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EVOLUTION IN BACTERIAL PLASMIDS AND LEVELS OF SELECTION

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ABSTRACT

Gene flow between different reproductive units such as bacterial plasmids and chromosomes presents unusual problems for evolutionary analysis. Far more than in eukaryotes, reproductive advantages at several levels of selection—genes, transposons, plasmids, cells, and clones—must be considered simultaneously to understand plasmid evolution. No level consistently prevails in conflict situations, and some reproductive units carry genes that restrain their own reproduction or survival, apparently to enhance the reproduction or survival of the higher-level reproductive units that carry them.

Despite gene flow between plasmids and chromosomes, genes for certain functions show strong tendencies to occur on plasmids while others consistently occur on chromosomes. Functions generally associated with plasmids are diverse, but all are useful only in locally restricted contexts; it is argued that the selective consequences of the greater horizontal (within generation) transmission of plasmids are responsible for this pattern. The tendency for prokaryote transposons, which are also horizontally mobile, to carry genes similar to those commonly on plasmids supports this argument. The apparent trends in eukaryote plasmids and transposons to lack these same characters also accords with predictions of the local adaptation hypothesis, because genes on these genetic units are generally no more horizontally mobile than chromosomal genes. There are theoretical reasons to expect that plasmid genes tend to evolve more rapidly than chromosomal genes.

“The selfish interests of genes have manifestly produced ‘vehicles’ in the forms of organelles, cells, individuals and yet higher units. If evolution is to predict as well as describe, then selfish interests must be understood in the framework of the constraints and opportunities generated by these ‘vehicles’” (Buss, 1987, p. 182).

INTRODUCTION

AS IN OTHER FIELDS of biology, the development of evolutionary theory has been both guided and limited by the idiosyncracies of certain groups of organisms. The

most familiar organisms for the great majority of evolutionary biologists have been multicellular, higher eukaryotes, especially vertebrates and insects. A logical stage in the ontogeny of a field such as evolutionary theory is to attempt to extend its concepts to organisms with sets

of idiosyncracies that differ from those of the groups for which the theory was originally designed. The expectation is that some beliefs will be strengthened by independent confirmations, while others may have to be adapted or modified to encompass new phenomena.

An extraordinary amount of detailed information has accumulated in the past twenty years on the genetics and reproductive behavior of bacterial plasmids, rings of extrachromosomal DNA which are present in most bacteria (Broda, 1979; Helinski et al., 1985). These replicating units pose a number of problems for conventional evolutionary theory, but the generally poor communication between molecular and evolutionary biologists has kept these problems from receiving the attention merited by our now detailed knowledge of their biology. The objective of this paper is to examine several aspects of plasmid natural history in the light of modern evolutionary theory. First, key aspects of plasmid natural history will be reviewed from an evolutionary perspective. Then I will attempt to explain why genes for certain types of functions usually occur on plasmids rather than chromosomes, while others occur almost exclusively on chromosomes, by discussing why some kinds of genes are expected to reproduce more effectively on plasmids and others more effectively on chromosomes. Predictions of the new "local adaptation" hypothesis I propose are then tested by examining data on bacterial transposons, viruses, and eukaryotic plasmids and transposons. Finally the complex interplay between selection acting at different levels of organization (genes, transposons, plasmids, chromosomes, cells, and clones) is examined and its implications for evolutionary theory are discussed.

NATURAL HISTORY OF BACTERIAL PLASMIDS AND THEIR REPLICATION

Plasmids range in molecular weight from about 1 to about 500 kilobases (Kb) (Broda, 1979; Darnell, Lodish, and Baltimore, 1986; 1 Kb is the approximate size of one typical gene, and bacterial chromosomes range from about 285 to 11,000 Kb; the *E. coli* chromosome has about 3570 Kb—Herdman, 1985). Plasmids often represent about 1 percent of a bacterium's genome, though in some species up to 10 to 30 percent is in plasmids (Banfalvi, Kondorosi, and Kondorosi, 1985; Panopoulos and Peet,

1985). Plasmids are very common and diverse. Most strains of *E. coli* contain one or more plasmids, with the average being about four kinds of plasmid per strain and a maximum of 11 in some samples (Silver, Aaronson, Sutton, and Schneerson, 1980). It is difficult to categorize plasmids, but Duckworth, Glenn, and McCorquodale (1981) noted more than 250 kinds in *E. coli* alone.

The most important (and unusual) fact about plasmids for the evolutionary analyses here is that they sometimes merge partially or completely with chromosomes. Bacterial genes move between plasmids and chromosomes (Rowbury, 1977; Hartl and Dykhuizen, 1984), from one plasmid to another (Godwin and Slater, 1979; Broda, 1979), and even from one bacterial species or genus to another (Campbell, 1981). Given common population sizes, measured rates of interchange (e.g., Lewin, 1977), and the astronomical numbers of bacterial chromosomes and plasmids that have existed over the course of evolutionary history, it is probable that most if not all bacterial genes have been in both plasmids and chromosomes repeatedly during their evolutionary lives. Gene flow between plasmids and chromosomes must be particularly common for genes on transposons, which insert themselves in both plasmids and chromosomes, and in bacterial species like *E. coli*, which harbor plasmids that are able to incorporate themselves temporarily in chromosomes, then sometimes take along chromosomal genes when they break free again (Broda, 1979). To a very appreciable degree, chromosomes and plasmids share a common gene pool. Nevertheless, there are clear trends for some kinds of genes to occur on plasmids, and others on chromosomes (see below).

In addition to being inherited from one bacterial generation to the next, many plasmids can also move "horizontally" from one cell to another by conjugating, a process that is initiated by gene products of the plasmid itself or of other plasmids in the cell. Occasionally, chromosomal genes are also transferred during conjugation but, in general, plasmid genes are much more horizontally mobile than those on chromosomes. Horizontal transmission is usually intraspecific, but occasionally occurs between different species and even different genera (Broda, 1979). Rates of transfer vary greatly with plasmid type and with donor and

receiver strains of bacteria. Under some conditions virtually all plasmid-less cells in a bacterial culture receive plasmids soon after a few plasmid carriers are introduced (e.g., Broda, 1979; Hopwood, Lydiate, Malpartida, and Wright, 1985), though transmission under other conditions is much less common (Freter, Freter, and Brickner, 1983).

Thus, neither prokaryotic species, their chromosomes, nor their plasmids constitute genetically discrete units or individuals in the sense in which these terms are used in most evolutionary discussions (Maynard Smith, 1982). Large taxonomic groups such as genera and families are connected by at least occasional gene flow (Reaney, 1976; Broda, 1979). In addition, bacterial cells normally grow in clones, so selection can also operate at the level of the entire clone, favoring characters not advantageous to individual reproduction (e.g., Jackson and Hughes, 1986). Bacteria and their plasmids are thus challenging topics for evolutionary theory.

Vertical Transmission

Probably all plasmids have one or several "replicons" (short segments of only 1 to 3 Kb where DNA replication originates), which are necessary for initiation of their own replication during the cell cycle (Scott, 1984; Nordstrom, 1985a). Many cellular enzymes (those coded on the chromosome) are necessary for plasmid replication (Broda, 1979; Scott, 1984). Other plasmid genes function in partitioning or distributing a plasmid or plasmids to each daughter cell (Scott, 1984). Some plasmids with high "copy numbers" (number of plasmid copies per chromosome) apparently lack a partition locus, and rely on the high probability that each daughter cell will contain at least one copy (Kasner and Rownd, 1985; Nordstrom, 1985b). Different plasmids use different partitioning mechanisms (Austin and Abeles, 1985), suggesting that the ability may have arisen more than once.

Still other plasmid genes inhibit bacterial cell division until enough copies of plasmid DNA are available to provide copies for each of the new cells (Kline, 1985; Nordstrom, 1985b). This inhibition may be important since the replication of at least some plasmids is not coupled temporally with that of the chromosome or cell division (Broda, 1979). All plasmids

studied to date carry genes that exert inhibitory control over their own replication (Nordstrom, 1985a). Occasionally a plasmid fails to be included in both daughter cells, and a lineage "cured" of the plasmid is created. One plasmid gene apparently functions in plasmid maintenance by killing newly generated cells lacking the plasmid (Gerdes et al., 1986).

Horizontal Transfer

Horizontal transfer of plasmids is promoted by a variety of plasmid gene products. These include (1) cell extensions (sex pili), which can serve as grappling hooks to snare other cells or which function in the actual DNA transfer to new cells during conjugation (Bradley, 1985); and (2) a set of gene products that anchor the plasmid at the transfer site, open the ring and unwind it, replicate it, and transfer one copy to the other bacterium (for example, 13 different genes on the F plasmid of *E. coli* are involved in these functions—Willets and Wilkins, 1984; Laine, Moore, Kathir, and Ippen-Ihler, 1985). Additional examples of horizontal transfer products include the following: *Streptococcus* plasmid genes for "aggregation substance" (or perhaps a complex of substances—Tortorello and Dunny, 1985), which causes a clumping response to occur in the presence of "sex pheromone" (possibly degradation products—Clewell, 1981) from nearby plasmid-less cells (Clewell, 1981); crown gall plasmid sequences, which permit incorporation of some copies of plasmid DNA into the host plant cell's genome and subsequent production of compounds (opines) that increase the rates of conjugation of other bacteria present in the gall (Nester and Kosuge, 1981); a very short segment on *Streptomyces* plasmids that enables the plasmids to transfer between mycelia (Hopwood et al., 1985); and possibly an ability in *E. coli* to migrate toward plasmid-less cells (Collins and Broda, 1975).

Some plasmids (often smaller in size) do not have the genes needed to carry out conjugation, but use structures and enzymes produced by "conjugative" plasmids in combination with their own "mobilization" genes to transfer themselves. In general the effectiveness of mobilization varies with the identities of the plasmids involved. Occasionally plasmids are transferred horizontally by physically joining ("cointegrating") with a conjugative plasmid (Willets and Clewell, 1985).

Not all cells carrying conjugative plasmids are able to perform conjugation, and the frequency of competent cells in a clone can fall from more than 50 percent to as little as 0.02 percent in only 7 generations after a cell first receives a plasmid (Lewin, 1977, and Broda, 1979, on R, F, and Col plasmids). Such transfer inhibition is coded by plasmid genes, and may have evolved independently in different plasmids (Lewin, 1977; Broda, 1979). It appears that control of horizontal transmission of these plasmids is designed to allow them to sweep through a plasmid-less population in epidemic fashion (Broda, 1979), but then to reduce conjugation and its attendant costs (see below). As the fraction of cells in a culture still lacking plasmids falls, the benefits to the plasmid of mating competence will also fall, until they equal their costs to the plasmid through reduced bacterial reproduction (Levin and Lenski, 1983). At this point selection on the plasmid favors repression of mating competence. This interpretation is complicated, at least in the case of the ColE1 plasmid, by the fact that the protein that represses plasmid replication also makes the plasmid better able to persist in the host bacterium (Summers and Sherratt, 1985).

The alternative tactics of conjugative and non-conjugative plasmids are reflected in the distribution of plasmid sizes, which is apparently bimodal; the peaks around 4 to 8 Kb and 70 to 100 Kb correspond, in general, to non-conjugative and conjugative plasmids (Broda, 1979).

Protection against Invasion

Apparently all plasmids carry genes that inhibit the invasion of their bacterial cell by certain other plasmids. This "surface exclusion" is probably an adaptation to promote the plasmid's own vertical (as well as horizontal) transmission. Plasmid replication within a cell is limited by the number of other plasmids of the same type ("incompatibility type") present there (Broda, 1979; Scott, 1984); and only plasmids of the same incompatibility type are kept out by surface exclusion. There are about 20 to 30 incompatibility types in gram negative bacteria (Broda, 1979), and also multiple types in gram positive bacteria (Clewell, 1981); evolutionary diversification of incompatibility types may have resulted from competition within al-

ready existing types (Eberhard, 1980; Levin and Lenski, 1983). The presence of a plasmid of one incompatibility type has little effect on the replication of those of other incompatibility types (up to 13 types occur in a single cell — Panopoulos and Peet, 1985). In the F plasmid two different genes code for surface exclusion (Willetts and Wilkins, 1984). Different surface exclusion mechanisms may have evolved independently (Broda, 1979).

Another trait with similar effects is the ability of *Streptococcus* plasmids to selectively inhibit the production of "sex pheromone" substance, and thus avoid causing other cells carrying the same type of plasmid to clump and to prepare to transfer their plasmids (Clewell, 1981). *Streptomyces* plasmids also exclude same-type plasmids, but the mechanism is as yet unknown (Hopwood et al., 1985).

Negative Effects of Plasmids on Cells and Clones

Plasmids probably often reduce the reproductive potential of cells in which they reside. Plasmid-free strains in general out-reproduced plasmid-bearing strains when direct competition was staged experimentally, using identical strains with and without antibiotic resistance plasmids, under antibiotic-free conditions in mouse gastro-intestinal tracts that lacked other bacteria (Duval-Iflah, Raibaud, and Rousseau, 1981), in unmodified human gut (Anderson, 1974), and in chemostats (Melling, Ellwood, and Robinson, 1977; Godwin and Slater, 1979; Cairns et al., unpubl.— cited in Slater and Bull, 1978; see also Levin and Lenski, 1983; Bouma and Lenski, 1988). In some cases both bacterial strains persisted (Grabow et al., 1974; Melling, Ellwood, and Robinson, 1977), and in the mice and chemostats, plasmid-carrying strains were maintained at constant, low-level populations, suggesting density-dependent controls (persistent mixed populations may be due to biochemical interdependence between different bacteria, a phenomenon known to be important in some soil bacteria — see Slater and Bull, 1978). Elimination or severe reduction of plasmid-bearing populations often occurred relatively rapidly, in 4 to 30 days in chemostats (Melling, Ellwood, and Robinson, 1977; Godwin and Slater, 1979), and in 10 to 60 days in gnotobiotic mice (Duval-Iflah, Raibaud, and Rousseau, 1981); in some cases the frequencies of plasmid-bearing cells were reduced by up

to four orders of magnitude in as little as 12 generations (Scholz et al., 1985).

Negative effects of plasmids on bacterial reproduction are also suggested by the fact that plasmids carrying genes for certain functions (e.g., antibiotic resistance) are usually absent or less common in environments in which the function is superfluous, for example, in habitats lacking antibiotics [see Broda (1979), Koch (1981), and Hughes and Datta (1983) on antibiotic resistance; Robinson and Tuovenin (1984) on heavy metal tolerance; Dowling and Broughton (1986) on root nodulation]. It appears that natural selection often favors minimization of genome size in prokaryotes (Cavalier-Smith, 1985), and evolutionary loss of chromosomal and plasmid genes that are no longer useful is often quite rapid [see Brubaker (1985), and Herdman (1985) on chromosomal genes in parasitic bacteria; Robinson and Tuovenin (1984) on heavy metal resistance plasmid genes; Broda (1979) on loss of tandem repeats of antibiotic resistance genes; Cavalier-Smith (1985) on loss of superfluous genes, in general].

There are a variety of reasons why plasmids can be disadvantageous to the bacteria carrying them. Many plasmids code for conjugative pili, and there are entire families of viruses that are only able to attack bacteria by using such pili (Lewin, 1977). Some pili are as long as the bacterial cell, and a large pool of additional unpolymerized pilus material is present in the outer membrane of some cells (Broda, 1979), so pilus products may represent appreciable metabolic costs to the cell. For instance, when the ColIb-P9 plasmid is activated (derepressed) to transfer itself, it decreases the growth rate of the cell and produces deleterious changes in the cell envelope (Hardy, 1975). Plasmid gene products sometimes inhibit cell division until sufficient plasmid copies are available (Kline, 1985; Nordstrom, 1985b), or kill offspring cells lacking plasmids (Gerdes et al., 1986). Conjugation itself takes approximately 15 minutes (in one case—Lewin, 1977), and thus the vertical transmission of the chromosome can be slowed by as much as about one generation in rapidly dividing cells. The additional DNA synthesis needed in plasmid-carrying cells may represent an appreciable drain on the cell's resources under some conditions (Slater and Bull, 1978;

but see Scott, 1984). Larger plasmids tend to slow host reproduction more than smaller ones (Zünd and Lebek, 1980).

An additional disadvantage to bacteria that will occur when they have useful genes on plasmids (instead of chromosomes) is that horizontal transmission of plasmid genes could increase competition from nearby bacteria. Since many bacteria colonizing sites where given plasmid genes are useful probably lack these plasmid genes, bacteria may often aid their own competition by passing these genes to neighboring, plasmid-less cells.

Despite the probably frequent disadvantages of carrying plasmids, there are few and only weak signs of chromosomal traits designed to reduce or eliminate a cell's complement of plasmids. The tendency to repulse conjugative plasmids (i.e., the strength of surface exclusion) in *E. coli* cells carrying F or R plasmids is reduced when cells grow more slowly or are starved for amino acids (Lewin, 1977; Broda, 1979). But reduced exclusion may simply result from reduced overall vigor of more slowly growing or starved bacteria. Chromosomal nucleases may be involved in the facultative elimination of the plasmid R1818 under conditions of thymine starvation (Tweats, Pinney, and Smith, 1974). This case is confusing, however, because genes on this plasmid are supposedly also involved in their own destruction (Tweats, Pinney, and Smith, 1974), a seemingly unlikely situation.

Summary

Plasmids carry a distinctive subset of "plasmid-selfish" genes that promote both vertical and horizontal transmission of the plasmid as a unit (23 different genes are involved in these functions on the conjugative F plasmid—Willetts and Wilkins, 1984). Plasmids are 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. Some plasmids have, in fact, been thought to be no more than intracellular parasites (Salysers, 1984; Datta, 1985). By conferring certain reproductive properties on plasmids, the plasmid-selfish genes set many of the rules of the game for evolutionary analyses at the levels of gene, transposon, cell and clone reproduction.

WHY ARE SOME GENES BUT NOT OTHERS
USUALLY ON PLASMIDS?

Despite the common gene pool shared by plasmids and chromosomes, the distribution of genes between them is far from random. "Plasmid-selfish" genes, which increase plasmid fitness without increasing the fitness of the cells carrying them (e.g., genes for horizontal transfer, surface exclusion, and partitioning mechanisms) tend to occur on plasmids rather than on chromosomes, for seemingly obvious reasons. In addition, mapping studies have shown that there are many other genes, with effects that are beneficial to the bacterium as a whole, which also show consistent tendencies to occur on plasmids rather than on chromosomes. The major categories of these plasmid-biased functions are (1) antibiotic resistance; (2) the ability to tolerate or metabolize unusual environmental pollutants, such as heavy metals and arsenates; (3) the ability both to produce toxins (bacteriocins) that kill closely related bacterial competitors and to tolerate these toxins; (4) the ability to invade plant cells and induce gall formation; (5) the ability to invade and nodulate plant roots and fix atmospheric nitrogen; (6) the ability to catabolize certain substrates such as lactose, citrate, chlorinated aromatics, hydrogen sulfate, urease; and (7) the ability (in disease organisms) to attack hosts by means of a variety of mechanisms such as adherence, toxin production, resistance to host defenses (Broda, 1979; Foster, 1983; Hartl and Dykhuizen, 1984). On the other hand, genes for most of the cell's structural proteins and basic metabolic processes ("housekeeping genes") tend *not* to occur on plasmids (Koch, 1981).

Trends for genes with certain functions to occur on plasmids clearly result from the independent convergences on plasmid locations by many genes. Even for genes with the same function, different genes residing on plasmids often have very different modes of action. For instance, some plasmid antibiotic resistance genes code for extracellular inactivation mechanisms, some alter enzyme or ribosomal targets, some change membrane characteristics that affect uptake or discharge, some change transcription rates, some reroute biochemical pathways to avoid blocked reactions, and some increase the ease with which gene dosages can be changed (amplification and deamplification) (Broda, 1979; Koch, 1981; Foster, 1983).

The evolutionary advantage of having these types of genes on plasmids is often thought to result from advantages to bacterial populations or species; the idea most commonly cited by molecular biologists is that plasmids provide a scattered reserve of "optional" functions that enables populations or species to respond to new environmental contingencies (Reaney, 1976; Hopwood, 1978; Broda, 1979; Campbell, 1981; Koch, 1981; Datta, 1985; Willetts and Clewell, 1985; Shapiro, 1985). This and other explanations (Betley, Miller, and Mekalanos, 1986) that are based on the supposition that natural selection acts for the good of populations (assemblies of clones) or of species, without reference to the reproductive interests of lower-level reproductive units such as genes, plasmids, cells, and clones, are probably wrong, since the numbers of competing higher-level units and their rates of turnover are much smaller than those of lower-level units (Williams, 1966). In addition, the gene flow between prokaryotic species reduces their discreteness, making their selection as units even less probable.

The "optional" hypothesis also fails to explain why some traits which, on the basis of their ubiquity appear not to be optional, nevertheless occur on plasmids [e.g., Brubaker (1985) and Aronson, Beckman, and Dunn (1986) for virulence in enteric bacteria and insect pathogens; Nester and Kosuge (1981) on plant gall bacteria].

The Local Adaptation Hypothesis

Many of the characters that tend to occur on plasmids are adaptations to local variations in environmental conditions that occur only sporadically in time or space. For instance, antibiotic compounds 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, sites of modern medical treatment of man and domestic animals and sites of industrial production of medicines. I will argue that this kind of sporadic selection makes the maintenance of local adaptations more likely when genes are on plasmids than when they are on chromosomes. The basic arguments will be given using antibiotic resistance as an example, then applied to other traits.

Consider identical genes for antibiotic resistance, one on a plasmid and the other on a chromosome, both occurring in equal, low numbers in the same habitat which contains some antibiotic. The plasmid gene would have a selective advantage for several reasons:

(1) Most of the bacterial strains that colonize the site containing antibiotic are unlikely to carry a plasmid with the resistance gene because of the competitive disadvantages of cells that carry such plasmids in most other habitats where antibiotic is not present (Broda, 1979; Koch, 1981; Hughes and Datta, 1983). These new bacteria will probably be receptive to horizontal transmission, and benefit from the plasmid-coded local adaptation. Whenever the plasmid gene's rate of horizontal transmission is greater than the reduction in its vertical transmission that results from its negative effects on host reproduction plus the plasmid's occasional failure to be included in offspring cells, the plasmid gene would propagate more rapidly than the chromosomal gene. These conditions are especially likely when bacterial growth rates slow as the environment becomes saturated (see Stewart and Levin, 1977; Levin and Stewart, 1980). The plasmid gene could eventually invade even those cells containing the chromosomal resistance gene, and spread from there into further cells. Thus, the resistance gene on a plasmid would be able to make more copies of itself in such an environment than a copy of the same gene on a chromosome.

(2) The plasmid's greater horizontal mobility would also mean that the plasmid resistance gene would inhabit cells that contain a greater variety of chromosomal and plasmid genomes than would the chromosomal gene. Since the plasmid gene would have a greater likelihood of associating itself with superior genes for other functions, it would tend to win out in clone-level competition. This is the conventional argument for the advantage of sexual over asexual reproduction (Williams, 1975). In sequential replacements of clones, as observed in intestinal *E. coli* (Caugant, Levin, and Selander, 1981), this would be an important advantage.

(3) A final, probably less general advantage is that several plasmid genes for antibiotic resistance (as well as plasmid catabolic genes) are known to add and subtract gene copies in the form of tandem repeats (Broda, 1979); ampli-

fied numbers evolve quickly and thus increase the amount of plasmid gene product when needed, then are rapidly eliminated by selection when not needed (Broda, 1979; Clewell, 1981). This tandem-repeat amplification undoubtedly confers increased fitness on the cells and clones (e.g., greater concentrations of antibiotic are tolerated when the gene is amplified) (Lewin, 1977; Broda, 1979), especially when gene expression is constitutive, as is generally the case for resistance plasmids in gram negative bacteria (Burman, 1975, 1977). The resistance gene on the 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, Pandit, and Sarkari, 1985).

There is positive feedback in selection favoring local adaptation genes being on plasmids. The advantage of being on a plasmid will be augmented if the plasmid already carries another gene or genes coding for adaptations to the same or similar local conditions. The tighter the temporal and spatial association between the occurrence of the different special environmental conditions for which different genes confer advantages, the greater the advantage of this linkage. Even if the special conditions are only weakly associated, a second gene could benefit from hitchhiking, at least if the rate of recombination is low enough. When two genes both confer adaptations to the same conditions, the piling-on advantage could be very powerful. This probably accounts for the remarkably rapid additions of genes to some resistance plasmids, such as a hospital-associated plasmid in Europe that added resistance genes to each of four additional antibiotics and grew from 45 to 65 megadaltons in the space of only about 10 years (Datta, 1985), the "type 29" *Salmonella typhimurium* of cattle that acquired resistance to seven different drugs in about 1.5 years in Britain (Broda, 1979), and the mercury resistance transposon on the plasmid CS229 in hospitals which also rapidly added multiple antibiotic resistance genes (Robinson and Tuovinen, 1984). The usually tight linkage of plasmid genes that code for different products used in the same biochemical process (e.g., Willetts and Wilkins, 1984, on F plasmids) is in accord with this argument.

When bacteria disperse away from a site where a local adaptation is advantageous, they

will often carry locally adapted plasmids, and will thus tend to suffer reductions in their reproductive potential because of the disadvantages of carrying these plasmids; when "cured" strains arise they will outcompete the plasmid-bearing strains.

Heavy Metal Resistance and Catabolic Genes

The arguments just given are also applicable to other plasmid-biased traits. The ability to resist heavy metals and other poisons, which probably usually have patchy distributions (Robinson and Tuovenin, 1984), and the ability to degrade such unusual substrates as lactose, chlorinated aromatics, and urease almost certainly represent adaptations that are only locally useful.

Virulence Factors

Virulence characters are those which are 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. They are obviously only locally useful in facultative pathogens such as *E. coli*, *Proteus*, *Streptococcus*, and *Bacteroides*, which maintain symbiotic populations from which virulent strains are derived. Virulent strains of the species must adapt to different biochemical problems (Broda, 1979; Clewell, 1981; Cavalieri, Bohach, and Snyder, 1984). *E. coli*, for example, continually "leak" from vertebrate intestine (Maejima, Deitch, and Berg, 1984), where they are usually symbiotic, into the iron-poor environment of the circulatory system; a virulent strain living there needs improved iron uptake mechanisms, and these are coded on plasmids (Crosa, 1984).

At first glance, however, virulence in obligatory pathogens seems not to be a local adaptation, because virulence factors are needed in all populations. Such factors tend to be present throughout the bacterial species in which they occur (Bulla, Rhodes, and St. Julian, 1975, on insect pathogens; Brubaker, 1985, on vertebrate pathogens). Most bacteria that cause invasive diseases in animals grow poorly or not at all outside their hosts (Bulla, Rhodes, and St. Julian, 1975; Brubaker, 1985; Aronson, Beckman, and Dunn, 1986).

These virulence factors, however, 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 populations of disease bacteria with slightly different virulence-coding plasmids. For instance, there are literally thousands of strains of insecticidal *Bacillus thuringiensis* and its relatives which invade the insect hemocoel where they multiply rapidly (Falcon, 1971), and no single isolate is active against all host species (Aronson, Beckman, and Dunn, 1986). Different protoxins coded by plasmid genes are active against different insect groups (Aronson, Beckman, and Dunn, 1986). In vertebrate pathogens, different variants of hemolysin plasmid genes have different levels of toxicity in different host species (Gaastra and van Graaf, 1982; Welch and Falkow, 1984; Goebel et al., 1985). In enterotoxigenic *E. coli* that cause diarrhea in man and farm animals, the fibrillar antigens that mediate adhesion to the intestinal epithelium are coded on plasmids, and the antigens are distinct and host-specific (Willshaw, Smith, McConnell, and Rowe, 1985).

Different strains of pathogenic bacteria in plants also often have limited host ranges. Many distinctive varieties of chemicals are produced by closely related pathogens (Mitchell, 1984), and in some cases it is known that host species or race specificity is coded on plasmids (Panopoulos and Peet, 1985).

In sum, virulence products of obligate pathogens, which tend to be coded on plasmids, probably often represent adaptations to taxonomically local conditions. It is also possible that different individuals of a given host species have different defenses, and that adaptations to individual differences represent even more locally restricted advantages.

A further possible advantage of horizontal transmission of virulence factors occurs at the level of possible mutualistic behavior among clones. Some host defenses are more likely to be overcome by larger numbers of pathogens, so horizontal transmission of key genes for successful invasion could raise the chances of success for all invading bacteria. Epidemics of some plant pathogens may only occur when sufficient numbers of bacteria successfully initiate infections in a few plants (Hirano and Upper, 1983).

Plant Gall and Nodule Factors

Plasmids in *Agrobacterium* and *Rhizobium* bacteria induce physiological processes in plants

that promote bacterial reproduction, including invasion of plant tissue and production of plant growth hormones resulting in the formation of galls or nodules (Nester and Kosuge, 1981). *Agrobacterium* plasmids, which insert part of their genome into the plant DNA, also cause the plants to produce substances (opines) that both serve as nutrients for other bacterial cells in the gall and promote conjugative plasmid transfer among them (Nester and Kosuge, 1981). These plasmids also code for the otherwise unusual ability to utilize opines as the sole source of carbon and nitrogen (Nester and Kosuge, 1981). *Rhizobium* plasmids code for nitrogen fixation, which aids in plant growth, presumably thereby increasing possibilities for further bacterial invasion, nodulation, and reproduction.

The two or three *Agrobacterium* species (Elkan, 1981) produce tumors in over 600 species in 61 families of plants (Lippincott and Lippincott, 1975; Panopoulos and Peet, 1985). The two *Rhizobium* groups (Elkan, 1981) also grow in a wide variety of plants (>1130 species in the families Leguminosae and Ulmaceae) (Stowers, 1985). Some populations of these bacteria are extremely diverse; for example, Dowling and Broughton (1986) mention 40 strains of *R. meliloti* isolated from 100 plants in only 100 m².

Not all bacterial strains invade all host species equally well, and plasmids often code this host specificity (Nester and Kosuge, 1981; Moore and Cooksey, 1981; Panopoulos and Peet, 1985; Gheysen, Dhaese, Van Montagu, and Schell, 1985; Dowling and Broughton, 1986). Plasmids from both genera tend to be particularly good at invading the host species from which they were originally isolated. Some plasmids of both genera even show specificities for certain varieties or cultivars of the host plant species (Nester and Kosuge, 1981; Denarie, Boistard, and Casse-Delbart, 1981), and in some plants different sites on the same plant are more successfully attacked by different plasmid strains of *Agrobacterium* (Gheysen et al., 1985).

Plasmids of the less-studied gall bacteria *Pseudomonas savastanoi* code a diversity of cytokinins. Such diversity is associated with host specificity in other groups (see Aronson, Beckman, and Dunn, 1986, on *Bacillus thuringiensis*; Panopoulos and Peet, 1985, on other plant pathogens; Cavalieri, Bohach, and Snyder, 1984, on ver-

tebrate pathogens), suggesting that these plasmids may also confer host-specific adaptations.

The host- and site-specificity of plasmid functions suggest a locally adaptive selective regime similar to that described for virulence factors. In addition, both gall and root nodule bacteria live free in the soil outside of their host plants. Different plasmid strains of *Rhizobium* have different abilities to survive in different soil types and compete with other bacteria present there (Dowling and Broughton, 1986). Different *Agrobacterium* strains also have different competitive abilities in different soil types (Moore and Cooksey, 1981).

In sum, the characters promoting invasion and intra-plant reproduction are clearly adaptations for life in the local vicinity of certain plants. In addition, the inter-clone mutualistic effects favoring massive invasions noted for plasmid virulence factors may also operate in root nodule bacteria. Infections with large numbers of bacteria are much more effective in inducing nodulation than smaller inoculations (Dowling and Broughton, 1986).

Bacteriocins

Some plasmids code for both the production of a powerful toxin (bacteriocin) and specific immunity to the same toxin. Often only a few cells in a clone (typically about 0.01 percent for some Col plasmids — Broda, 1979) actually produce toxin in substantial quantities; in doing so they kill themselves. As a result, however, they promote the reproduction of their clone-mates (that is, all neighbors carrying the same plasmid) by killing closely related cells nearby that do not carry the bacteriocin plasmid (Hardy, 1975; Broda, 1979). Different bacteriocin plasmids (19 different types are known in *E. coli* alone — Broda, 1979) specify different toxins which have a variety of different modes of action. 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).

Despite their apparently powerful selective advantage and their high frequencies in at least some natural populations, bacteriocins probably also confer only local adaptations. Most bacteriocins are degraded by proteases such as those in the vertebrate intestine (a habitat of

many enterobacteria) and also in dental plaque and saliva (where some *Streptococcus* and *Staphylococcus* live) (Hardy, 1975). Anaerobic conditions such as those typical of intestines also decrease the effectiveness of bacteriocins, which 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 (Chao and Levin, 1981; Levin, 1988). In addition, a few bacteriocin genes, such as those of ColV, and *Streptococcus* groups A and D, increase their carriers' virulence (Hardy, 1975), and their presence on plasmids may be at least partly explained by the arguments in the section on virulence.

Infection experiments using enterobacteria in humans, mice, and pigs, and also *Streptococcus* in the human throat, to evaluate the competitive advantages of bacteriocin plasmids have given mixed results. Possession of a bacteriocin plasmid tends to improve a bacterial strain's chances for establishment and longer residence in a host, but there are many exceptions (Hardy, 1975). Some bacteriocin-sensitive strains coexist stably with bacteriocinogenic strains (Hardy, 1975). It may be that bacteriocin plasmids confer different degrees of advantage when their carriers cohabit hosts with different combinations of bacterial species or inhabit different sites in their hosts.

Modulation of bacteriocin production also suggests that the poisons are only locally useful. When densities of cells containing bacteriocinogenic Col factors are increased or the cells are starved of thymine, the percentage of competent cells producing toxins in large quantities rises from 0.01 percent to 1 percent (Broda, 1979). In the epiphytic *Erwinia uredovora*, bacteriocin production is correlated with gene amplification, as occurs with antibiotic resistance (Thiry et al., 1985). Non-induced stages show only low amplification.

The relatively high frequency of bacteriocin plasmids in nature may be partially explained by the apparent difficulty of losing them once they are acquired (Kasner and Rownd, 1985; Clewell and Gawron-Burke, 1986); loss may often have to be preceded by inactivation of the toxin or acquisition of other resistance genes (Kasner and Rownd, 1985).

Summary

Theoretical considerations predict that a subset of the bacterial genome—plasmid-selfish genes, which promote the reproduction of plasmids as units, and local adaptation genes, which promote the reproduction of the cell as a unit in certain limited habitats—will reproduce more effectively when on plasmids than when on chromosomes. These arguments can account for all of the major documented trends in which genes that code for certain functions tend to occur on plasmids rather than on chromosomes. Genes whose utility is not locally restricted (“housekeeping genes”) would not benefit from being on plasmids because of the combination of disadvantages that plasmids impose on the cell's growth and replication, and because of the occasional failure of plasmids to be included in new daughter cells.

The focus here on major trends in gene functions has excluded from consideration plasmid genes with a variety of other phenotypic effects (e.g., Koyama and Yura, 1975; Chernin and Mikoyan, 1981). Some of these, such as UV resistance in enterobacteria, *Pseudomonas*, and *Streptococcus* (Broda, 1979; Clewell and Gawron-Burke, 1986) and restriction-modification immune systems to defend against viral attack (Levin, 1988), may also prove to be local adaptations. Restriction-modification systems may have the unusual property of being under frequency-dependent selection favoring rarer forms (Levin, 1988); thus given genes may derive an advantage from horizontal transmission *away* from the sites where they have become common.

Also excluded were plasmids that have no known phenotypic effects. Such “cryptic” plasmids seem to be very common (e.g., 47 of 84 plasmids from bacteria collected before the use of antibiotics in medicine were cryptic—Hughes and Datta, 1983). Perhaps some cryptic plasmids will be found to carry genes for local adaptations; others are probably simply parasites, carrying no genes useful to the cell as a whole (Salysers, 1984; Datta, 1985). Levin and Stewart (1980) and Levin (1986) argue that most cryptic plasmids, and especially non-conjugative cryptic plasmids, cannot be maintained unless they code for selectively advan-

tageous functions, though the possibility of purely parasitic plasmids is not ruled out (Levin, 1986). The sheer numbers of plasmids and the variety of genes they carry suggest that additional explanations may be needed.

The arguments concerning local adaptations were developed here for extreme cases (local vs. general adaptations) but these are obviously only the ends of a continuum. As the degree of spatial limitation of a gene's selective advantage decreases, the advantages of being on a plasmid should also decrease. And as a plasmid becomes more common, the disadvantage to housekeeping genes of being on the plasmid should decrease. It is thus possible that the basic arguments of the local adaptation hypothesis may also explain the scattered apparent exceptions to the general trends (genes for supposedly local adaptations that occur on chromosomes—e.g., Broda, 1979; Konisky, 1980; Hughes and Datta, 1983; Aronson, Beckman, and Dunn, 1986; Betley, Miller, and Mekalanos, 1986; and plasmids that are apparently fixed and present in all strains of some bacteria—e.g., Denarie, Boistard, and Casse-Delbart, 1981). Detailed studies of these cases could provide important tests of the ideas presented here.

It is interesting to note that similar ideas about horizontal versus vertical transmission have emerged from analyses of human cultural traits (Cavalli-Sforza and Feldman, 1981; Boyd and Richerson, 1985). Locally useful traits tend to be transmitted horizontally (between individuals of the same generation), while more generally useful traits tend to pass from parents to offspring.

TESTS OF THE LOCAL ADAPTATION HYPOTHESIS

Prokaryote Insertion Sequences and Transposons

The local adaptation hypothesis predicts that the relative horizontal mobility of other genetic elements should also correlate with their tendency to carry genes for only locally useful functions. Insertion sequences are short stretches of DNA that are able to cause copies of themselves to be cut free from DNA molecules such as chromosomes and plasmids and to become integrated into other DNA molecules (Arber, Sengstag, Caspers, and Dalrymple, 1985; Chan-

dlar and Galas, 1985). Transposons are insertion sequences that carry an additional stretch of DNA coding for one or more additional functions. Genes carried on transposons can be either chromosomal or plasmid in origin. Because they can move into plasmids, both insertion sequences and transposons of prokaryotes are more horizontally mobile than are other components of chromosomal DNA.

As predicted, there is apparently a strong trend for transposon genes to code for the same types of local adaptations typically found on plasmids. Transposon genes include resistance to antibiotics, heavy metals, toxin production, virulence, and catabolism of novel substrates (Chakrabarty, 1976; Doolittle, 1982, and included references; Foster, 1983; Robinson and Tuovenin, 1984; Cavalieri, Bohach, and Snyder, 1984; Crosa, 1984; Chandler and Galas, 1985; Schmitt et al., 1985; Bowen and Pemberton, 1985; Panopoulos and Peet, 1985; Aronson, Beckman, and Dunn, 1986). This trend is so strong that transposons have even been *defined* (mistakenly, at least in terms of the definition above) as insertion sequences that carry antibiotic resistance genes (Finnegan et al., 1982). The ability of transposons to amplify gene-copy numbers may also contribute to explaining why transposons carry local adaptation genes, or at least those for antibiotic resistance (Campbell, 1981).

Viruses

Despite the strong selection for compactness in viral genomes (Cavalier-Smith, 1985), genes for local adaptations in bacteria are known to occur in viruses. Viral transduction of plasmid antibiotic resistance genes in *Streptococcus* may have been important in their spread (Clewell, 1981); some temperate bacteriophages carry virulence characters (Brubaker, 1985); "shiga-like" endotoxins in *Shigella dysenteriae* are coded on bacteriophages (Betley, Miller, and Mekalanos, 1986); and some staphylococcal endotoxin genes are carried in phages (Betley, Miller, and Mekalanos, 1986). Betley, Miller, and Mekalanos (1986) concluded that a major theme in bacterial endotoxin genetics was the localization of genes on accessory elements of any of several kinds that are more horizontally mobile than are chromosomes. In sum, the evidence

is not as conclusive as with transposons, but viruses do carry a number of the types of genes predicted by the local adaptation hypothesis.

Plasmids of Eukaryotes

Eukaryote cells also have extranuclear fragments of DNA that are similar to bacterial plasmids in that they replicate independently (having their own replicons). In contrast to plasmids in bacteria, eukaryote genes on these plasmids probably do not usually have greater horizontal mobility than chromosomal genes, since (1) eukaryote chromosomal genes generally participate in sexual reproduction (giving high rates of chromosomal gene reassortment and recombination), and (2) opportunities for transfer of plasmids without concomitant chromosomal DNA transfer between genetically different cells are quite rare in most groups (fungi and slime molds with cytoplasmic fusions may be exceptions). Eukaryotic plasmids seem not to code for characters that would promote their horizontal transmission (Esser et al., 1986). Thus, the local adaptation hypothesis predicts that eukaryote plasmids and transposons, in contrast to their prokaryote counterparts, generally should *not* show a trend to carry the local adaptation traits rather than housekeeping traits.

As predicted, most eukaryote plasmids are not known to carry any genes for local adaptations (Rush and Misra, 1985; Sederoff and Levings, 1985; Esser et al., 1986). Since current knowledge is still fragmentary, it must be admitted that genes for such traits may yet be discovered. More conclusive is the fact that housekeeping genes do occur on a number of plasmids as predicted. Of the 41 fungal plasmids listed by Esser et al. (1986) from 25 species of fungi, 8 are known to be homologous to mitochondrial DNA sequences, 3 are known to carry genes for tRNA or rRNA, and one carries coding regions for subunits of cytochrome c oxidase (Rush and Misra, 1985). Plasmids associated with mitochondria in the plants *Sorghum*, *Zea*, *Vicia*, and *Brassica* produce male sterility (Sederoff and Levings, 1985), a character that is probably a "mitochondrion-selfish" adaptation favoring mitochondrial transmission to the next generation (Eberhard, 1980; Cosmides and Tooby, 1981). A plasmid in *Zea* carries part of a chloroplast gene for a thylakoid membrane protein used in photosynthe-

sis (Sederoff and Levings, 1985). Thus, housekeeping genes are not uncommon on eukaryote plasmids, whereas local adaptations are conspicuous by their absence.

The "killer" plasmids in yeasts and other fungi are a possible exception (Tipper and Bostian, 1984; Sakaguchi, Hirochika, and Gunge, 1985). These plasmids act like bacteriocin plasmids in prokaryotes, producing both a toxin that is effective against close relatives of the carrier organism, and an immunity to the toxin. Some of these plasmids are RNA molecules, others are DNA. The killer ("M") plasmid cannot maintain itself in the host unless a second, slightly larger plasmid ("L") is also present in the cell.

Although killer plasmids differ from typical viruses in that none is known to be transferred extracellularly (there is one possible exception—Tipper and Bostian, 1984), it appears that they are probably modified viruses (Hopwood, 1978; Tipper and Bostian, 1984). The L and M complementarity is similar to that in "helper viruses," which are limited to short lengths by the imprecision of RNA polymerase enzymes. Both M and L plasmids occur in yeast cytoplasm within virus-like particles consisting of protein capsids (Tipper and Bostian, 1984). In addition to capsid formation, they replicate in two distinct steps as in reoviruses (Tipper and Bostian, 1984). Both M and L plasmids can be transferred horizontally between different species and genera by protoplast fusion, transformation, and rare mating (Sakaguchi, Hirochika, and Gunge, 1985). Yeast killer plasmids have no homology with any chromosomal or mitochondrial sequences of their hosts. Viruses sometimes transform into plasmids, at least in prokaryotes (Broda, 1979), and if gene transfer from killer plasmids to yeast chromosomes is rare (I know of no data on this point other than the lack of homology with chromosomal DNA), it could be that these eukaryote plasmids are descended from viruses that have not been incorporated into chromosomes.

Eukaryote Transposons

The local adaptation hypothesis predicts that eukaryote transposons will more likely carry housekeeping genes than local adaptation genes. Available evidence is again in accord. None of the best-studied eukaryote transpo-

sons, including *copia* and *copia*-like elements, *FB*, *TE*, *hobo*, *P*, and *I* in *Drosophila*; *Ds*, *Ac*, *Mul* in maize; *Tam1* in snapdragon; and *Ty* in yeast are known to carry genes with local adaptation effects. The most common traits are manifestation and regulation of the transposon's own mobility (inhibition as well as stimulation), control of the expression of adjacent genes, and chromosomal instability (Freeling, 1984; Syvanen, 1984; Dellaporta and Chomet, 1985; Finnegan, 1985). Type 1 ribosomal insertions are transposable in *Drosophila* and carry genes for ribosomal proteins (David et al., 1981). *Copia*-like elements of *Drosophila* and IAP elements in mice have some of the conserved functional sequences characteristic of proviruses (Finnegan, 1985).

Summary

Data on the composition of prokaryote transposons are relatively abundant, and give a strong confirmation of the local adaptation hypothesis. Knowledge of eukaryotes is much less complete, and although their mobile elements show the expected trends, the support at present is more suggestive than convincing. More conclusive tests will be possible as additional data become available. Fungi are particularly interesting in this context, as some routinely form heteroplasmons by cytoplasmic fusion while others do not (Fincham and Day, 1965). Such fusions may give plasmids greater horizontal mobility than chromosomes (depending on relative rates of reproduction and dispersal within heteroplasmons). Assuming some gene flow between eukaryote chromosomes and plasmids, species with plasmids having more horizontal mobility would be predicted to have more local adaptation genes on their plasmids.

EFFECTS OF PLASMIDS ON RATES OF GENE EVOLUTION

The evolutionary establishment of bacterial genes with new functions may be quite rare. Artificial selection for new metabolic abilities in bacteria has usually not resulted in changes in structural genes; instead, regulatory changes occurred that permitted already existing enzymes from different pathways to be utilized in new functions (Mortlock, 1982). In some cases structural genes on chromosomes and plasmids for metabolic abilities and for antibiotic

resistance may have been "borrowed" ready-made from other bacterial strains or species via plasmids and/or transposons (Mortlock, 1982; Labigne-Roussel, Witchitz, and Courvalin, 1982). Addition of a gene or genes to a pre-existing plasmid has been a common mode of evolution in antibiotic resistance plasmids (Datta and Hughes, 1983). In some cases, however, no possible predecessors are known for new genes (Mortlock, 1982; Foster, 1983).

Occasionally, nevertheless, novel structural genes must arise to carry out new functions. I will argue that genes on plasmids are more likely to evolve rapidly in response to environmental changes than those on chromosomes. Consider the fates of identical mutant genes conferring slight antibiotic resistance, one on a plasmid and the other on a chromosome, in an environment containing sublethal concentrations of antibiotic. The plasmid gene would, because of its greater horizontal mobility, cohabit cells with a greater variety of both chromosomal and plasmid genes, giving it a greater range of genetic backgrounds, and thus improving its chances of co-occurring with other genes compatible with its altered properties, and giving it a greater range of genes with which to recombine. In addition, some plasmids have greater rates of recombination than chromosomes (Cohen, 1976; Janniere, Niaudef, and Erlich, 1985) (plasmid recombination occurs via different pathways in at least some cases—Janniere, Niaudef, and Erlich, 1985, on *E. coli* and *Bacillus subtilis*).

Plasmids may also have greater densities of transposons (see below; see also Nies, Meyer, Kratz, and Wiedemann, 1985), and these elements tend to cause both mutations and recombinations (Syvanen, 1984; Arber et al., 1985). Recombinations resulting in gene duplication or other large additions or subtractions of large DNA segments appear to have been especially important in plasmid evolution (Cohen, 1976; Kopecko, Brevet, and Cohen, 1976; Datta and Hughes, 1983).

The result is that the antibiotic resistance gene that was on a plasmid would be more likely to vary, and it would also be more likely to occur in cells with other compatible and superior variants of this and other genes, making the plasmid gene's products more likely to acquire improved resistance than those of the chromosomal gene (Koch, 1972; Felsenstein,

1974; Malmberg, 1977). The plasmid advantages would hold even if, as is to be expected, copies of the plasmid gene were occasionally transferred to a chromosome. This argument that new functions are more likely to arise on plasmids contradicts the assumption of Wheelis (1975) that new bacterial genes originate on chromosomes.

The argument that mutability per se, as well as an increased range of mutational variety, are advantageous is somewhat unconventional. Selection has apparently acted to hold mutation rates in bacteria to very low levels (Cox, 1976), and increased mutability is known to be generally associated with decreased fitness at several different reproductive levels: at the level of genes, a given allele is effectively eliminated each time it is mutated to a different allele; at the levels of individual plasmids, chromosomes, and the cells which contain more mutable DNA, the reproductive units are less likely to survive and reproduce (e.g., Charlesworth, 1987). At the level of large clones, however, and especially those that are only imperfectly adapted to their environments, those clones that generate larger ranges of variants are more likely to end up with some descendants possessing improved adaptations (Chao, Vargas, Spear, and Cox, 1983).

Relatively rapid evolution of plasmid genes has been demonstrated by Nies et al., 1985. Aerobactin gene copies in *E. coli* and *Klebsiella pneumoniae* in medical centers also apparently evolved more rapidly when on pColV-K30 plasmids than when on chromosomes (Valvano, Wolf, Crosa, and Crosa, 1985).

LEVELS OF SELECTION

As already noted by other authors (Hardy, 1975; Dawkins, 1976; Broda, 1979), analyses of plasmid evolution entail simultaneous consideration of selection acting at several different levels of reproduction, including genes, transposons, plasmids, chromosomes, cells, and clones. The concept of different levels of selection is not new (e.g., Weismann, 1904, in Buss, 1987), and has been used in analyses of other characters (Lewontin, 1971; Alexander and Borgia, 1978; Vrba and Eldredge, 1984; Buss, 1987). But the necessity of simultaneous consideration of so many different levels is unusual. Recent developments in fields ranging

from embryology to cultural evolution to organelle biology suggest that such hierarchical analyses are destined to become more common (Buss, 1987; Cavalli-Sforza and Feldman, 1981; Boyd and Richerson, 1985; Eberhard, 1980; Cosmides and Tooby, 1981; Werren, Nur, and Wu, 1988). Similar analyses of different reproductive levels may be necessary in eukaryote genomes since significant rates of exchange of genetic materials are now known to occur within organelles, between organelles, and between organelles and nuclear DNA (Hohn and Dennis, 1985). In addition, eukaryote nuclear genes are regularly transferred in linkage groups or "genetic chunks" (Lewontin, 1974; Alexander and Borgia, 1978).

One general lesson for evolutionary theory to be learned from multilevel analyses of plasmid evolution is that self-sacrifice can and does evolve at certain levels for the reproductive good of higher levels. In conventional analyses of the effects of natural selection at levels such as individuals, clones, populations and species, there is a general consensus that the effects at lower levels (individuals and clones) are usually much more important (Williams, 1966; Alexander and Borgia, 1978; Leigh, 1977, 1983; Vrba and Eldredge, 1984). But bacterial cells and clones do not have the small sample sizes, slow turnover, and unlikely balances between extinction and migration that make selection at higher levels, such as populations and species, usually important only under unusual conditions (Williams, 1966; Wilson, 1980).

Altruistic plasmid genes that facultatively repress the replication of their own plasmids are present in all plasmids studied to date (Nordstrom, 1985a). In addition, horizontal transmission of plasmids is also generally facultatively repressed by other plasmid genes (Lewin, 1977; Broda, 1979). These inhibitory genes slow down short-term plasmid replication, but are probably advantageous at the cell (individual bacterium) level. Similarly, transposons of both bacteria and *Drosophila* often carry genes to repress their own spread through the genome (Syvanen, 1984). This repression probably functions to benefit the entire cell, because transposon insertions into host genes often have disastrous effects on host survival (Syvanen, 1984; Charlesworth, 1987), although competition from other potentially competing (and probably also cell-damaging) transposons may

also have been selectively important (Lowe and Berg, 1983). The *Ty* transposons of yeast limit their insertion to sites where they are less likely to cause cell-level damage (Syvanen, 1984; see, however, Finnegan, 1985). Interestingly, transposon-coded repression is less intense when a transposon first invades a cell, then gradually becomes stronger (Lowe and Berg, 1983). This is the same pattern seen in plasmid-coded inhibition of plasmid horizontal transmission, and can result in a quick epidemic spread (in this case, through the genome) which is followed by relative stasis.

The evolution of plasmid recombination rates may offer an additional illustration of the imposition of higher level reproductive interests. Recombination is more frequent and follows different pathways in plasmids than in chromosomes in some species. Janniére, Niaudef, and Erlich (1985) suggest that elevated recombination rates in plasmids may be an adaptation. They argue that unsuccessful variants in plasmids are energetically less costly than those in chromosomes, presumably because they will occur only in the plasmid-carrying portion of the population. This hypothesis is unlikely to be correct since species- or population-level benefits are invoked, but it is clear that under certain circumstances more frequent recombination in plasmids could be adaptive. If plasmid genes confer adaptations to conditions that are likely to vary relatively rapidly over time (for instance, if host defenses change during the course of an infection, so that it is advantageous to the bacterium to evade defenses by changing its own characteristics, as in the AIDS virus), a clone that produced more variants would be more likely to generate a variant adapted to the next set of conditions. Selection at this level would act in conflict with that at the levels of both plasmids and cells, since recombination would tend to break apart functionally linked genes. Levin (1986) makes a similar argument with respect to the abilities of conjugative plasmids to carry chromosomal genes between cells. The basic logic is similar to the "lottery ticket" idea used by Williams (1975) to explain the evolution of sexual reproduction in eukaryotes. Greater plasmid recombination rates could also be favored in cases where they result in increased numbers of certain genes in the presence of environmental challenge (e.g., amplification of resistance

genes in the presence of antibiotics — see Broda, 1979) by favoring those bacteria with larger numbers of repeats of these genes.

These self-sacrificing traits contrast with the often conflictive interactions between social reproductive units of other kinds [genes in eukaryotic organelles and nuclei — see Eberhard (1980), Cosmides and Tooby (1981); cells in multicellular organisms — see Buss (1987); individuals in animal societies — see Wilson (1975); and parasites and their hosts — see Ewald (1983), Ewald and Schubert (in press)]. The relatively benign relationships between plasmids and transposons and the genomes of the cells that carry them are probably due to two factors:

(1) Incompatibility and surface exclusion adaptations of plasmids generally prevent reproductively competing and genetically distinct plasmids from anything other than very transient occupation of the same higher-level reproductive unit (cell); this contrasts with some organelles, some multicellular organisms, most animal societies, and many pathogens (Eberhard, 1980; Cosmides and Tooby, 1981; Buss, 1987; Wilson, 1975; Ewald, 1983). The plasmids are thus less likely to evolve competitive intracellular interactions that are damaging for the entire cell.

(2) Plasmids are nearly absolutely dependent on higher-level reproductive units (cells) for both vertical and horizontal transmission (in contrast to most viruses and other pathogens, and most animals in societies).

If the chain of causes is traced one step further back, surface exclusion probably evolved because incompatibility interactions of plasmids result in plasmids of the same incompatibility types being reproductive competitors. These incompatibility reactions are apparently associated with the plasmids' control of their own replication and partitioning (Nordstrom, 1985b) that usually involves folded RNA or repeated DNA sequences (Scott, 1984). Thus, the suite of plasmid-cell interactions may be largely an indirect result of the molecular details related to the control of plasmid replication.

CONCLUSIONS

The evolutionary origin of plasmids is uncertain. Some may be descendants of the mul-

tiple nucleic acid molecules present in the cells of earlier eras (e.g., Dyson, 1985), while at least some are probably descended from viruses (Broda, 1979). The presence of accessory genetic elements in bacterial cells has resulted in new modes of evolution that would not otherwise have been possible. Modern prokaryotes are undoubtedly more variable and evolutionarily flexible than they would have been without plasmids, and genes for new bacterial functions may tend to evolve on plasmids rather than on chromosomes. It seems probable that plasmids or other horizontally mobile elements played an important role in the transition from prokaryotes or other simple organisms to eukaryotes.

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