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1 **Plasmid-mediated horizontal gene transfer is a coevolutionary process**

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3

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6

7 Keywords: Coadaptation, coevolution, experimental evolution, conjugative plasmid,  
8 mobile genetic element, accessory genome

9

10 **Abstract**

11 Conjugative plasmids are key agents of horizontal gene transfer that accelerate  
12 bacterial adaptation by vectoring ecologically important traits between strains and  
13 species. However, while many conjugative plasmids carry beneficial traits, all  
14 plasmids exert physiological costs-of-carriage on bacteria. The existence of  
15 conjugative plasmids therefore presents a paradox, since non-beneficial plasmids  
16 should be lost to purifying selection, whereas beneficial genes carried on plasmids  
17 should be integrated into the bacterial chromosome. Several ecological solutions to  
18 the paradox have been proposed, but none account for coadaptation of bacteria and  
19 conjugative plasmids. Drawing upon evidence from experimental evolution, we argue  
20 that horizontal gene transfer via conjugation can only be fully understood in a  
21 coevolutionary framework.

22

23 **Mechanisms of horizontal gene transfer**

24 Horizontal gene transfer (HGT) is a major process in the evolution of bacteria. The  
25 uptake of ready-made genes or operons from the 'mobile gene pool' facilitates rapid  
26 adaptation to novel environments, without the reliance upon rare, beneficial mutations  
27 arising spontaneously in the population [1]. As such, HGT is often associated with  
28 evolutionary and ecological innovation, conferring new phenotypic traits (or suites of  
29 traits) and thereby access to novel ecological niches [2, 3]. The effectiveness of this  
30 mode of adaptation is acutely demonstrated by the rapid global spread of antibiotic  
31 resistance throughout bacterial populations [4]. Importantly, because HGT can occur  
32 between taxonomically distinct bacterial lineages, and even between kingdoms [5], it  
33 blurs the boundaries between clades and obscures phylogenetic relationships. Yet  
34 conversely, since species-specific traits, i.e. those that distinguish sister clades, often

1 arise through HGT, it is equally an important driver of bacterial speciation [2, 3]. As  
2 a consequence of HGT, microbial diversity should be viewed less as a reticulate tree,  
3 and more as a thicket of interconnecting branches [6].

4  
5 HGT is mediated by three different mechanisms: **transformation**, **transduction** and  
6 **conjugation** (for items in bold see glossary) [7]. It is curious that despite HGT  
7 underpinning bacterial adaptation, only one of these mechanisms, transformation, is  
8 under the control of bacteria. Both transduction and conjugation are mediated by  
9 semi-autonomous vectors: temperate phages and conjugative elements respectively  
10 (of which conjugative plasmids are the most significant) [7]. Because these vectors  
11 encode genes controlling their own replication and transmission they must be  
12 considered as evolving agents subject to natural selection in their own right, with  
13 fitness interests that need not necessarily be aligned with those of their bacterial host.  
14 There is therefore opportunity for both conflict and collaboration between bacteria  
15 and HGT vectors, generating reciprocal selection and thus the potential for on-going  
16 adaptation and counter-adaptation. In this essay, we argue that to better understand  
17 vector mediated HGT, a coevolutionary rather than simply evolutionary approach  
18 should be taken. We focus on conjugative plasmids, for which a large body of theory  
19 has been developed to understand their population biology and identify the ecological  
20 conditions for their maintenance.

21

## 22 **The plasmid paradox**

23 Conjugative plasmids are a diverse group of (mostly) circularized DNA molecules  
24 that exist independently of the host bacterial genome. Plasmid genomes consist of a  
25 backbone containing essential genes controlling core plasmid functions as well as a  
26 suite of non-essential accessory genes [Box 1]. It is these accessory genes that  
27 provide the currency of HGT, encoding traits that are potentially beneficial to the  
28 bacterial host. Accessory genes can be divided into three key functional groups: those  
29 conferring virulence, by allowing their hosts to inhabit and exploit other organisms  
30 [8], resistance to toxins such as antibiotics [9] and heavy metals [10], and metabolic  
31 functions such as nitrogen fixation in rhizobia [11]. It is notable that many accessory  
32 gene encoded traits are expressed outside of the cell, i.e. the gene products are  
33 secreted, thereby leading to the hypothesis that HGT may play a key role in microbial  
34 sociality [12]. Accessory genes are themselves often carried on smaller mobile

1 elements embedded within the plasmid [10, 13], allowing them to mobilize within and  
2 between plasmids, as well as integrate into the host chromosome.

3

4 A great deal of attention has been focused on establishing the theoretical ‘existence  
5 conditions’ for conjugative plasmids [14-17]. The carriage of plasmids exerts a high  
6 physiological burden on the host cell. The upkeep and repair of plasmid DNA [18]  
7 and the production of plasmid proteins [19] uses up raw materials within the cell,  
8 occupies cellular machinery such as ribosomes [18] and disrupts the cellular  
9 environment [20]. In addition to being energetically costly, production of conjugative  
10 pili also exposes the cell to attack from pilus-specific bacteriophage [21]. **Positive**  
11 **selection** for beneficial, plasmid-borne accessory traits could outweigh this cost.  
12 However, consistent positive selection on beneficial traits is predicted to ultimately  
13 favor the integration of these traits into the host chromosome and the subsequent loss  
14 of the plasmid backbone [15]; a process facilitated by the location of accessory genes  
15 on mobilizable elements within the plasmid genome. In the absence of positive  
16 selection, conjugative plasmids are predicted to be lost from the population by  
17 **purifying selection** unless plasmids are capable of very high rates of conjugative  
18 transfer [15, 22]. Whether such rates are achievable in nature has been hotly debated  
19 [16, 22, 23]. Moreover, plasmids persisting through conjugation alone would be  
20 expected to experience strong selection to jettison extraneous genetic material  
21 including their complement of accessory genes [24].

22

23 Explaining the existence and ecological persistence of beneficial conjugative plasmids  
24 therefore presents a paradox: in the absence of positive selection, highly conjugative  
25 plasmids should evolve high transmission rates and lose their accessory genes,  
26 whereas under consistent positive selection beneficial accessory traits should be  
27 integrated into the bacterial chromosome. How then is the rich diversity of plasmid  
28 vectors and their accessory elements maintained? A number of long term bacteria–  
29 plasmid co-culture experimental evolution studies (summarized in Table 1) provide a  
30 test-bed for theoretical predictions.

31

### 32 **Resolving the plasmid paradox: a role for coevolution?**

33 A consistent finding across co-culture studies is that costly plasmids are not easily lost  
34 from bacterial populations, and can be maintained for hundreds of generations, even

1 in the absence of positive selection [24-29]. This pattern cannot be accounted for by  
2 high conjugation rates alone, because non-conjugative plasmids are also maintained  
3 over these long timescales [24, 26, 27, 29]. Nor can this pattern be explained by  
4 stringent segregation systems, such as post-segregational killing mechanisms, as these  
5 were lacking in several studies [24, 26]. The surprising stability of bacteria-plasmid  
6 associations can be attributed to evolutionary adaptation. In the vast majority of long-  
7 term co-culture experiments, persistence is associated with a reduction in the burden  
8 of plasmid carriage [24-27, 29-34] (although notable exceptions exist [28]). This  
9 weakens the strength of purifying selection against plasmid carriage, and therefore  
10 reduces the rate at which plasmids are removed from the population.

11

12 A number of co-culture studies have attempted to determine the extent to which co-  
13 adaptation of both bacteria and plasmid, rather than simply adaptation by one party or  
14 the other, contributes to higher than expected plasmid stability [24-26, 30, 32]. By  
15 comparing costs-of-carriage between evolved and ancestral plasmids in both evolved  
16 and ancestral host genetic backgrounds, the relative contributions of bacterial and  
17 plasmid evolution can be deduced [Box 2]. Reduction in costs-of-carriage could, in 4  
18 of the 5 studies, be attributed to coadaptation, with both host and plasmid adaptations  
19 contributing to improved fitness [24-26, 32]. For example, following 1100  
20 generations without positive selection for plasmid-encoded traits, Dahlberg & Chao  
21 [25] observed, in 5 of 6 evolved bacteria-plasmid clones, complete amelioration of the  
22 cost-of-carriage, i.e. no difference in fitness was detected between evolved bacteria  
23 with or without their co-evolved plasmid. Further assays measuring the fitness of  
24 constructed bacteria-plasmid clones suggest that improved fitness resulted from  
25 adaptations by both bacteria and plasmids: reduced costs-of-carriage were observed  
26 for evolved plasmids in the ancestral genetic background (indicating plasmid  
27 adaptation), and for the ancestral plasmid in the evolved bacterial genetic background  
28 (indicating bacterial adaptation).

29

### 30 **Mechanisms of amelioration**

31 Co-culture studies therefore suggest that bacteria-plasmid coadaptation could broaden  
32 the conditions favouring plasmid persistence. Such studies highlight 3 key  
33 mechanisms by which amelioration can occur: changes in conjugation rate, loss of  
34 plasmid genes and changes in plasmid gene expression.

1

2 *Conjugation rate*

3 Dahlberg & Chao [25] observed that in two populations, evolved plasmids entirely  
4 lost the ability to conjugate, while another population had a reduced conjugation rate  
5 associated with the evolution of suppression by the bacterial host. Conjugation is  
6 thought to impose a cost to the host, which must invest energy in pili formation and  
7 plasmid replication [34], thus a positive relationship is expected between the cost-of-  
8 carriage and conjugation rates. Such a correlation has been demonstrated by Turner *et*  
9 *al.* [34] who found that plasmids which evolved lower conjugation rates imposed  
10 lower fitness costs in the ancestral bacterial background, while those that had evolved  
11 increased conjugation rates imposed greater costs. Reduced conjugation rates  
12 represent a shift towards higher investment in vertical transmission, and thereby  
13 closer alignment of bacterial and plasmid fitness interests, because plasmid fitness is  
14 more dependent upon bacterial growth rate. These findings stand in stark contrast to  
15 theoretical predictions that plasmid maintenance in the absence of positive selection  
16 requires high conjugation rates [22]. The evolution of reduced conjugation rates  
17 however suggests that co-adaptation may lead to the domestication of plasmid  
18 genomes and a reduction in HGT.

19

20 *Loss of plasmid genes*

21 Amelioration of the cost-of-carriage may also be achieved through the loss of the non-  
22 essential portion of the plasmid genome. When not under positive selection,  
23 accessory genes represent 'excess baggage'; increasing the number of genes requiring  
24 transcription and translation by the host [24]. The loss of accessory genes has been  
25 found to occur during co-culture, and has been shown to lead to a reduced cost-of-  
26 carriage [24]. In one case, amelioration by the plasmid was due to a large deletion,  
27 encompassing  $\frac{1}{4}$  of the plasmid genome as well as a tetracycline resistance cassette  
28 [24]. Large deletion events can therefore be a rapid route to amelioration of the cost-  
29 of-carriage, but the loss of accessory traits from the population would ultimately  
30 negate the role of plasmids in HGT. However, co-culture studies also demonstrate  
31 that, like their plasmid vectors, accessory traits are not easily lost. Interestingly, in the  
32 same study, an ampicillin resistance marker was maintained in the absence of  
33 selection [24]. This difference is likely to be due to the deleted region corresponding  
34 to a mobile **integron**, which was therefore more easily excised. Dahlberg & Chao

1 [25] note that although plasmids lacking antibiotic resistance markers did arise in  
2 experimental populations, they remained at low frequencies through out the  
3 experiment. A longer-term study, following four different multi-drug resistant  
4 plasmids in *Escherichia coli* found that antibiotic resistance was maintained for  
5 between 500 to 1000 generations before genes conferring resistance to different  
6 antibiotics were gradually lost [27]. Therefore accessory gene loss appears to be  
7 unexpectedly rare. Where it does occur, the association of loss events with mobile  
8 elements may allow retention of such genes within the wider mobile gene pool,  
9 simply because those accessory genes most likely to be excised are also those most  
10 likely to integrate elsewhere.

11

### 12 *Reduced gene expression*

13 Gene expression represents a key cost of carrying additional DNA [35-37]; therefore  
14 down regulation of plasmid genes could play a role in amelioration. Transcription is  
15 also likely to present a target for host associated amelioration, as bacteria are able to  
16 exert control over plasmid gene expression [38], potentially stabilizing bacteria-  
17 plasmid associations [39]. Only a single study has investigated the effect of long term  
18 co-culture on plasmid gene expression [33]. Heuer et al. [33] allowed an antibiotic  
19 resistance plasmid to evolve over 1000 bacterial generations in populations of  
20 *Pseudomonas putida*, under a regime in which the plasmid was switched regularly  
21 between host strains. Following 1000 generations under antibiotic selection the cost  
22 of carriage was reduced. Plasmid core genes, including those involved in conjugation  
23 and stability, as well as some accessory genes were down-regulated. Conversely,  
24 plasmid-borne antibiotic resistance genes that were under positive selection were  
25 expressed at a higher level in coevolved bacteria-plasmid clones. Changes in gene  
26 expression are likely to be important for HGT, as reduced expression lowers the costs  
27 associated with accessory genes while allowing their retention and thereby their  
28 maintenance in the population.

29

### 30 **Specificity of coadaptation**

31 Following long-term co-culture of a conjugative R1 plasmid in *E. coli* under positive  
32 antibiotic selection, Dionisio *et al.* [32] observed that evolved plasmids ameliorated  
33 the cost-of-carriage in all populations. Indeed, plasmids from two populations, when  
34 placed into the ancestral bacterial genetic background, actually increased bacterial

1 fitness relative to plasmid-free cells. Surprisingly, this amelioration was maintained  
2 even when evolved plasmids were placed into a naïve *Salmonella* strain [32]. The  
3 mechanism underlying this fitness increase is uncertain, but demonstrates the  
4 potential for generalized plasmid adaptations, whereby adaptations evolved in one  
5 host background can confer improved fitness in alternative hosts. Similar findings  
6 have been reported in studies specifically selecting on plasmid host range. De Gelder  
7 *et al.* [31] show that adaptation of a conjugative plasmid to a novel host (under  
8 positive antibiotic selection) resulted in an expansion of host range, ameliorating the  
9 cost of plasmid carriage in both the ancestral host, as well as a second, naïve novel  
10 host species. A further study demonstrates that regular switching of bacterial host  
11 species resulted in greater amelioration in the ancestral background, relative to  
12 plasmids co-cultured with a single host species [33].

13

14 Generalist plasmid adaptations are not, however, consistently observed across studies.  
15 Modi & Adams [24] describe one evolved plasmid genotype which imposed a smaller  
16 burden on its coevolved host, but when returned to its ancestral host, imposed a  
17 significantly greater burden than the ancestral plasmid [26]. This illustrates the  
18 potential for evolution of specialized coadaptation between host and plasmids, as  
19 opposed to more generalist adaptation observed by Dahlberg & Chao [25], where  
20 adaptations in the evolved plasmid improved fitness in both the evolved and ancestral  
21 bacterial genetic backgrounds. Understanding what drives the evolution of plasmid  
22 specificity will be important in predicting the fate of plasmids in bacterial  
23 communities, and the taxonomic breadth of HGT between strains and species via  
24 conjugation.

25

### 26 **Integration of beneficial genes into the bacterial genome**

27 Under consistent positive selection for plasmid borne traits, theory predicts that  
28 accessory genes will be integrated into the host chromosome [15]. This outcome has  
29 been reported in just one co-culture study. Modi *et al.* (1992) [29] observed  
30 chromosomal integration of a previously plasmid bound ampicillin resistance marker,  
31 located on a Tn3 transposon, in two independent populations. However, contrary to  
32 theory, this occurred in populations grown in the absence of ampicillin, and therefore  
33 not as a consequence of positive antibiotic selection. The absence of integration  
34 events in studies conducted under positive selection indicates that this is perhaps not



1 as widespread a response to selection as predicted [30-34, 40], at least not under  
2 laboratory conditions.

3

#### 4 **Virulent plasmids: the potential for reciprocal antagonism**

5 It should be noted, that coevolution does not always tend towards amelioration of  
6 plasmid burden. In one study, plasmid-bacteria coevolution appeared to be highly  
7 antagonistic under conditions in which multiple plasmids were able to co-infect  
8 bacterial hosts [28]. The resulting within-host competition drove the evolution of  
9 extreme virulence in evolved plasmids when moved into their ancestral hosts, such  
10 that evolved plasmids were lethal in some instances. Reciprocal counter adaptations  
11 were observed in evolved bacterial populations which showed evidence of evolved  
12 resistance to plasmid infection, indicating the potential for antagonistic ‘arms race’  
13 coevolution between plasmids and their hosts.

14

#### 15 **Concluding remarks**

16 Co-culture studies have demonstrated that coadaptation has a major role to play in  
17 explaining the maintenance of plasmids and their accessory genes in bacterial  
18 populations. Under laboratory conditions, coevolution frequently leads to the  
19 amelioration of plasmid burden and consequently significantly broadens the range of  
20 ecological conditions favoring plasmid persistence. The evolution of generalist  
21 plasmids with improved fitness across a range of bacterial genetic backgrounds in  
22 some studies suggests that coevolution can potentially enhance the success of  
23 subsequent HGT event. Conversely, often the mechanisms underlying amelioration,  
24 such as reduced conjugation rate or accessory gene loss, suggest a shift towards  
25 vertical transmission and domestication, and therefore potentially reduced rates of  
26 HGT. Understanding the interaction between coadaptation and HGT requires future  
27 studies to explore a much wider range of ecological conditions to identify those  
28 factors that favour and those that counteract plasmid domestication (see box 3).  
29 Crucially, to date co-culture studies have largely focused on pairwise bacteria–  
30 plasmid associations under constant laboratory conditions, while in nature HGT  
31 occurs in much more complex environmental and community contexts. Several  
32 theoretical models explore the effects of heterogeneous environments [9], spatial  
33 structure [17] and population dynamics [15] on plasmid persistence. However, these  
34 models ignore the potential role of co-adaptation. In order to properly understand the

1 fate of conjugative plasmids and their role in HGT, future theoretical and empirical  
2 work (Box 3) should be directed at bridging this gap.

3

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8 manuscript.

9

#### 10 **Glossary**

11 **Purifying selection:** this acts to remove deleterious alleles from the population.

12 **Positive selection:** this acts to increase the frequency of beneficial alleles in the  
13 population.

14 **Transformation:** is the uptake of DNA from the environment by bacteria.

15 **Transduction:** is the transfer of DNA between cells via a phage vector.

16 **Conjugation:** is the transfer of DNA by direct cell-to-cell contact often mediated by  
17 conjugative plasmids.

18 **Integron:** a mobile genetic element carrying an integrase, which allows acquisition  
19 (or loss) of genes by homologous recombination.

20

Plasmid type <sup>1</sup>	Selection for plasmid borne traits?	Bacterial Generations	Change in cost of carriage <sup>2</sup>	Which party adapted? <sup>3</sup>	Study
<i>Pairwise host – plasmid co-culture</i>					
C	No	1100	↓	c	[25]
N	No	650	↓	c	[24]
N	No	773	- n/a (plasmid lost) -		[29]
N	No	773	↓	c & p	[26]
N	Yes	500	↓	b	[30]
C	Yes	420	↓	c	[32]
<i>Multihost–single plasmid co-culture</i>					
C	Yes	1000	↓		[33]
C	Yes	500	↓		[31]
N	Yes	1000			[40]
<i>Long term persistence</i>					
C & N	No	4000			[27]
<i>Within-host competition of coinfecting plasmids</i>					
C	No	400	↑		[28]
<i>Enforcing horizontal and or vertical modes of plasmid transmission</i>					
C	Yes	500	↓		[34]

Table 1. Summary of co-culture studies and their outcomes

<sup>1</sup> Conjugating (C) or non-conjugating (N)

<sup>2</sup> ‘↓’ denotes a reduction in the cost-of-carriage, ‘↑’ denotes an increase

<sup>3</sup> ‘c’ denotes coevolution, ‘p’ denotes plasmid evolution and ‘b’ denotes

1

2

1 **Box 1. What makes a plasmid?**

2 Plasmid genomes are modular in structure, such that genes are broadly arranged into  
3 discreet operons encoding specific functions [41]. This structure is a consequence of  
4 frequent genetic recombination, forming a mosaic of genes from different sources.  
5 Plasmids can be subdivided into a core ‘backbone’ of genes encoding plasmid  
6 functions, and ‘accessory’ genes encoding traits beneficial to the bacterial host  
7 (discussed in the main text). ‘Backbone’ genes encode the following key functions:  
8 replication, segregation and conjugation.

9

10 Replication is the only function required to meet the basic definition of a plasmid.  
11 The replication region generally consists of an origin of replication (*ori*) as well as  
12 proteins that recruit the host’s own DNA replication machinery (i.e. polymerase  
13 molecules, tRNAs and ribosomes) to carry out replication. Genes regulating plasmid  
14 replication are also common on plasmids, to ensure that the number of plasmid copies  
15 in the host remains stable.

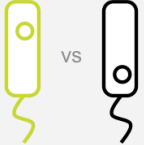
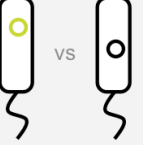
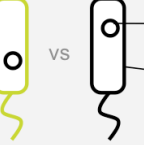
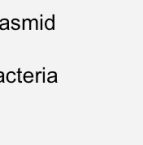
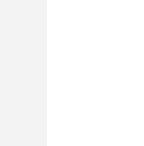

16

17 Segregation systems act to minimise the loss of the plasmid during cell division.  
18 High copy number plasmids often lack such systems and rely on diffusion to ensure  
19 plasmids are present in both mother and daughter cells. However low copy plasmids  
20 often take a proactive approach to minimise mis-segregation. Active partitioning  
21 (*par*) systems mimic the mitotic process. Plasmids encode proteins that bind to a  
22 centromere-like region and direct plasmid molecules towards the poles of the dividing  
23 cell. Alongside this, many plasmids also utilise post-segregational killing. These  
24 encode a toxin-antitoxin system producing a stable toxin and a less stable antitoxin  
25 molecule: if the plasmid is lost, the antitoxin degrades quicker than the toxin in the  
26 cell, leading to cell death.

27

28 Conjugation genes allow the plasmid to transmit horizontally through cell-to-cell  
29 transfer. Conjugative plasmids encode genes for ‘mate pair formation’ – the  
30 formation of a physical link between donor and recipient cells, often in the form of a  
31 pilus. A second, sometimes separate, set of genes allows the one strand of the  
32 plasmid DNA to move into the recipient cell and become established [41]. Many  
33 ‘mobilizable’ plasmids forgo the need to carry their own mate pair formation genes  
34 however, piggybacking on the actions of coinfecting conjugative plasmids [41].

1 **Box 2. Measuring coevolution**

Competition experiments	(a)		(b)		(c)	
						
Patterns of relative phenotypic change	1	↑	↑	=	=	= Plasmid adaptation
	2	↑	=	↑	↑	= Bacterial adaptation
	3	↑	↑	↑	↑	= 'Generalized' coadaptation
	4	↑	↓	↓	↓	= 'Specialized' coadaptation

2  
3 Figure I Coevolutionary changes can be detected through a series of comparisons between the different  
4 combinations of evolved (green) and ancestral (black) plasmid and bacteria, to the ancestral plasmid  
5 and bacteria. The pattern of change (arrows) and stasis (=) in fitness relative to the ancestor can be  
6 used to disentangle whether evolutionary or coevolutionary changes have occurred.

7  
8 Coevolution can be inferred where changes in fitness (or other traits) are associated  
9 with adaptation in both plasmid and bacteria, following long-term co-culture. In  
10 figure I a series of competition experiments are shown in grey which can be used to  
11 unravel these interactions: (a) overall change is measured by competing the evolved  
12 (green) bacteria-plasmid against the ancestral (black) genotype, (b) adaptation in the  
13 plasmid is estimated by measuring fitness of the evolved plasmid in the ancestral  
14 background and (c) adaptation in the bacteria is measured by measuring fitness of the  
15 evolved bacteria carrying an ancestral plasmid.

16  
17 Whether evolutionary or coevolutionary changes have occurred can then be inferred  
18 from the pattern of fitness change relative to the ancestor, where arrows denote  
19 change and = denotes no difference from ancestor. In Figure I, four hypothetical  
20 scenarios illustrate this point: (1) Where a difference is observed in comparisons (a)  
21 and (b), but not (c) this implies that no significant adaptation has occurred in the  
22 bacteria. Therefore the change is driven primarily by plasmid evolution. (2) In  
23 contrast, if no adaptation in the plasmid (b) is detected, this implies that the change is  
24 due to bacterial evolution. (3) If an increase in fitness is seen in all 3 comparisons,  
25 then this represents 'generalized' coadaptation, as adaptation has occurred in both  
26 plasmid and bacteria but is not specific to the coevolved partner. (4) If the change in

1 fitness in the coevolved bacteria-plasmid pair (a) is opposite to that measured in the  
2 plasmid (b) and bacteria (c) alone, this may indicate ‘specialized’ coevolution, as the  
3 increase in fitness is specific to the presence of the coevolved partner.

4

1 **Box 3. Future directions**

2

3 *The genetic basis for coevolution:* Deletion of sections of the plasmid genome – for  
4 instance, those encompassing accessory traits – is just one mechanism that plasmids  
5 can employ to reduce the physiological burden on the host. Selection can also focus  
6 on genes encoding core functions such as conjugation [34], segregation or more subtle  
7 changes such as reducing gene expression [33], which compensate for the presence of  
8 these additional genes. Understanding how frequently, and under what circumstances  
9 these different mechanisms occur will be an important step in understanding and  
10 predicting the fate of horizontally transmitted traits in microbial communities.

11

12 *Coevolution in complex environments:* Whether plasmids are beneficial or costly to  
13 their bacterial hosts is determined by the selective environment (e.g. the presence or  
14 absence of antibiotics). Heterogeneity in the direction of selection can theoretically  
15 favor the maintenance of beneficial traits on mobilizable plasmids [9], and such  
16 heterogeneity is predicted, by coevolutionary theory, to affect the maintenance of  
17 coadaptation across populations [42]. The interplay between ecological and  
18 evolutionary factors is likely to be crucial to understanding HGT in natural  
19 populations.

20

21 *Coevolution in the meta-community:* Many plasmids are promiscuous in terms of host  
22 range, and are likely to compete with other genetic elements with which they share  
23 hosts. Coevolution with multiple host species may impede adaptation to any given  
24 host because the intergenomic linkage between co-adapted genes will be continuously  
25 broken down. Competition and conflict with other mobile elements may drive greater  
26 antagonism between hosts and plasmids [28]. What impact therefore does community  
27 context have on bacteria-plasmid coevolution?

28

29 *Levels of coevolutionary selection:* The mobilizable elements on which beneficial  
30 accessory traits are themselves often located are likely to be subject to selection in  
31 their own right. HGT may therefore be a tripartite coevolutionary process between  
32 bacteria, conjugative plasmids and mobilizable elements; at what level reciprocal  
33 selection acts is likely to depend upon the environmental and community context.

34

35

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