Why Do Bacterial Plasmids Carry Some Genes and Not Others?

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Previous explanations of why bacterial genes for certain "optional" traits tend to occur on plasmids rather than chromosomes are based on an outdated misunderstanding of natural selection. They also fail to explain why certain characters that are ubiquitous in some bacterial species tend to occur on plasmids. This paper shows that all major classes of traits usually associated with plasmids rather than chromosomes confer adaptations to locally restricted conditions. A new "local adaptation" model of plasmid evolution, based on simultaneous application of modern selection theory at the levels of gene, plasmid, cell, and clone reproduction, shows that genes coding local adaptations will reproduce more successfully when on plasmids than when on chromosomes, due to plasmids' greater horizontal mobility.

Plasmid genes often encode "optional" characteristics which are selectively advantageous to bacteria in some environments but not others. The function of plasmids is commonly thought to be to provide a scattered reserve of variants that enables a population or species to adapt to new environmental contingencies without burdening most individual cells with often superfluous genes (Reanney, 1976; Hopwood, 1978; Broda, 1979; Koch, 1981; Campbell, 1981; Datta, 1985; Willetts and Clewell, 1985; Shapiro, 1985). This and other explanations (e.g., Betley et al., 1986) that are based on the supposition that natural selection acts for the good of populations or species are probably wrong.

Advances in evolutionary theory have shown that the effects of a trait on lower levels of reproduction (e.g., individual or genic) rather than those on higher levels such as populations or species generally determine whether the trait is favored by natural selection (Williams, 1966; Dawkins, 1976, 1982; Alexander and Borgia, 1978, Leigh, 1983, 1987; Ewald and Schubert, in press). This means that because plasmids often have negative effects on the reproduction of individual bacterial cells, they cannot be maintained by the occasional benefits they confer on populations or species.

In addition, some typically plasmid-borne traits are not optional in the usual sense and are in fact found in most isolates from nature (e.g., Brubaker (1985) and Aronson et al. (1986) on virulence in enteric bacteria and insect pathogens; Nester and Kosuge (1981) on plant gall bacteria). Some large plasmids (>250 MDa) are never absent from Agrobacterium tumefaciens or the nitrogen-fixing Rhizobium meliloti, suggesting that the genes they carry are essential for these cells' growth (Denarie et al., 1981). "Optional character" hypotheses do not account for these traits being on plasmids.

This paper presents an alternative explanation of why genes for some characters but not others tend to occur on plasmids, using analyses of differential reproduction of genes, plasmids, and the cells and clones which contain them. The terms "function" and "adaptation" are used here only in the restricted sense appropriate for evolutionary discussions: the function of a trait refers to a context in which the trait confers reproductive advantage upon its bearers (vs nonbearers), with the result that the trait becomes more common in the population. It is important in evolutionary discussions, which attempt to distinguish cause and effect, to differentiate the primary functions of a trait which contributed to its original
spread and its maintenance via differences in the reproduction of bearers and nonbearers, from second-order effects which are incidental consequences of the trait's becoming established (Williams, 1966; Gould and Vrba, 1982; Vermij, 1987). This distinction between function and effect has not been made carefully in some previous discussions of the evolution of plasmids and other mobile elements. To use an example that has generated recent controversy, when transposons and insertion sequences propagate themselves within a genome, they often generate new gene combinations and mutations; these arc occasionally advantageous and have undoubtedly influenced the course of evolution in different groups. But this does not justify claiming that the generation of new combinations (which usually reduce their bearer's reproduction) is their function (Campbell, 1981; Syvanen, 1984) (see Charlesworth, 1987). Just because a consequence of a trait is to increase certain kinds of change does not mean that the function of the trait is to produce these changes.

GENERAL CONSIDERATIONS

Bacterial genes move between plasmids and chromosomes (Rowbury, 1977; Hartl and Dykhuizen, 1984), as well as from one plasmid to another (Godwin and Slater, 1979; Broda, 1979). Given common population sizes and rates of interchange (e.g., Lewin, 1977), and the probably very long evolutionary lives of many genes in bacteria, it is probable that most bacterial genes have been in both plasmids and chromosomes repeatedly during their evolutionary lives. This must be especially true for genes associated with insertion sequences and for bacterial species with F-like plasmids which integrate into the chromosome. Chromosomes and plasmids thus share, to a substantial degree, a common gene pool. To determine why some types of genes and not others tend to occur on plasmids, one must ask why some genes have done better (made more copies of themselves) when located on plasmids than when on chromosomes.

Bacterial genes are not randomly distributed between chromosomes and plasmids. Two general classes of traits tend to be coded on plasmids rather than chromosomes: “plasmid-selfish” characters such as conjugation, mobilization, inhibition of bacterial cell division, and plasmid surface exclusion and partitioning, which promote the survival and replication of the plasmid as a unit; and optional characters such as resistance to antibiotics, heavy metals, and other poisons, virulence, metabolism of unusual substrates, plant gall formation, root nodulation and N fixation, and bacteriocins (Broda, 1979), characters which favor the survival and reproduction of the entire cell in certain environments. The reason plasmid-selfish characters tend to occur on plasmids is clearly because they favor plasmid (rather than chromosome) reproduction; they make plasmids resemble independent organisms in some respects (Novick, 1980; Saliers, 1984; Datta, 1985). These traits set the “rules of the game” for analyses of why other, non-plasmid-selfish genes also tend to occur on plasmids.

The question of why optional genes tend to occur on plasmids is posed here at the level of gene reproduction, but its answer involves analyses at three higher levels of reproduction: plasmids vs chromosomes within cells; cells that do or do not have plasmids in addition to their chromosomes; and clones with or without plasmid-carrying cells. The reproductive advantages or disadvantages of a given gene at these different levels are of course to some extent synonymous. But some traits can increase the reproduction of units at one level while decreasing reproduction at others. Hierarchical living systems show “emergent” concerns at different levels (Buss, 1987), as well as conflicts of reproductive interest between different levels (Buss, 1987; see also Alexander and Borgia, 1978; Eberhard, 1980; Cosmides and Tooby, 1981; Leigh, 1987). Thus consideration of possible synergisms and conflicts between selection at all four levels is necessary to understand plasmid evolution. Previous analyses which did not carefully separate and weigh advantages at these different levels (e.g., Reanney, 1976; Koch, 1981; Campbell, 1981) produced confusing results.
NEGATIVE EFFECTS OF PLASMIDS ON CELL AND CLONE REPRODUCTION

Plasmids have been thought to be to some extent parasitic on the bacteria in which they reside, reducing the reproductive potential of their carriers as a result of promoting their own maintenance and propagation (Salyers, 1984; Datta, 1985). Most evidence supporting these ideas is of two types: experimental demonstrations that plasmid-carrying strains usually lose in competition with similar bacteria when under more or less artificial conditions (Anderson, 1974; Godwin and Slater, 1979; Mell- ing et al., 1977; Zünd and Lebek, 1980; Duval-Iflah et al., 1981; Fretter et al., 1983; Scholz et al., 1985); and demonstration that bacteria found in natural environments lacking conditions for which plasmid genes would confer special advantages (e.g., environments lacking antibiotics) tend to lose or to lack plasmids carrying those genes (Grabow et al. (1974), Koch (1981), and Hughes and Datta (1983) on antibiotic resistance; Robinson and Tuovi- nen (1984) on heavy metal tolerance; Dowling and Broughton (1986) on root nodulation; see also Cavalier-Smith (1985) on superfluous genes in general). The combination of these lines of evidence suggests that plasmids per se often have negative effects on bacterial fitness.

PLASMIDS AND LOCAL ADAPTATIONS: A NEW MODEL

Although rates of horizontal transfer vary between plasmids and in different bacteria (Broda, 1979), genes on plasmids are generally more likely to be transferred horizontally and thus cohabit cells with different chromosomal and plasmid genes than those on chromosomes (Wheelis, 1975; Lewin, 1977). The more frequent horizontal transmission of plasmid genes results in a wider variety of genetic settings in which they can act, giving the plasmid genes more of the selective advantages of sexual reproduction (Felsenstein, 1974; Williams, 1975; Maynard Smith, 1978; Leigh, 1987). One expected result is that plasmid genes will be able to evolve more rapidly than chromosomal genes in response to environmental change.

In addition, genes coding for adaptations to variations in environmental conditions that occur only sporadically in time or space should reproduce more rapidly when on plasmids. Arguments supporting this claim will be developed using antibiotic resistance as an example, then extended to other functions.

Antibiotics are generally restricted to the immediate vicinity of antibiotic-producing organisms such as certain soil fungi, actinomycetes, and other bacteria (Broda, 1979; Martin and Demain, 1980) and, recently, to sites of modern medical treatment and industrial antibiotic production. Several factors contribute to the maintenance of a locally advantageous character like antibiotic resistance on plasmids rather than chromosomes. Consider two otherwise equal strains of bacteria carrying an antibiotic resistance gene, one with the gene on a chromosome and the other with the gene on a plasmid. The resistance gene produces a phenotype that has a selective advantage only in the conditions that prevail in certain locations where antibiotic is present. At such sites both strains will be favored over bacteria lacking resistance genes, but the resistance genes on plasmids will outreproduce those on chromosomes for several reasons:

1. New bacterial strains colonizing the site are unlikely to carry the resistance gene (e.g., Broda, 1979; Koch, 1981; Hughes and Datta, 1983). These new bacteria, which are more likely to lack a plasmid of the incompatibility type that carries the resistance gene will thus be more likely to be receptive to horizontal transmission and more likely to benefit from the plasmid-coded local adaptation. Thus advantages at both the cell and the plasmid levels would make a resistance gene on a plasmid able to propagate itself more rapidly than if it were on a chromosome.

2. The resistance gene on plasmids will inhabit cells containing a much greater variety of other chromosome and plasmid genomes than will the genes on chromosomes. Plasmid genes will thus have a greater likelihood of as-
sociating themselves with superior genes for other functions and winning out in competition between clones. This could be an important advantage if sequential replacements of clones such as those observed in intestinal *Escherichia coli* (Caugant et al., 1981) are common modes of evolution in bacteria.

3. Some antibiotic resistance genes on plasmids are amplified quickly when needed and rapidly eliminated when not needed (Broda, 1979; Clewell, 1981), conferring increased fitness on the cells and clones carrying them. The resistance gene on transposon Tn9 is amplified more readily when the gene is on a plasmid (fewer other gene products are needed) than when it is on a chromosome (Mahajan et al., 1985). This expected advantage will be especially strong when gene expression is constitutive, as is generally true for resistance plasmids in gram-negative bacteria (Burman, 1977).

4. There is an additional positive feedback in the advantages to local adaptation genes of being on plasmids, since the advantage of being on a plasmid is greatly increased if the plasmid already carries another gene or genes coding for adaptations to the same or similar local conditions. The tighter the association between the different conditions for which the different genes confer advantages, the greater this additional linkage advantage. Even if the conditions are very weakly associated, a second gene could benefit from hitchhiking along with another, at least if the rate of recombination is low enough. When different genes both confer adaptations to the same conditions, the “piling on” advantage could be very powerful and probably accounts for the remarkably rapid additions of genes to some resistance plasmids such as the hospital-associated plasmid that added four antibiotic resistance genes and grew from 45 to 65 MDa in the space of only about 10 years (Datta, 1985), and the mercury resistance transposon on the plasmid CS229 in hospitals which also rapidly added multiple antibiotic resistance genes (Robinson and Tuovinen, 1984).

The same type of reasoning explains the reverse side of the coin—the tendency for genes of general usefulness (“housekeeping genes”) to not occur on plasmids (Koch, 1981). Horizontal transmission will not confer the reproductive advantages just mentioned on genes whose products are useful in all potentially habitable environments.

**COMMON PLASMID FUNCTIONS AS LOCAL ADAPTATIONS**

Some other plasmid functions in addition to antibiotic resistance clearly seem to be local adaptations: the ability to metabolize or otherwise resist heavy metals and other poisons, which probably usually have patchy distributions (Robinson and Tuovinen, 1984); virulence characters in facultative pathogens; and the ability to degrade unusual substrates (Wheelis, 1975; Broda, 1979; Ghose et al., 1985). Some plasmid functions, however, such as virulence in obligate pathogens, plant gall, and nodule formation and N fixation, and production of bacteriocins do not appear to fit this characterization. I will argue, nevertheless, that they too are only locally adaptive.

**I. Virulence Factors**

At first glance, virulence (that subset of characters which is useful in establishing infections by dealing with conditions associated with the host, but which do not have any known use to the bacterium in other contexts) does not seem to be a local adaptation in bacteria which are obligate pathogens. For instance, those bacteria which cause invasive diseases in animals generally grow poorly or not at all outside their hosts (Bulla et al., 1975; Brubaker, 1985; Aronson et al., 1986), and virulence factors tend to be present throughout these bacterial species (Bulla et al., 1975; Brubaker, 1985). Closer examination shows, however, that these virulence factors probably confer only local advantages.

Many of these bacteria are not host-specific, and some attack a wide spectrum of hosts. Different host species appear to harbor disease bacteria with slightly different virulence-coding plasmids. For example, there are “literally thousands of” strains of insecticidal *Bacillus*
thuringiensis and its relatives, and "no single isolate is active against all pest species" (Aronson et al., 1986); different plasmid-coded protoxins are active against different insect groups. Different hemolysin genes on plasmids in vertebrate pathogens also have different levels of toxicity in different host species (Gaastra and van Graaf, 1982; Welch and Falkow, 1984; Goebel et al., 1985). In enterotoxic E. coli that cause diarrhea in man and farm animals, plasmid-coded fimbrial antigens that mediate adhesion to the intestinal epithelium differ and are host-specific (Willshaw et al., 1985). These different virulence factors thus represent adaptations to taxonomically "local" conditions.

Different strains of bacterial pathogens of plants also often have limited host ranges, and in some cases it is known that host specificity is coded on plasmids (Panopoulos and Peet, 1985). It is also possible in both plants and animals that different individuals of the same host species may require different adaptations by pathogens.

A further possible advantage of horizontal transmission of virulence factors occurs at the level of mutualisms between clones. Some host defenses are more likely to be overcome by massive invasion than by small numbers of disease organisms (e.g., Hirano and Upper, 1983). Horizontal transmission of virulence factors could raise the chances of successful infection for all invading bacteria.

II. Plant Gall and Nodule Factors

The better-studied genera Agrobacterium and Rhizobium are similar to the pathogens just discussed, as host specificity is known to be encoded on plasmids. The two or three Agrobacterium species (Elkan, 1981) produce tumors in 142 genera in 61 families of plants (Lippincott and Lippincott, 1975), while the two Rhizobium groups (Elkan, 1981) also grow in a wide variety of plants (>1130 species in the families Leguminosae and Ulmaceae) (Stowers, 1985). Not all bacterial strains invade all host species equally well, and plasmids often code this host specificity (Nester and Kosuge, 1981; Denarie et al., 1981).

Plasmids of the less-studied Pseudomonas savastanoi code a diversity of cytokinins; since such diversity is associated with host specificity in other groups (see Aronson et al. (1986) on B. thuringiensis; Cavalieri et al. (1984) on hemolysin plasmids; Panopoulos and Peet (1985) on other plant pathogens), this suggests that these plasmids may also confer host-specific adaptations.

The host specificity of plasmid functions suggests a locally adaptive selective regime similar to that just described for virulence factors. Mutualism between clones may also operate, as infections by larger numbers of Rhizobium are more likely to produce nodulation (Dowling and Broughton, 1986).

In addition, both gall and root nodule bacteria have an alternate habitat (free in the soil outside of host plants). Different strains differ in their abilities to survive and compete with other bacteria in different soil types, and plasmid genes are known to be responsible for some of these differences (Dowling and Broughton, 1986; see also Moore and Cooksey, 1981, on Agrobacterium). In sum, characters promoting invasion and intraplant reproduction are clearly adaptations for life in the local vicinity of certain plants.

III. Bacteriocins

As a group, bacteriocin plasmids are relatively common, at least in some bacteria: 10 of 32 E. coli isolates collected prior to the antibiotic era produced bacteriocins, and this may be an underestimate (Hughes and Datta, 1983). If bacteriocin plasmids are so common and are advantageous in competition with other closely related bacteria (Broda, 1979), could they still represent only local adaptations?
The probable answer is yes, since most bacteriocins are degraded by proteases such as those common in important habitats like the vertebrate intestine and dental plaque (Hardy, 1975). Bacteriocins are also less effective in anaerobic conditions such as those of vertebrate intestines. These same bacteriocins are more active in urine, blood, and the peritoneal cavity (Hardy, 1975). Thus the advantage of having bacteriocin plasmids probably depends to a substantial degree on local conditions.

Modulation of bacteriocin production suggests that the poisons are costly and only locally useful. When densities of cells containing bacteriocinogenic Col factors are increased or the cells are starved of thymine, the percentage of cells producing toxin in large quantities rises from 0.01 to 1% (Broda, 1979). In Erwinia uredovora bacteriocin production is correlated with gene amplification, and noninduced stages show only low amplification (Thiry et al., 1985).

DISCUSSION

The goal of this article is not to explain all plasmid characters, but to understand why certain subsets of bacterial genomes—genes that confer local rather than general adaptations—tend to occur on plasmids rather than chromosomes. Excluded from consideration were the large number of cryptic plasmids as well as other plasmid genes with a variety of other phenotypic effects (e.g., Koyama and Yura, 1975; DiJoseph et al., 1973; Chernin and Mikoyan, 1981). Some plasmid-coded traits, such as uv resistance in Streptococcus (Clewell and Gawron-Burke, 1986), may also prove to be local adaptations. Some cryptic plasmids may also be found to carry genes for local adaptations, while others may be parasitic. Levin and Stewart (1980) and Caugant et al. (1981) argue that most cryptic plasmids, and especially nonconjugative cryptic plasmids, cannot be maintained unless they code for selectively advantageous functions, but this may not be true for some transposons (Biel and Hartl, 1983).

The local adaptation theory was presented above in terms of extreme cases (local vs general adaptations), but these are obviously the ends of a continuum. Genes conferring advantages which are less locally restricted would derive less advantage from being on plasmids. And if a given plasmid became more and more common, the disadvantages to a housekeeping gene of being on that plasmid would gradually decrease. Eventual transfer of one or more such genes could tend to "fix" the plasmid, making it subject to more chromosome-like selection. This may have occurred for a large Rhizobium plasmid (Dennar et al., 1981).

Plasmid-selfish selection is also obviously important, and some plasmids may simply be parasites, carrying no genes useful to the cell (Datta and Hughes, 1983, Salyers, 1984; Datta, 1985). The sheer numbers of plasmids and the variety of genes they carry suggest that still other explanations may be needed.

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