid carriage, and this cost may be a result of any one of a number of proposed mechanisms (Enne et al. 2004; San Millan and MacLean 2017). Thus, costly plasmids are expected to be lost from a population due to competition with plasmid-free cells. For instance, the non-transmissible plasmid pNUK73 in *P. aeruginosa* is costly to its host compared to its plasmid-free ancestor, and does not readily persist in the absence of selection (San Millan et al. 2014b).

Many studies have suggested that carriage of AMR plasmids is generally costly to the host in the absence of antibiotic, although some evidence suggests this cost can be reduced over time (Dahlberg and Chao 2003; Dionisio et al. 2005; Starikova et al. 2013; San Millan et al.

2014; Johnson et al. 2015; Vogwill and MacLean 2015; Porse et al. 2016; Di Luca et al. 2017). This trend holds across plasmid types and bacterial species (Table 1), with fitness reductions ranging from 1-28%. Non-AMR plasmids also often incur a cost (Hall et al. 2015). While plasmid carriage is typically costly, Vogwill and MacLean (2015) noted that the fitness costs associated with plasmids are generally less than the costs associated with chromosomal resistance mutations, suggesting that plasmid-borne resistance may sometimes be preferred to chromosomal resistance. However, plasmids by no means always confer fitness costs. For example, a pCT plasmid encoding an extended-spectrum- β -lactamase (ESBL) gene in *E. coli* strains SW103 and J-53, as well as *Salmonella enterica* serovar Typhimurium SL1344, showed no measurable decrease in fitness, whereas a sulphonamide resistance-encoding plasmid p9123 increased the fitness of its *E. coli* host by $3.3 \pm 1.5\%$ in the absence of selection (Enne et al. 2004; Cottell et al. 2012)

Host genetic background may be an important determinant of plasmid fitness. That is, a single plasmid could decrease fitness in a strain with a certain genetic background, produce no noticeable fitness cost in another, and be beneficial in another strain (Table 2). For example, Humphrey et al (2012) found that the plasmid RP1, when tested on five unique *E. coli* strains, had variable fitness effects, suggesting that the fitness cost of a plasmid is dependent on host genotype ((Humphrey et al. 2012); Figure 1). Indeed, Vogwill and MacLean indicate in a recent meta-analysis (2015) that both host genetic background and plasmid have some influence on the cost of carriage. Interestingly, such epistatic interactions do not appear to be limited to interactions between a plasmid and its host, but can also include interactions between multiple plasmids in a single host strain (San Millan et al. 2014a). Here, two costly plasmids, a low-cost

conjugative plasmid and a high-cost resistance plasmid, showed less severe fitness decreases when present together, than were observed for each plasmid alone.

Mechanisms Underlying the Cost of Plasmid Carriage

While costs of plasmid carriage have been described widely, the mechanisms responsible for those costs are largely unclear. Several possible explanations have been proposed, ranging from the metabolic load introduced by plasmid replication and the expression of plasmid-borne genes, to the disruption of essential host genes by the integration of plasmid genes, alteration in host gene expression, and other metabolic effects, such as the introduction of novel efflux pumps which may pump out important biomolecules (Pacheco et al. 2017; San Millan et al. 2017).

Here, a brief overview of each mechanism will be provided, but see San Millan and MacLean (2017) for an extensive review on the topic. The metabolic burden of plasmid carriage can come from the replication of plasmid genes. For example, replication of either the entire plasmid or specific plasmid genes diverts resources, such as biomolecules, machinery, or energy-rich compounds, away from the pool set aside for the replication of the host genome, which may stress the cell and reduce fitness (Baltrus 2013; San Millan and MacLean 2017). Consistent with this hypothesis, some studies have indicated that the deletion of large portions of a plasmid, which may also be associated with the loss of an antibiotic resistance marker, reduce fitness costs associated with those plasmids (Porse et al. 2016).

However, the expression of these same genes may also play a role in the resultant fitness cost associated with plasmid carriage, and is thought to perhaps be the most likely candidate for plasmid-associated fitness costs (San Millan and MacLean 2017). Expression of plasmid-borne

genes can negatively impact fitness due to sequestration of ribosomes and issues stemming from codon usage and limited tRNA supplies. For example, a tetracycline resistance gene has been shown to induce fitness costs associated with the non-conjugative plasmid pBR322, and *van* genes have been identified as the main cause of fitness costs associated with some vancomycin resistance plasmids in *Enterococcus* (Lee and Edlin 1985; Valenzuela et al. 1996; Foucault et al. 2010). Similarly, it has been suggested that production of the conjugative machinery of the T4SS system can contribute to fitness costs (Koraimann and Wagner 2014). Both the expression and the assembly of this system can induce stress responses in *E. coli*, which can in turn interfere with the host and impose a fitness cost (Zarhl et al. 2006). Nonetheless, it is important to note that non-conjugative plasmids – which lack transfer machinery – are also typically costly. Given that half of all plasmids are thought to be non-transmissible, but still are costly to their hosts, conjugation machinery cannot be the only contributor to fitness deficits (San Millan et al. 2014b).

Changes to the expression of host-encoded genes can also influence the fitness costs associated with plasmids. Indeed, there is good evidence that it is the mis-expression of chromosomal genes that is responsible for fitness costs associated with pQBR103 in *Pseudomonas fluorescens*: about one fifth of chromosomal genes are mis-regulated following plasmid uptake, and compensation is accompanied by restoration of normal expression levels (Harrison et al. 2015). Here, mutations to *gacA/gacS*, genes responsible for the global regulatory system, resulted in the decreased expression of about 17% of both plasmid and chromosomal genes. Inhibition of these genes by mutations to *gacA/gacS* likely results in a decreased translational demand on the cell, reducing the cost of the plasmid, as the mutations result in the sustained inhibition of post-translational modification of RNA-binding proteins.

Other more specific mechanisms have been proposed to explain the fitness costs associated with plasmid carriage. These include the integration of plasmids into the host chromosome, interference of plasmid genes and proteins with the host's normal functioning, as well as the efflux of important host proteins (Helling et al. 1981; Harrison and Brockhurst 2012; Hong et al. 2014; San Millan et al. 2015). Integration can induce fitness costs as a result of the plasmid integrating into and interrupting important host genes (Jackson et al. 2011; Rankin et al. 2011; San Millan et al. 2015; Wu et al. 2015). Costs associated with plasmids may also result from the interference of plasmid-encoded elements with host structure and function; for example, plasmid-encoded molecules may interact negatively with the host's native proteins or be responsible for initiating processes like replication or protein expression, resulting in changes to host function and resulting in fitness decreases (San Millan and MacLean 2017).

Plasmid Loss by Segregational Loss and Integration

Even in cases where plasmid carriage is not costly, plasmids are expected to slowly be lost from a population due to segregational loss, whereby a plasmid is lost by chance during cell division. Moreover, for plasmids carrying beneficial functions, a particular plasmid-borne gene can be integrated into the host chromosome and the rest of the element removed, or the entire element may be integrated, so that there is no segregational loss. Both of these processes can lead to population-wide plasmid loss over time.

Segregational instability is the term used to describe plasmid loss which occurs during cell division, whereby plasmids are not accurately segregated between daughter cells, resulting in plasmid-free individuals (Popov et al. 2009). This is of concern for low-copy number plasmids,

where fewer plasmids are present in the host. Since this loss may be problematic to the continued survival of the plasmid, specific mechanisms have evolved to ensure accurate plasmid distribution between cells, such as *parI* and *parII*, which are part of the active partitioning systems for some plasmids (Million-Weaver and Camps 2014). However, if these systems are inefficient and segregational loss does occur frequently enough, even in the face of such mechanisms, and this rate is lower than that of horizontal transfer, plasmid loss within the population is the expected outcome in the absence of plasmid selection (Hernández-Arriaga et al. 2014; San Millan et al. 2015). Estimates of the rate of segregational loss vary widely. For example, a segregational loss rate of 10^{-3} per cell per generation was estimated for an R1 plasmid derivative, which was much less frequent than a previous estimate of 1-5% per cell and generation found in other studies (Lau et al. 2013). Higher rates were estimated for the pACY184 plasmid in *E. coli* B, at approximately 0.02 per cell per generation without selection, and 0.01 per cell per generation with a certain level of selection (Lenski and Bouma 1987).

Given that plasmids are at risk for loss over time, mechanisms have evolved that reduce the frequency of plasmid-free bacteria in a population. One such example is that of the post-segregational killing (PSK) system, which kills individual cells which lack the plasmid (Hernández-Arriaga et al. 2014). This is especially important for plasmids maintained at low-copy numbers (Dmowski and Jagura-Burdzy 2013). PSK, which can also be referred to as the toxin-antitoxin system (TA), works by either removing cells without plasmids or by increasing plasmid replication to ensure copy number maintenance (Hayes and Van Melderren 2011; Dmowski and Jagura-Burdzy 2013). These systems are typically arranged so that there is a chromosomally-encoded toxin which is constantly produced by the cell under normal conditions, and the plasmid-encoded antitoxin is also expressed, interfering with or inactivating the toxin

(Brendler et al. 2004; Hernández-Arriaga et al. 2014). However, if the plasmid is accidentally lost during a cell division event, that antitoxin is no longer produced, whereas the toxin is still expressed, which then can either severely inhibit growth of, or kill, the plasmid-free host. Nevertheless, plasmid loss is still observed in systems with an active PSK system (San Millan et al. 2014b). Additionally, observations of TA and PSK showed that plasmid levels remained steady regardless of whether the TA genes were encoded on the plasmid or on the host chromosome, suggesting that the TA system may have evolved to prevent colonization of other plasmids, rather than to intrinsically improve plasmid stability (Hernández-Arriaga et al. 2014). Moreover, as Harrison and Brockhurst (2012) note, there are examples of plasmids persisting in the absence of these TA systems, so these systems must not be the sole reason for plasmids remaining in their hosts.

The importance of segregational loss may be in part determined by plasmid size and copy number. Small high-copy number plasmids (up to 100kb in size, and >10 copies/cell) are generally thought to be randomly distributed between daughter cells during division, as they lack adequate partitioning and PSK mechanisms, though there is some evidence of plasmid localization at the poles of dividing cells to aid in partitioning (Million-Weaver and Camps 2014; San Millan et al. 2016; Wang 2017). Because of their higher copy numbers, chances are low that either daughter cell will be plasmid-free. This mode of plasmid partitioning may facilitate adaptive evolution, as the genes on these plasmids also exist in high copy number in the cell. This increases the chance of gaining beneficial mutations, and depending on the number of copies divided amongst the cells, selection may act more or less efficiently on the mutated copies (San Millan et al. 2016).

Unlike small, high-copy number plasmids, larger, lower-copy number plasmids require specific mechanisms to ensure propagation of the plasmid, such as the tight regulation of replication and copy number control, and frequently encode toxin-antitoxin systems (Carattoli 2013; Tsang 2017). Carrying beneficial genes, such as antibiotic resistance genes, can provide a basis of plasmid selection, increasing maintenance of the plasmid and making it more likely that the plasmid may coevolve with the host over time (Stalder et al. 2017). The presence of these sometimes-beneficial genes increases the survivability of plasmids because they may be more readily expressed on a plasmid than on the chromosome, and having these benefits can increase the niche range of the host, mitigating fitness costs (Eberhard 1989; San Millan et al. 2014b). Beyond this, however, strategies employed by the plasmid can increase persistence. For example, large conjugative resistance plasmids are thought to have three distinct coevolutionary patterns that act to increase persistence, which include mutations to replication genes, mutations to the global transcriptional regulatory system of the host, as well as the acquisition of a toxin-antitoxin encoding transposon from another plasmid with an established system (Porse et al. 2016). Porse et al. (2016), for example, found that persistence was accompanied by changes in the plasmid backbone (i.e. deletions of costly portions of the plasmid), the acquisition of a toxin-antitoxin transposon, and changes to the pattern of transferability, in order to improve the vertical, rather than horizontal, inheritance of the element.

Non-transmissible plasmids, as they generally lack the ability to persist through high transfer rates, are thought to increase stability through the compensation of plasmid-associated fitness costs and plasmid selection (San Millan et al. 2014b). For example, positive selection of the plasmid pNUK73, a costly non-transmissible plasmid in *P. aeruginosa*, is required to offset the potential loss of plasmid by segregational loss (San Millan et al. 2014b). However, there is

some evidence that non-transmissible plasmids may very rarely accomplish horizontal gene transfer, which can help stabilize the plasmid in a population (Pena-Miller et al. 2015).

The Plasmid Paradox

We expect that plasmids, whether costly or beneficial to their hosts, to either be lost over time or integrated into the host chromosome, but we do not expect that they will persist. Yet, there is much evidence indicating that plasmids, whether costly or not costly, selected for or not, can continue to persist over long periods of time (Table 3; Harrison and Brockhurst 2012; MacLean and San Millan 2015). This long-term persistence has been coined the ‘plasmid paradox’ (Harrison and Brockhurst 2012). In laboratory studies, where plasmid persistence is typically measured through serial transfers of the strain of interest in non-selective media over time, plasmids are often retained. While plasmid persistence is often observed, the level of selection required by the plasmid to persist varies, from no selection whatsoever to varying levels of antibiotic selection (Bergstrom et al. 2000; De Gelder et al. 2007; Heuer et al. 2007; Fischer et al. 2014; San Millan et al. 2014b). For example, the ESBL-encoding plasmid pCT persisted in *E. coli* for approximately 70 generations in the absence of any antibiotic selection (Cottell et al. 2012). Consistent with the lack of requirement for selection, the removal of a resistance gene from pCT did not change the level of plasmid persistence at the end of a selection experiment (Cottell et al. 2012). Similarly, a multi-drug resistant plasmid in *E. coli* was relatively stable over time, regardless of selection for that plasmid using antibiotics (Porse et al. 2016). However, other studies have indicated that varying levels of antibiotic selection are required for persistence - for example, antibiotic selection was needed for the maintenance of the non-transmissible plasmid pNUK73 in *P. aeruginosa* PAO1 (San Millan et al. 2014b; Johnson et

al. 2015; Löhr et al. 2015). Over deeper evolutionary time, plasmids have persisted in louse symbionts for upwards of 35 million years (Boyd et al. 2017). In this case, it appears that essential genes encoding for vitamin B5 synthesis have moved to the plasmid, suggesting that this configuration may have some benefit for both the plasmid and organism (Boyd et al. 2017).

At least part of the plasmid paradox can be resolved by host-plasmid co-evolution (Harrison and Brockhurst 2012; MacLean and San Millan 2015). The costs of plasmid carriage can be ameliorated by mutations to either the plasmid or the host; the accumulation of such mutations over time is referred to as compensatory evolution (Harrison and Brockhurst 2012). We note that the genetic background effects referred to above differ from compensatory evolution only in the timing by which compensatory mutations arise – prior to plasmid acquisition in the case of background effects, and afterwards in the case of compensatory evolution (Wong 2017). Compensatory evolution is easily achievable in the lab; studies tracking changes in fitness over time have generally found that under non-selective conditions, plasmids can remain in the population and the plasmid-related fitness costs may be reduced, returning to almost wild-type fitness (San Millan et al. 2014b; Porse et al. 2016). For example, Yano et al (2016) showed that plasmid persistence in the absence of selection may be a direct result of host-plasmid coadaptation. Here, mutations to TrfA1, a protein for replication initiation of the plasmid pMS0506, were associated with increased persistence. Additionally, compensatory evolution can occur quite rapidly when the mega-plasmid pQBR103 is transferred to *Pseudomonas fluorescens*, rescuing the host from previously deleterious effects associated with this plasmid. Compensation occurs via mutations to the global regulatory genes *gacA* and *gacS* (Harrison et al. 2015). Importantly, the presence of compensatory mutations does not only ameliorate the fitness cost of the plasmid, thus allowing it to be maintained even in the absence of selection, but may

308 also provide a means by which plasmid loss would be detrimental to the cell (Schrag et al. 1997;
309 Wong 2017). That is, the compensatory mutation(s) may only be beneficial so long as the element
310 for which it compensates is present, and are deleterious if the plasmid is lost. The fitness effects
311 of compensatory mutations in the absence of plasmids, however, have not been thoroughly
312 investigated.

313 Fewer studies have investigated the mechanisms and mutations contributing to
314 compensation, but in one study a 25-kb deletion on a plasmid was typically found in strains that
315 had reduced fitness costs but still maintained the plasmid (Porse et al. 2016). In the case of
316 Dahlberg and Chao (2003), the absence of resistance markers was found occasionally after
317 periods of coevolution, suggesting loss of the gene may be behind the reduced fitness cost for
318 carriage of that plasmid in some individuals. Conversely, San Millan et al (2014b) did not find
319 any plasmid mutations from six clones containing a plasmid, but instead found single
320 compensatory chromosomal mutations to either a helicase or a protein kinase gene. These results
321 concur with work done by Helling et al (1981) and Bouma and Lenski (1988), which
322 demonstrated that compensatory mutations were located on the host chromosome rather than on
323 the plasmid.

324 While host-plasmid co-evolution helps to explain the persistence of plasmids in the face
325 of costs and of segregational loss, it is worth emphasizing that beneficial plasmid-encoded genes
326 which may be on an otherwise costly plasmid, may integrate into the host chromosome, creating
327 chromosomal variants with the gene of interest. Nevertheless, host chromosome integration is
328 rare in the lab (Table 3), and at least some plasmids persist as independent entities for millions of
329 years (Boyd et al. 2017). The occurrence of co-adaptation does not adequately explain why

integration is an exception, rather than the rule, since strong selection should favour integration of beneficial genes (Bergstrom et al. 2000).

There is some evidence for selection against integrants, which may help to explain some cases of long-term plasmid persistence. While integration is possible, it is frequently unstable in the instances when it does occur, and there is evidence that cells prefer the presence of separate replicons for each element, rather than a larger integrated one (Mavingui et al. 2002; Heap et al. 2012). The instability of integration, beyond the potential introduction of fitness costs, may be a result of disrupting the symmetry between the origin and termini of replication (*oriC* and TER sites). This asymmetry may interfere with cellular activity and cause the cell to favour excision of the inserted DNA or to introduce inversions to restore balance (Alokam et al. 2002). For example, the insertion of *TN10* into *rrnH/G* and *proB* of *S. paratyphi* A resulted in an inversion to compensate for the asymmetry between *oriC* and TER. However, it has been noted that inversions in certain locations in the genome are deleterious, and that inversions and other rearrangements may not survive in wild-type strains as they do in culture (Liu and Sanderson 1995; Alokam et al. 2002).

Alternative explanations for the persistence of plasmids as independent replicons argue that a plasmid's ability to move is precisely what keeps it from being integrated, and thus lost as an independent element. Under one model, plasmids 'hitchhike' on increasingly fit genetic backgrounds, which allows them to outpace the fitter strains with chromosomally encoded plasmid-genes (such as an antibiotic resistance gene, for example). This occurs in a cyclical manner, as described by Bergstrom et al (2000). First, it is assumed there is a focal gene, for example, an antibiotic resistance gene, which can either be encoded by the plasmid, or integrated into the host chromosome; this may create chromosomal variants with the initially plasmid-

353 encoded gene. This allows the host to retain the advantageous gene and it may gain a selective
354 advantage, as it lacks the costly element itself, which is then at risk for loss in the population. If a
355 new strain arrives within the area that has some novel beneficial characteristic, and it begins to
356 outcompete the other strains, the plasmid at risk for loss can transfer to this strain as it sweeps
357 through the population. The beneficial plasmid gene then has a potential to integrate into the
358 chromosome, beginning the cycle again, where the plasmid faces removal from the population
359 unless it can hitchhike along with another ascendant strain. A graphical representation based on
360 this process described by Bergstrom et al (2000) shows the changes in fitness for different
361 variants carrying a focal gene originally found on a plasmid over time (Figure 2). However,
362 while this model is attractive given that plasmid persistence appears to be common, it is only
363 valid so long as the most fit, ascendant strain does not already encode the beneficial gene which
364 may have been found on the plasmid in the first place.

365 The cross-ecotype model attempts to explain how plasmids persist beyond purifying
366 events, such as a selective sweep (Bergstrom et al. 2000). Briefly, an ecotype is a distinct
367 population inhabiting a given environment, which presumably diverged from the original
368 population due to ecological speciation (Koeppel et al. 2013). Using again an example of a focal
369 antibiotic resistance gene which can either be chromosomally or plasmid encoded, it can be
370 assumed that chromosomal variants are preferred to the same gene on a plasmid. However, the
371 chromosomal variant may be lost over evolutionary time due to mutation or deletion events
372 (Bergstrom et al. 2000). While overall the chromosomal variant is preferred and will outcompete
373 genotypes containing the plasmid-encoded variant, they are unable to transfer across ecotypes,
374 unlike their plasmid-encoded counterparts. As such, recolonization of the plasmid and the focal
375 gene is possible, even between ecotypes, if one assumes that the more fit chromosomal variant

has been lost by chance over time. If, for example, that chromosomal variant is still present in the population, it is unlikely that the plasmid-encoded variant will successfully recolonize the population if it is still costly to maintain. This model thus provides one explanation for why certain types of genes, like an antibiotic resistance gene, are commonly found on plasmids – they are unlikely to be lost completely due to the ability to transfer across ecotypes, when compared to chromosomally encoded variants. Similarly, it has been argued that plasmids may exist as local adaptations, and that their ability to be readily transferred between bacteria can allow for the recipient to adapt to the environment if the plasmid provides a benefit in those conditions (Eberhard 1989).

The presence of high plasmid transfer rates has also been proposed as a possible solution to the plasmid paradox. Models suggest that plasmid persistence is likely so long as the plasmid is occasionally beneficial and horizontal transfer rates are sufficiently high to overcome plasmid fitness costs and segregational loss (Svara and Rankin 2011). In these models, when the rate of horizontal transfer for a plasmid is low (transfer rate $\beta = 0.01$ (cells/mL $\cdot$ min)⁻¹), chromosomal resistance genes are favoured under short and strong selection, whereas when rates are higher ($\beta = 0.1$ (cells/mL $\cdot$ min)⁻¹), plasmid-encoded resistance genes will be favoured (Svara and Rankin 2011). Notably, however, they do not consider integration as a possibility for plasmid loss (Slater et al. 2008). Gordon (1992) and Wan et al (2011) have estimated that the plasmid transfer rate in nature is between 1.3×10^{-15} cells per ml h⁻¹ to 3.0×10^{-8} cells per ml h⁻¹, based on experimental data with various laboratory and environmental *E. coli* strains, but the exact rate is unknown. Moreover, transfer rates may be higher in biofilms than in planktonic cells (Ghigo 2001; Stalder and Top 2016). More broadly, high transfer rates do not provide a good explanation for the persistence of non-conjugative and non-mobilizable plasmids, as these plasmids are unable to

transfer on their own (Harrison and Brockhurst 2012). Moreover, Hall et al. (2017) suggest that persistence through high transfer rates is a costly endeavour which might not always be successful and therefore is not the best long-term strategy (Hall et al. 2017).

Finally, there is some suggestion that the large size of some plasmids may help to explain their persistence. Chromids are very large plasmids – comparable in size to a bacterial chromosome – that are found in about 10% of bacterial genomes (Harrison et al. 2010). These elements share chromosomal and plasmid characteristics, in that they can carry essential housekeeping genes and share the codon usage properties of a chromosome, while carrying plasmid genes such as replication and partitioning genes, as well as other accessory genes (Harrison et al. 2010; Hülter et al. 2017). The replication of chromids is thought to be coordinated with that of the host chromosome (Hülter et al. 2017).

Essential housekeeping genes may remain on chromids for many reasons. This configuration may allow the host to maintain a larger genome overall, while allowing the chromosome to remain smaller. Under this configuration, genetic material can be more quickly replicated. Moreover, replicative stress on the cell may be reduced since replication can start later during the cell cycle, when cell size and resource availability may be greater. Further, while less evidence supports the following possibility, it is suggested that this configuration allows for the coordinated regulation of different chromid genes when the host adapts to different niches, such that different environments can induce different groups of genes to be expressed or not depending on the conditions the host is exposed to. For example, the effect of the loss of a 1.35 Mb megaplasmid and 1.68 Mb chromid was measured in *Sinorhizobium meliloti*. While the host could survive in the absence of these elements, a limitation in the number of usable carbon sources was observed, which suggested that there may be a limited niche range in the absence of

these elements (diCenzo et al. 2014). Further, the loss of the megaplasmid resulted in an increase in growth rate relative to the wild-type. Nonetheless, it continues to persist in the face of this cost, suggesting that it must confer some advantage to the host that may not be observed in the laboratory.

Conclusions

Plasmids have clear benefits in a number of challenging environments, encoding traits such as antibiotic resistance, virulence, and metal tolerance. In the absence of selection for such traits, however, plasmids often impose a fitness cost. We have suggested that the mechanisms underlying these fitness costs are incompletely understood; resource use and interference with host processes certainly contribute in particular cases, but the relative importance of these mechanisms across plasmids and hosts is still unclear. Moreover, the evolutionary processes that maintain plasmids in the face of segregational loss, fitness costs and integration require further investigation. Elucidation of the mechanisms underlying costs, and of the processes contributing to persistence, will both clarify fundamental plasmid biology, and assist in the management of plasmid-encoded traits such as antibiotic resistance.

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Tables:
Table 1: Fitness changes associated with plasmid carriage.

Paper	Plasmid(s)	Host Strain(s)	Fitness Measurement	Change in Fitness	Fitness After Selection
Starikova et al. 2013	<i>vanA</i> -encoding	<i>E. faecium</i> , <i>E. faecalis</i>	Mixed Culture	↓ 25-27%	Near wild-type; 400 generations
Johnson et al. 2015	pAC	<i>E. coli</i>	Mixed Culture	-0.10 to 0.35*	-
Porse et al. 2016	pKP33	<i>E. coli</i> (Ec37 and Ec38), <i>K. pneumoniae</i> (Kp08)	Growth Rate	↓ 6-12.5%	Near wild-type for <i>E. coli</i>
Dionisio et al. 2005	R1	<i>E. coli</i> K12 MG1655	Mixed Culture	1.013 - 2.051*	Near wild-type, with two more fit than wild-type; 420 generations.
San Millan et al. 2014b	pNUK73	<i>P. aeruginosa</i> PAO1	Mixed Culture	↓ 21.4%	↓ 6%, relative to plasmid-free clones; 300 generations
Di Luca et al. 2017	pG12-KPC-2, pG06-VIM-1	<i>E. coli</i>	Mixed Culture	↓ 1.1-3.6%	Mixed – near-wild-type fitness in some transformants, reduced in others; 312 generations
Silva et al. 2011	R124, R831, R16, R702, RP4	<i>E. coli</i> K12 MG1655	Mixed Culture	↓ 2.8-8%	-
San Millan et al. 2014a	pNI105, pBS228, Rms149, pAKD1, PAMBL-1, PAMBL-2	<i>P. aeruginosa</i> PAO1 and PAO1-GFP	Mixed Culture	↓ < 13.2-14.3%	Stable maintenance; 100 generations
Dahlberg and Chao 2003	R1, RP4	<i>E. coli</i>	Mixed Culture	↓ 6-21%	Up to 70% more fit than plasmid-bearing ancestors; 1100 generations
Enne et al. 2004	<i>Sul2</i> -encoding; p9123 P9938 P9118	<i>E. coli</i> K12 JM109	Mixed Culture	↑ 3.3 ± 1.7 % † ↓ 9.0 ± 1.5 % ↓ 4.7 ± 3.1 %	For P9938, fitness increase observed after initial decrease; observed after 20 generations.

* Relative fitness
† Indicates fitness increase

824 Table 2: Host-dependent fitness costs of plasmid carriage.

Paper	Plasmid	Host Species	Fitness Effects
Humphrey et al. 2012	RP1, pUB307, R46, pVE46, N3	<i>E. coli</i> 345-2RifC, 345-8, 343-9	Cost of RP1 varies with host Varying cost of N3 and R46 across hosts
Starikova et al. 2013	<i>vanA</i> plasmids	<i>Enterococcus faecium</i> 64/3	Host-dependent fitness cost of plasmids
Di Luca et al. 2017	pG12-KPC-2 and pG06-VIM-1	<i>E. coli</i> K56-65, K56-75, K56-43, K56-68, K56-75	pG06-VIM-1: fitness cost not dependent on host background pG12-KPC-2: fitness cost depends on host background
Johnson et al. 2015	pAC, pI1, and pACpI1	<i>E. coli</i> and <i>S. enterica</i>	Fitness of plasmid varies between species Host-dependent fitness cost of plasmids within species

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841 Table 3: Persistence of plasmids during laboratory selection.

Publication	Selection Conditions	Plasmid	Host Organism	Duration of persistence (generations)
San Millan et al. 2014 <i>b</i>	Antibiotics required	pNUK73	<i>P. aeruginosa</i> PAO1	300
Cottell et al. 2012	Persists without selection	pCT and pCT2	<i>E. coli</i>	70
Fischer et al. 2014	Persists without selection	IncI1 family	<i>E. coli</i>	385-651
De Gelder et al. 2007	Variable; depends on host	pB10	<i>Alpha-, Beta-, and Gammaproteobacteria</i>	200
Porse et al. 2016	Persists without selection; coevolution hypothesized	pKP33	<i>E. coli</i> Ec 37, Ec 38, <i>K. pneumoniae</i> Kp08	280

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Figure Captions:

Figure 1: Change in fitness (%) for plasmid RP1 across *E. coli* strains 345-2RifC, 343-9, 99-24, 99-40, and K12 JM109, relative to the wild-type strain. Figure produced by authors using data from Humphrey et al. 2012.

File: Figure1_RelativeFitness.jpg

Figure 2: Selective sweeps of a plasmid to increasingly fit, ascendant strains. Initially a strain with a chromosomal variant of a focal plasmid gene (solid black line) becomes more fit than a strain with a plasmid-encoded gene (dotted black line). The ascendant strain (dashed grey line), which contains novel beneficial characteristics, enters the population with increased fitness over both the strains carrying either the plasmid or chromosomally-encoded focal gene. The plasmid can transfer to this ascendant strain, potentially increasing fitness (black dashed line), which has increased fitness relative to the original ascendant strain, and the process may begin again. Figure produced by authors using information from Bergstrom et al. (2000).

File: Figure2_PlasmidSweep.jpg

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