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Ecological and evolutionary solutions to the plasmid paradox

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Ecological and evolutionary solutions to the plasmid paradox

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Abstract

Plasmids are common features of bacterial genomes, but theoretically they should not exist: Due to the costs associated with plasmid maintenance, non-beneficial plasmids should be purged by negative selection, whereas even under positive selection plasmid-encoded beneficial genes should be captured to the bacterial chromosome, followed by loss of the redundant plasmid. In the decade since we described this apparent “plasmid paradox” a range of ecological and evolutionary solutions have been shown to operate in bacterial populations and communities, explaining the widespread distribution and stable persistence of plasmids. We conclude, therefore, that the theoretical plasmid paradox has now been solved. The current challenge for the field, however, is to better understand how these solutions operate in natural bacterial communities to explain and predict the dynamics and distributions of plasmids and the horizontal gene transfer that they mediate in bacterial (pan)genomes.

Introduction

Plasmids are (usually circular) extrachromosomal genetic elements that encode their own replication, maintenance and, in the case of conjugative plasmids, the conjugative pilus that enables horizontal transfer of the plasmid to other cells [1]. In addition to encoding these plasmid-related functions, plasmids often also carry a cargo of accessory genes that confer a wide range of ecological traits upon the bacterial host, including resistance to stressors and metabolic capacities [2]. As such, plasmids play a key role in bacterial evolution by transferring these ecologically important genes between lineages [3].

Although plasmids are common features of bacterial genomes, theoretically they should not exist, a situation described as the “plasmid paradox” [4]: Plasmids place a metabolic burden upon host cells for their maintenance and expression and often cause other types of cellular disruptions leading to fitness costs (summarised in [5,6]). Consequently, plasmids should be purged from bacterial populations by negative selection [7]. Yet even when plasmid encoded accessory functions are sufficiently beneficial to outweigh these costs (and thus the encoding plasmid is positively selected), theory predicts that the accessory genes should be captured to the bacterial chromosome, thereby allowing loss of the redundant plasmid [7]. Addiction systems, such as toxin-antitoxin systems, are also no guarantee of long-term survival due the possibility of these systems also being captured by the chromosome, enabling loss of the plasmid without suffering ill effects of the toxin [8].

Over the past decade, new theory and experiments have shown that a range of solutions to the plasmid paradox operate in bacterial populations and communities (Figure 1). In this review, we summarise the ecological and evolutionary solutions explaining the widespread existence and stable maintenance of plasmids in bacterial genomes.

Ecological solutions

Solution 1: Infectious transmission

Rather than persisting long term in any given genome, plasmids may potentially survive at the population level by horizontal transmission, passing from cell to cell as an infectious element [9]. Early mathematical models of plasmid-host dynamics usually assumed, based upon the available data [10], that rates of plasmid conjugation were too low to sustain plasmid maintenance by infectious transmission [7]. However, more recent studies have shown that some plasmids have conjugation rates that are sufficiently high to enable their persistence in bacterial populations even in the face of high fitness costs and, consequently, strong negative selection. For example, Lopatkin *et al* [11] showed conjugation mediated persistence of nine antibiotic resistance plasmids from six major incompatibility groups in experimental *Escherichia coli* populations in the absence of antibiotics.

Even more remarkably, the environmental mercury resistance plasmid, pQBR57, was capable of driving itself to fixation in experimental *Pseudomonas fluorescens* populations through infectious transmission in both liquid broth [12] and soil habitats

[13] without mercury selection. Notably, similar dynamics were not observed in a congeneric host, *Pseudomonas putida*, where pQBR57 was less costly but went extinct due to a lower conjugation rate in this host [13]. In environments that experience occasional pulses of positive selection, even low rates of conjugation can enhance plasmid survival by enabling low level persistence during the periods between pulses, which then select for clonal expansion of plasmid carriers [14].

Higher rates of conjugative horizontal transmission are required to enable plasmid persistence as the fitness costs of plasmid carriage increase [15]. In addition, other sources of selection on bacterial populations can increase the relative importance of conjugation for plasmid survival. For example, predation by phages can limit plasmid persistence by reducing bacterial density and thus the opportunity for conjugation [16]. Similarly, conjugation was required for the persistence of an antibiotic resistance plasmid in bacterial populations that were being predated by a protist [17].

Infectious transmission through conjugation can, of course, only explain the persistence of the fraction of plasmids that are self-transferable and encode their own conjugative apparatus. Plasmids that are not self-transferable must either rely on other plasmids to mobilize them [18], or on other mechanisms of cell-to-cell transmission, such as packaging and transfer of plasmid DNA by phages [19] or natural transformation [20]. The relative contribution of these alternative transfer mechanisms to the persistence of non-self-transferable plasmids versus the other stability mechanisms we discuss below is unknown.

Solution 2: Plasmid properties vary across host backgrounds

Models of plasmid persistence typically assume that plasmid properties are inherent to the plasmid. However, key properties that determine plasmid persistence are increasingly being shown to vary between bacterial species and even between closely related conspecific bacterial strains. Plasmid segregation [21] and conjugation rates [22–25] vary extensively across genomic backgrounds. The causes of this variation in plasmid properties are largely unknown, but recent studies suggest that this variability among coexisting bacterial strains could affect plasmid stability in bacterial communities.

Using hospital isolates of *E. coli* and *Klebsiella pneumoniae* and a co-circulating antibiotic resistance plasmid, [23] have shown that the fitness effects of plasmid acquisition were on average costly but ranged from negative to positive effects on bacterial fitness among strains in the absence of antibiotics. A similar pattern has been independently observed in a diverse collection of *E. coli* environmental and clinical isolates for an unrelated antibiotic resistance plasmid [22]. Combining their plasmid fitness effects data with a simple mathematical model, Alonso-Del Valle *et al* [23] showed that introducing variable plasmid fitness effects among strains increased the stability of low cost plasmids. In effect, the variability of plasmid fitness effects meant that in some hosts, the plasmid causes no fitness cost (and indeed may increase fitness), correspondingly reducing the dependence upon infectious transmission for plasmid survival. Interestingly, this stabilising effect of variable plasmid fitness costs

strengthens with increasing bacterial community diversity. Variable fitness effects cannot, however, explain the persistence of high cost plasmids. Where mean fitness costs are high, the distribution of plasmid fitness effects may result in fewer geotypes able to serve as permissible hosts, and thus high cost plasmids are still predicted to critically rely on infectious transmission for their maintenance [23]. Nevertheless, variation in fitness effects may allow certain host genotypes to act as refugia for plasmids, enabling their maintenance in the community.

Solution 3: Interactions with other plasmids

Plasmid persistence can also be affected by the presence of other plasmids in the same genome. Coinfecting plasmids can affect transfer efficiency through mobilisation and co-integration [18,26–28] of both non-conjugative [29] and conjugative [30] plasmids.

Using plasmid coinfection experiments, San Millan *et al* [29] showed that for 5 out of 6 plasmids their fitness cost was lower in coinfection than expected from the cost of each plasmid when measured alone. This is an example of synergistic epistasis, and could lead to improved plasmid persistence through coinfection by weakening negative selection. This may help to explain why bacterial genomes contain multiple plasmids more often than is expected by chance [29].

A similar pattern of synergistic epistasis of plasmid fitness costs has been observed for two large conjugative plasmids [30]. However, this study also suggested that finding synergistic epistasis was somewhat dependent upon how fitness was measured, such

that it was more likely to be observed when competing plasmid bearing cells against plasmid free cells, than when coinfected cells were competed against single-infected cells. Nevertheless, a recent theoretical study modelling how plasmid coinfection affected stability found that epistatic interactions in particular are likely to determine the persistence of plasmids in bacterial populations, while variation in other plasmid properties (such as conjugation and segregation rates) were less important [31]. Thus, more experimental tests across a wider range of plasmid-host combinations are urgently needed to test the generality of the role for plasmid-plasmid interactions in determining plasmid stability.

Solution 4: Source-sink spillover transmission

In bacterial communities containing multiple species that vary in their proficiency as plasmid hosts, plasmids can persist in species that would otherwise be incapable of sustaining them due to spillover transmission from other more proficient host species (i.e., those species that do stably maintain the plasmid within their own population). In soil microcosms, whereas the mercury resistance plasmid pQBR57 went extinct in *P. putida* populations without mercury selection, the plasmid was consistently detected at appreciable levels in *P. putida* when grown in a community alongside a more proficient host, *P. fluorescens*, which acted as a source of plasmid spillover transmission [13].

Experiments in more complex communities by Cairns *et al* [32] suggest that spillover transmission of an antibiotic resistance plasmid occured preferentially to higher abundance taxa and close phylogenetic relatives of the donor species. Interestingly,

transmission to lower abundance taxa was enhanced in spatially structured environments, presumably by increasing cell-to-cell contacts and thus opportunities for conjugation. Moreover, by increasing plasmid abundance, low antibiotic concentrations increased the diversity of bacterial taxa acquiring the plasmid.

The impact of spillover transmission on plasmid spread in communities varies with the plasmid donor species, such that plasmids reach higher community-level abundances when introduced by a proficient plasmid host [33]. Conversely, plasmid transmission within a given species' population can be impaired when living in a community alongside less proficient plasmid hosts due to the dilution effect [26], which could potentially destabilise the plasmid if efficient infectious transmission is required for its stability in the source host population.

Spillover from proficient plasmid hosts into less proficient host species could increase the probability of plasmids adapting to better persist in less proficient host species, similar to host shifts in infectious disease [34,35]. For example by reducing their fitness cost in this new host or enhancing their conjugation rate from this host [36], thus broadening the host range of the plasmid.

Evolutionary solutions

Solution 5: Compensatory evolution

The fitness costs of acquiring a plasmid can be overcome by compensatory evolution to ameliorate the cost and thus reduce or negate negative selection on the plasmid.

Compensatory mutations have been observed to evolve repeatedly in laboratory populations of diverse bacteria-plasmid associations [8,30,37–43]. Compensatory mutations can occur on the plasmid [40,41] or the chromosome [30,37–39,43] or on both [8,43]. Theoretically, plasmid encoded compensatory mutations should be more successful if they also reduce plasmid costs in transconjugant cells as the benefits are carried in linkage with plasmid transfer [44]. However, chromosomal compensatory mutations appear to be more commonly observed across the studies performed to date [8,30,37–39]. This may simply be a consequence of the chromosome being larger and containing more genes than the plasmid, and thus offering a bigger mutational target, but it is possible that other selective forces or limitations may contribute to this pattern. For example, in diverse communities, variation in plasmid fitness effects across host backgrounds [23] may weaken selection for plasmid-encoded compensatory mutation.

Importantly, compensatory evolution occurs both with or without positive selection for plasmid encoded functions [30,37], confirming that even when a costly plasmid's net fitness effect is beneficial (because the benefit outweighs the cost), the fitness cost of plasmid maintenance remains and must be ameliorated to prevent plasmid loss driven by chromosomal capture. Indeed, positive selection has been shown to enhance compensatory evolution by increasing the population size of plasmid-carrying cells [38], which increases the likelihood of acquiring a compensatory mutation. Studies that have tracked the temporal dynamics of compensatory evolution suggest that there is, in effect, a race between the processes of plasmid loss, chromosomal capture, and compensatory evolution [37,38,45] . Nonetheless, compensatory evolution can be

extremely rapid, in some cases occurring during the outgrowth of transconjugant colonies within 48-72 hours [39].

A potential explanation for the speed of compensatory evolution is that amelioration often only requires a single mutation, although some examples of large-scale genetic changes, including large plasmid deletions, have been observed [40,46,47]. Comparing multiple independent populations shows that compensatory mutations tend to be focused within one or a few genetic targets [8,30,37–41]. This pattern of requiring only one or a few targeted mutations to ameliorate fitness costs suggests that these plasmids cause their fitness costs through specific genetic conflicts occurring between one or few plasmid genes and one or few chromosomal genes. The targets of compensatory mutations are often associated with the SOS response, and in particular helicase genes have acquired compensatory mutation across several host-plasmid pairs [38,41–43]. In addition, genes that interact deleteriously with incoming plasmids are often themselves horizontally acquired accessory genes already present in the chromosome [42,48,49].

Certain chromosomal compensatory mutations have been shown to ameliorate the fitness costs of multiple plasmids in a cell, potentially enabling these bacterial lineages to become hotspots of plasmid-plasmid recombination [42,50–52]. However, even after compensatory evolution to negate their fitness costs, the coexistence of plasmids in a cell may not be stable if they encode the same ecological function. This is because

positive selection has been shown to discriminate between the relative fitness benefits of coexisting plasmids, only retaining the most beneficial [50].

Compensatory evolution is a particularly important mechanism enabling the long-term maintenance and survival of non-conjugative plasmids, which unless spread horizontally by other mechanisms (e.g. packaging by temperate phages) rely entirely upon their efficiency of vertical transmission at cell division [38].

Solution 6: Piggybacking on niche adaptation

Compensatory mutations are defined as those that specifically reduce the plasmid fitness cost, and thus will not be selected in bacterial populations evolved without the plasmid where they provide no benefit. Recent experiments by Kloos *et al [53]* reveal a different class of mutations that are generally beneficial, and as such evolve in both plasmid-carrying and plasmid-free cells, but nonetheless have the effect of pleiotropically reducing the fitness cost of the plasmid. Using clinical isolates of *E. coli* with two different multidrug resistance plasmid, Kloos *et al* [53] showed that mutations in bacterial global regulators of metabolism reduced the plasmid fitness costs by causing a net downregulation of plasmid gene expression. Crucially, these mutations increased bacterial growth in the environment in which they evolved regardless of plasmid carriage, confirming that in addition to reducing plasmid fitness costs these mutations were generally adaptive in this niche.

Conclusion

253 The plasmid paradox has inspired a large body of research during the past decade. This
254 work demonstrates that there are multiple ecological and evolutionary mechanisms that
255 can explain the persistence of plasmids in bacterial genomes. These mechanisms may
256 also often work in concert, such that a plasmid may undergo compensatory evolution to
257 negate its fitness cost whilst also still transmitting horizontally by conjugation, with
258 potentially synergistic benefits for the plasmid which is no longer subject to negative
259 selection. While the theoretical plasmid paradox is now effectively solved, the challenge
260 for the field is to translate these insights to better understand plasmid dynamics in
261 natural microbial communities.
262
263 **Future directions**
264 With the plasmid paradox now solved, where next for plasmid ecology and evolution?
265 Below we outline some of the key open research questions for the field (see also
266 Outstanding Questions):
267 - **What is the relative importance of these plasmid stability mechanisms in**
268 **nature?** Most experimental work on plasmid stability to date has used relatively
269 simplified laboratory systems, which do not reflect the complexity of plasmid-host
270 interactions in natural communities and environments (although see [32,54] for
271 experimental set-ups that approach more natural levels of diversity and habitat).
272 Studies tracking the ecological and evolutionary dynamics of plasmid-host
273 interactions in natural communities are required to determine the relative
274 contributions of the various mechanisms of plasmid stability observed in the lab
275 to dynamics occurring in nature. A recent example of such a study tracked a

carbapenem resistance plasmid in hospitalised patients, demonstrating pervasive plasmid horizontal transmission between bacterial lineages and species, suggesting a potentially important role for spillover transmission in this natural setting [55].

- **What are the effects of these plasmid stability mechanisms on the dynamics of horizontal gene transfer?** Understanding how different mechanisms of plasmid stability impact rates of horizontal gene transfer and gene mobilisation will be important for predicting the spread of accessory genes in natural communities. For example, in environments where plasmids persist by infectious transmission, higher rates of interspecies gene mobilisation have been observed compared to communities in environments where plasmids persisted mostly by vertical transmission [56]. Conversely, in some studies, compensatory evolution has been associated with reduced conjugative ability [37,57], and thus potentially reduced rates of HGT. More generally, whether compensatory mutations are plasmid- or chromosomally encoded could alter rates of horizontal gene transfer in complex microbial communities, and in turn different compensatory mechanisms could be favoured depending on the host and plasmid community diversity [44].
- **What drives the distribution of plasmids in bacterial (pan)genomes?** Understanding the molecular causes of the fitness costs of plasmids across a wider diversity of plasmid-host interactions could enable the compatibility of plasmid-host pairs to be predicted from sequence data. This could in turn improve prediction of strains that pose particular risks of stably acquiring

plasmids and e.g. becoming multidrug resistant or acting as hubs of plasmid recombination and dissemination. For example, comparative genomic studies have revealed associations between plasmid carriage and chromosomal regulatory sequences that suggest past compensatory evolution, and may indicate other strains with the same allelic variants that are “pre-adapted” to be proficient plasmid hosts [58]. How these patterns of chromosome-plasmid compatibility interact with selection for the genes encoded on plasmids will shape the flow of plasmids and the genes they mobilise in bacterial pangenomes [59].

- **What kinds of genes become mobilised by plasmids and why?** Plasmids are notorious for encoding and transferring antibiotic resistance genes among lineages, thus contributing to the antimicrobial resistance crisis [60]. But plasmids also carry other kinds of functions, such as metabolic, virulence, and symbiosis genes [61,62]. One property that potentially unifies the kinds of traits that are more frequently plasmid versus chromosomally encoded is that their fitness benefits are strongly environmentally contingent, such that being encoded on plasmids might enable their recurrent gain, loss, and regain depending upon local selection pressures [63]. Moreover, genes with social effects, such as cooperative or spiteful traits, have been suggested to be enriched on plasmids [64]. Recent theory suggests that whether genes are plasmid or chromosomally encoded tends to be under positive frequency dependent selection, leading to priority effects such that once mobilised a moderately beneficial plasmid encoded trait will tend to prevent the invasion of a chromosomal version, especially when the trait is moderately beneficial across multiple bacterial species [65].

- **What are the effects of plasmids beyond horizontal gene transfer?** Plasmids play other roles in bacterial ecology and evolution besides the transfer of accessory genes [66] but these remain relatively poorly understood. Key examples include the manipulation of bacterial phenotypes through gene regulatory cross-talks [67], which can alter bacterial lifestyle (e.g. from planktonic to biofilm growth [68,69]) or physiology (e.g. from aerobic to anaerobic metabolism [22]). In addition, because plasmids are often present in multiple copies within the cell, this can alter the evolutionary dynamics and evolvability of plasmid-encoded versus chromosomally encoded genes [70].

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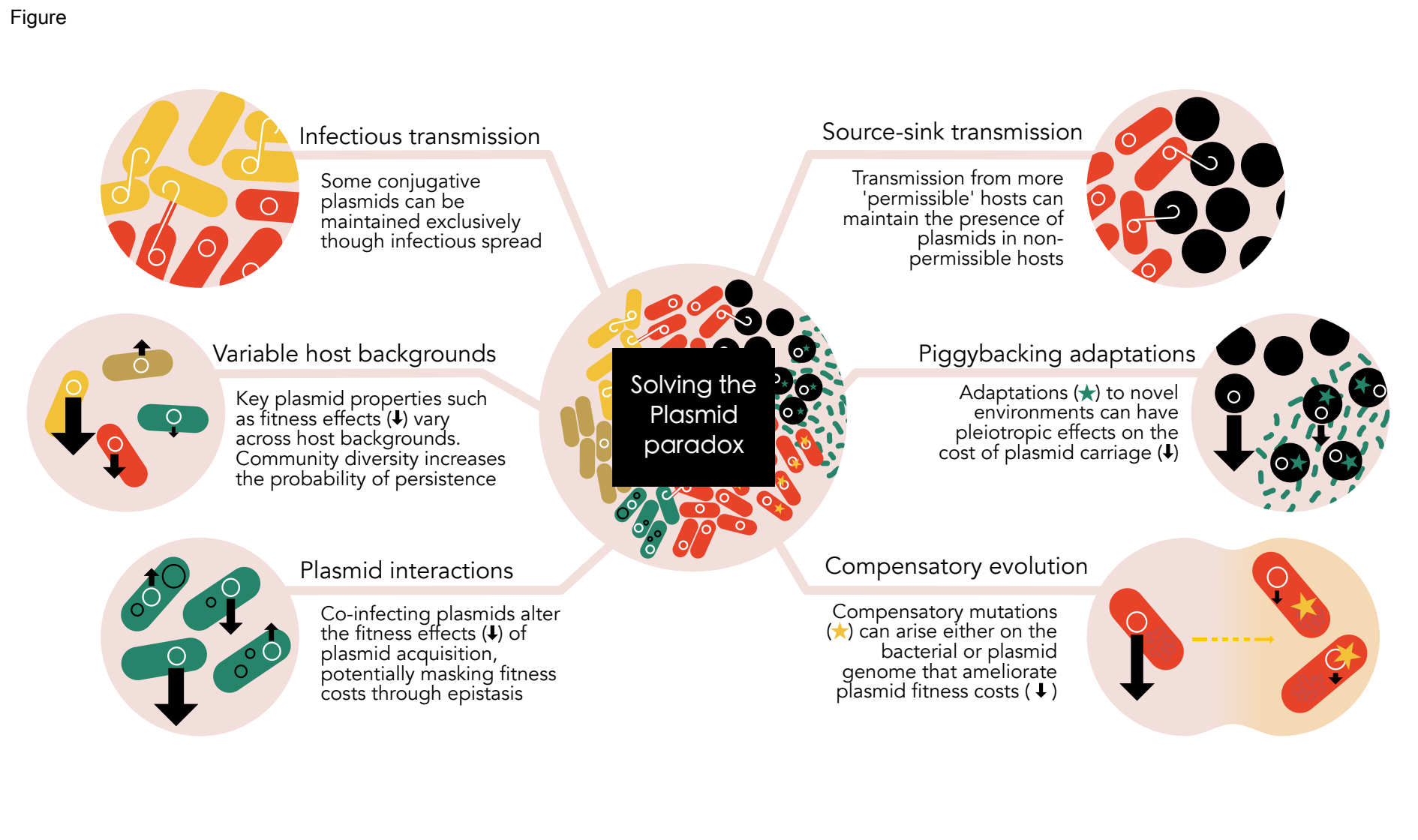
Figure Legend

Figure 1 | Ecological and evolutionary mechanisms of plasmid stability

Over the past decade theoretical and experimental studies have discovered a range of ecological and evolutionary mechanisms that enable plasmids to persist in bacterial populations and communities. Cells of the same colour belong to the same species, whereas those of a different colour belong to a different species or lineage. Plasmids are shown as white rings, and where these connect cells, this denotes conjugative transfer. Solid arrows denote fitness costs, and are scaled by the magnitude of the fitness cost. Dotted lines denote evolutionary changes.

Figure


Solving the Plasmid paradox
Infectious transmission
Some conjugative plasmids can be maintained exclusively though infectious spread
Source-sink transmission
Transmission from more 'permissible' hosts can maintain the presence of plasmids in non-permissible hosts
Variable host backgrounds
Key plasmid properties such as fitness effects (⬇) vary across host backgrounds. Community diversity increases the probability of persistence
Piggybacking adaptations
Adaptations (★) to novel environments can have pleiotropic effects on the cost of plasmid carriage (⬇)
Plasmid interactions
Co-infecting plasmids alter the fitness effects (⬇) of plasmid acquisition, potentially masking fitness costs through epistasis
Compensatory evolution
Compensatory mutations (★) can arise either on the bacterial or plasmid genome that ameliorate plasmid fitness costs (⬇)

- Plasmids are common in bacterial genomes, but theoretically they should not exist, giving rise to the plasmid paradox
- Recent studies show that multiple mechanisms can explain the long-term persistence of plasmids in bacterial genomes, solving the plasmid paradox
- Ecological solutions to the plasmid paradox include infectious transmission and variation in fitness costs between lineages
- Evolutionary solutions to the plasmid paradox include compensatory evolution and piggybacking niche adaptation

- What is the relative importance of these plasmid stability mechanisms in nature?
- What are the effects of these plasmid stability mechanisms on the dynamics of horizontal gene transfer?
- What drives the distribution of plasmids in bacterial (pan)genomes?
- What kinds of genes become mobilised by plasmids and why?
- What are the effects of plasmids beyond horizontal gene transfer?