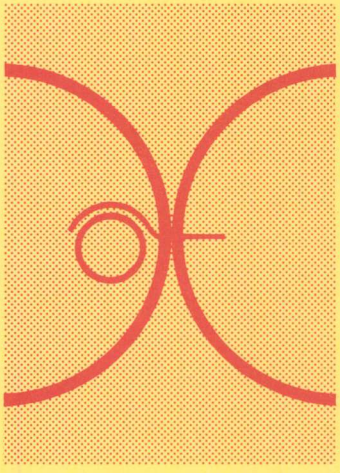
on conjugative plasmids

mathematical models of their population dynamics and population genetics



Nelly van der Hoeven

STELLINGEN

1. Voor zover bekend is de koppeling tussen basepaar triplet en aminozuur in alle levende wezens in principe identiek. Er zijn echter mutaties mogelijk, die een andere koppeling veroorzaken. Het feit dat de koppeling tussen basepaar volgorde en aminozuur desondanks uniform is, zou veroorzaakt kunnen worden doordat soorten met een andere koppeling niet in staat zijn via horizontale gen overdracht functionele genen te verwerven.

2. Een solitaire sluipwesp verhoogt haar reproduktiesucces, als zij de eerste gastheren, die zij in haar leven vindt, parasiteert, ongeacht of deze al eerder zijn geparasiteerd, en indien zij later in haar leven wel onderscheid maakt tussen wel en niet geparasiteerde gastheren. Derhalve kan uit het feit dat de sluipwesp *Leptopilina heterotoma* bij haar eerst gevonden gastheren geen onderscheid maakt tussen wel en niet geparasiteerd, en later wel, niet worden geconcludeerd dat zij het discrimineren moet leren.

3. Inteelt depressie is alleen relevant bij soorten waarbij weinig of geen inteelt voorkomt. Derhalve kan inteelt depressie niet gebruikt worden als verklaring voor outbreeding.

4. Indien homosexualiteit een genetische basis heeft, hebben de kerken door het tegengaan van homosexueel gedrag het voortbestaan van homosexualiteit bevorderd.

5. Het gebruik van de term "incompatibel" om aan te geven dat plasmiden bepaalde eigenschappen gemeen hebben, werkt verwarrend.

6. Het feit dan men ervoor gekozen heeft het Nederlandse equivalent voor het Engelse woord plasmid, "plasmide", onzijdig te laten zijn, doet vermoeden dat men een plasmide beschouwt als een soort chemische verbinding. Immers, andere woorden op -ide plegen in het Nederlands vrouwelijk te zijn.

7. De betekenis van de Latijnse oorsprong van het woord evolutie, evolvere, doet vermoeden dat de eerste gebruikers van deze term eerder dachten aan een gepredestineerde voortgang, die zich slechts hoeft te ontrollen, dan aan door toevalsprocessen bepaalde veranderingen in de loop van de tijd.

8. De huidige vorm van beoordeling van wetenschappelijk werk maakt dat onderzoekers er tegenwoordig vaak minder waarde aan hechten dat hun artikelen worden gelezen dan worden geteld.

9. De huidige onverhuurbaarheid van veel hoogbouw-flatwoningen toont eens te meer aan, dat de stokpaardjes van de jaren zestig tot de nachtmerries van de jaren tachtig kunnen worden.

10. Het spreekwoord "Als het kalf verdronken is, dempt men de put" getuigt van een vergaande vorm van optimisme.

Stellingen behorend bij het proefschrift van Nelly van der Hoeven

RIJKSUNIVERSITEIT TE GRONINGEN

ON CONJUGATIVE PLASMIDS

Mathematical Models
of their
Population Dynamics
and
Population Genetics

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. E. Bleumink
in het openbaar te verdedigen op
vrijdag 13 december 1985 des namiddags te 4.00 uur
door

NELLY VAN DER HOEVEN

geboren te Rotterdam

1985
DRUKKERIJ VAN DENDEREN B.V.
GRONINGEN

Promotor: Prof. dr. W. van Delden

Referenten: Dr. R. F. Hoekstra
Dr. J. Reddingius

Dit onderzoek is uitgevoerd op het Genetisch Instituut van de Rijksuniversiteit Groningen. Het is gesubsidieerd door BION-ZWO in het kader van de Werkgemeenschap Theoretische Biologie.

VOORWOORD

Gaarne wil ik op deze plaats al diegenen bedanken, die mij tijdens mijn studie en mijn promotieonderzoek terzijde hebben gestaan en hebben gestimuleerd. In de eerste plaats denk ik hierbij aan mijn ouders. Zij beiden hebben mij altijd aangespoord om te studeren, mij vrijlatend in mijn studiekeuze. Helaas heeft mijn vader de voltooiing van dit proefschrift niet meer mee mogen maken.

Hans Metz heeft, zowel als begeleider, als ook als vriend, een grote invloed op mijn studie gehad. Hoewel ik ook zonder hem waarschijnlijk Mathematische Biologie had gekozen, zou de pret erin dan niet zo groot zijn geweest. Ook vele andere Leidse vrienden hebben mijn wetenschappelijke vorming beinvloed.

Tijdens mijn promotieonderzoek vond ik in Rolf Hoekstra altijd een aandachtige luisteraar. Het idee om onderzoek te verrichten naar de invloed van extrachromosomale elementen op de evolutie van prokaryoten is van hem afkomstig. Ook Wilke van Delden en Hans Reddingius waren altijd bereid naar mijn denkbeelden te luisteren en nieuwe ideeën te suggereren.

I am especially grateful to Bruce Levin for his interest in my investigations. I will always remember my visit to his lab and the Gordon Conference with pleasure. Some of my research was inspired by his remarks, in particular Chapters 5 and 6.

In het bijzonder wil ik Saskia Walburgh Schmidt danken, die dit proefschrift, vol met akelige formules, nauwgezet heeft getypt. De tekeningen zijn gemaakt door Henk Mulder en de omslag is verzorgd door de Heer Leeuwinga.

Max, jij hebt als offer aan mijn proefschrift mij gedurende de laatste jaren alleen in de weekends kunnen zien. Met de voltooiing hiervan zal ook aan deze toestand, waar slechts de PTT en de NS wel bij voeren, een eind worden gemaakt.

Aan de nagedachtenis van mijn vader
en aan mijn moeder.

CONTENTS

CHAPTER 1:

INTRODUCTION

In this thesis some mathematical models will be formulated to analyse the effects of selection on the population dynamics of conjugative plasmids. In the Introduction some general properties of plasmids will be summarized. Thereafter, the questions, investigated in this thesis are given. In Chapter 7 some still unsolved problems about the influence of selection on the structure and function of plasmids are considered.

PLASMIDS

The genetic information of bacteria is encoded in a single circular chromosome. In addition to this chromosome many bacteria also contain smaller autonomous circular DNA molecules. These pieces consist, just as the bacterial chromosome, out of two complementary DNA strands, and are called plasmids (Novick et al., 1976). Sometimes, they can be incorporated into the bacterial chromosome, in which case they are called episomes (Novick et al., 1976). On a plasmid several genes may be situated. Some of these genes are plasmid specific and encode for functions necessary for the plasmid, like plasmid replication, whereas other genes are not necessary for plasmid maintenance. Some plasmids have genes with a clearly discernible phenotypic effect on their bacterial bearer. Many plasmids for instance, encode for resistance against antibiotics or against heavy metals. Plasmids of which no phenotypic effect is discovered, are called cryptic (Novick et al., 1976).

A particular plasmid type may be present with a number of copies in a single bacterial cell. The number of copies (copynumber) can range from one or two up to about 800 (Projan, Carleton & Novick, 1983). Large plasmids often have a lower copynumber (mostly less than 10) than smaller plas-

mids.

Most plasmids are not limited to a single host species: Plasmids, which are indistinguishable with the used identification methods can be found in geographically separated bacterial populations and in different bacterial species (for instance, Grindley, Humphreys & Anderson, 1973b; Roussel & Chabbart, 1978; Jørgensen & Sørensen, 1979; Polak & Novick, 1982). Some plasmids can be transferred to a wide range of bacteria in mating experiments in the lab (for instance, Datta & Hedges, 1972; Chandler & Krishnapillai, 1974; Appendix B of Bukhari, Shapiro & Adhya, 1977). Some plasmids are not even restricted to prokaryotes, but can also be successfully introduced into simple eukaryotes (Goursot et al., 1982).

PLASMID REPLICATION AND SEGREGATION

In order to be maintained in a bacterial cell line, plasmids have to replicate. Each plasmid copy has an origin of replication at which the replication starts either unidirectional or bidirectional (see for instance Scott, 1984). The initiation of replication is in most cases plasmid-regulated. For their replication plasmids may partly make use of the replication enzymes of their bacterial host. The rate of replication is mostly plasmid determined (Nordström, Ingram & Lundbäck, 1972; Timmis & Winkler, 1973), but properties of the bacterial host can also have some influence (Macrina, Weatherly & Curtiss, 1974; Cress & Kline, 1976). Each plasmid copy has to replicate on the average once per cell cycle in order not to disappear from the cell line (average <1) or to increase unlimited in number (average >1). If this replication would be a random process with a mean of exactly 1, the number of plasmid copies per bacterial cell would vary considerably. However, plasmid copy numbers appear to be very stable (Barth, Richards & Datta, 1978). Besides, slight deviations from the mean value of 1 will either lead to the disappearance of the plasmid or to an unlimited growth in numbers. Therefore, there has to be

some control on the number of plasmid copies to prevent deviations from the average. It appears that this control leads to a constant number of replications per unit of time, independent of the number of copies already present (Gustafsson & Nordström, 1980; Pritchard & Grover, 1981). Replications occur during the whole cell cycle, independent of the replication of the bacterial chromosome (Gustafsson, Nordström & Perram, 1978; Steinberg & Helmstetter, 1981). For each replication, the replication is initiated in a random copy (Gustafsson & Nordström, 1975). However, plasmids which have just been replicated, cannot immediately start with another round of replication (Gustafsson, Nordström & Perram, 1978).

For a stable inheritance of plasmids in a bacterial cell line, it is not only essential that the plasmids replicate, but also that each daughter cell of a plasmid bearing bacterium contains at least one plasmid. For plasmids with a high copy number, random segregation will already ensure that almost all daughter cells have at least one plasmid copy. For low copy number plasmids, random segregation will result in a high percentage of plasmid-free daughter cells. However, low copy number plasmids do not segregate at random (Miki, Easton & Rownd, 1980; Nordström, Molin & Aagaard-Hansen, 1980; Austin & Abelis, 1983a, b). There are strong indications that each daughter cell obtains half of the number of the plasmid copies of the mother cell. Nordström, Molin & Aagaard-Hansen (1980) found that the rate of loss of plasmids is even lower than should be expected if the initiation of plasmid replication was distributed according to a Poisson distribution (a constant probability of replication initiation per unit of time and per plasmid-bearing cell) and the partitioning was strictly even (in case of an odd number of plasmid copies, one daughter cell obtains one plasmid copy more than the other one). This extreme stability could be caused by a higher than usual replication rate in case of very low number of plasmids (Nordström & Aagaard-Hansen, 1984). Ogura and Hiraga (1983) discovered that the moment of cell division can be delayed if only one plasmid

is present. Such a delay will also reduce the plasmid loss rate. A recent review of plasmid replication is given by Nordström, Molin & Light (1984).

PLASMID INCOMPATIBILITY

As mentioned in the previous section the number of plasmid replications per cell cycle is regulated in a fairly accurate way. However, if two different plasmids occur in the same bacterial cell, both using the same mechanism of regulating their replication, the total number of replication initiations is regulated, but at each initiation each copy has the same probability of being replicated. This will lead to a random increase of the relative frequency of one of the plasmids.

If both plasmid types use the same partitioning mechanism, both daughter cells will obtain an equal number of plasmid copies, but the two types will be distributed randomly over the daughter cells. It may occur that one of the daughter cells obtains only copies of one plasmid type. Gradually, bacteria bearing both plasmid types will disappear and more and more bacteria containing only one plasmid type will arise.

Two plasmid types which use either the same mechanism to regulate their replication or the same partitioning mechanism, or both, cannot be maintained for several generations in the same bacterial cell line (unless there is a selection pressure for bacteria carrying both). Two such plasmids are therefore called incompatible (for a review, see Timmis, 1979). The rate of segregation into different cell lines of two incompatible plasmids starting in the same bacterium has been calculated by Ishii, Hashimoto-Gotoh & Matsubara (1978), Novick & Hoppensteadt (1978) and Cullum & Broda (1979). That rate depends on the copy number of the plasmid. If two plasmids use the same replication and/or segregation mechanism, there is a particular relationship between them. Their genes coding for replication and segregation have, in that case, almost the same nucleotide sequence (Grindley, Humphreys &

& Anderson, 1973b; Broda, 1979a). The relation between two incompatible plasmids may be asymmetric: Copies of one of them may be preferentially replicated. Plasmids are ordered according to this property into incompatibility groups (Datta, 1979). A list of incompatibility groups, and the plasmids belonging to them is given in Bukhari, Shapiro & Adhya (1977) appendix B.

CONJUGATION

Some plasmids are capable of infectious transmission from one bacterium to another. When a plasmid-bearing bacterium collides with another bacterium, one strand of the DNA of a plasmid copy can be transferred. This process is called conjugation, and plasmids, capable to induce their own transfer are called conjugative plasmids. The first plasmid was discovered by its ability to induce recombination. It was called a Fertility factor (F-plasmid). Nowadays a wide range of different conjugative plasmids is known (Bukhari, Shapiro & Adhya, 1977, Appendix B). Several different conjugative systems are known. Incompatible plasmids often use the same transfer system (Bradley, 1980a, b).

Conjugative plasmids induce their bacterial host to form plasmid specific pili, some kind of extracellular filamentous organelles (Bradley, 1980a). These pili are one of the characteristics of a transfer system. Plasmids using different transfer systems encode for different pili. Pili play an important role in the pair formation between donor cell and recipient (Ou & Anderson, 1970; Tomoeda, Inuzuka & Date, 1975). They can also serve as an attachment site for pilus specific bacteriophages (Brinton, Gemski & Carnahan, 1964; Caro & Schnös, 1966; Bradley, 1976, 1980a). In the presence of such a bacteriophage, therefore, the possession of pili is disadvantageous for a bacterium.

When pair formation between two bacteria has been successful, one strand of the DNA of a plasmid copy will be transferred. This transfer always takes place in the same direction, starting with the origin of transfer, *ori*T. For the F

plasmid, the order of transfer is such that directly after *oriT*, the gene complex encoding for replication regulation is transferred (Rowbury, 1977). The genes coding for transfer themselves are transferred last (Walker & Pittard, 1972; Broda et al., 1972; Guyer & Clark, 1977). After the strand of plasmid DNA is transferred completely, the cell to cell contact between donor and recipient will be dissolved. In the recipient a complement to the transferred strand is synthesized and the now double stranded DNA is recircularized (Ohki & Tomizawa, 1968; Vapnek, Lipman & Rupp, 1971). The recipient itself becomes a potential donor bacterium. However, if the contact is broken before the total plasmid strand is transferred, the transfer region will not be transmitted, and the recipient will not be able to induce transfer itself (Guyer & Clark, 1977).

Sometimes, a plasmid which does not code for conjugation itself can be transferred in case its bacterial host also contains a conjugative plasmid. This may be the result of a covalent union between both plasmids. In that case the non-conjugative plasmid is as it were dragged along with the conjugative one (e.g.: Hooykaas, Den Dulk-Ras & Schilperoort, 1980). In this way some conjugative plasmids can also mobilize and carry along parts of the bacterial chromosome (Holloway, 1979).

Some non-conjugative plasmids possess an *oriT* region on their DNA. Such plasmids can use the transfer products encoded by a conjugative plasmid, to become mobilized and transferred (Warren, Twigg & Sherratt, 1978). In that case the non-conjugative plasmid is transferred alone. Such plasmids often do not need all transfer genes of the conjugative plasmids (Van der Pol, Veltkamp & Nijkamp, 1978; Warren, Saul & Sherratt, 1979; Willetts & Maule, 1979). Because these plasmids can be mobilized by the transfer gene products of another plasmid, they are called mobilizable plasmids (Clark & Warren, 1979).

Levin, Stewart & Rice (1979) have investigated whether the transfer rate of plasmids from donor to recipient satisfies a simple mass action model. They experimentally ver-

ified that the number of transfers per unit of time is indeed proportional to the product of the donor and recipient concentration. Experiments of Cullum, Collins & Broda (1978a) indicate, however, that the transfer rate per donor and per recipient decreases with increasing bacterial concentration. They explain this by the finding that the efficiency of pair formation decreases in a crowded environment (Collins and Broda, 1975) and by the finding that a plasmid-bearing bacterium can donate a plasmid only once per generation. In an environment with a surplus of recipients, the transfer rate can become almost one per donor generation.

The transfer rate appears to depend not only on the bacterial concentrations, but also on the number of generations the plasmid-bearing bacterium already carries its plasmid (Ozeki, Stocker & Smith, 1962; Stocker, Smith & Ozeki, 1963). A newly infected host is not capable to donate a plasmid during the first one or two generations. After this initial "incubation time" the descendants of the infected bacterium become very efficient donors. This lasts several generations, but then the ability to induce transfer is repressed, and transfer occurs only rarely. Finnegan & Willetts (1971, 1972, 1973) and Grindley et al. (1973a) have investigated the genetics of transfer repression of F-like plasmids. In order to enable transfer, the transfer gene complex (*traY* to *traZ*) has to be translated. The translation of this complex is positively controlled by the product of the *traJ* gene. This gene in its turn, is negatively controlled by the combination of the products of two other genes (*finO* and *finP*). After infection of a fresh host, *traJ* immediately gets translated. Its gene product enables the translation of the *tra* gene complex, which, in its turn, makes conjugational transfer possible. However, at the same time the *finO* and *finP* gene products are synthesized, repressing the transcription of *traJ*. First *traJ* and afterwards the other *tra* gene products are then gradually diluted by subsequent cell divisions. (For a review, see Willetts & Skurray, 1980). Freter et al. (1983) have estimated the transfer rate both from the original host and from newly infected bacteria. It appears

that for the transfer repressing plasmid R1 the transfer rate from the newly infected host is 10^5 to 10^8 times as high as from the original donor. After several generations, the transfer rate drops to 10^{-1} to 10^{-4} times of that of the newly infected bacterium.

SURFACE EXCLUSION

Bacteria already containing a plasmid are often much less efficient recipients for other plasmids than plasmid-free bacteria (Lederberg, Cavalli & Lederberg, 1952). This phenomenon is called surface or entry exclusion. It is caused by certain plasmid encoded proteins in the cell membrane of the plasmid bearing cells (Achtman, Kennedy & Skurray, 1977; Kennedy et al., 1977). Surface exclusion does not depend on the presence or absence of pili (Achtman, Willetts & Clark, 1971).

Not all plasmids exclude each other equally strongly. According to this property they can be ordered into groups of plasmids excluding each other mutually strongly, the so called surface exclusion groups. This classification has, however, several disadvantages. One of them is that surface exclusion is not necessarily mutual. It can only be detected if it is directed against transferable plasmids, so that only the conjugative plasmids can be classified in this way. Besides, it is, of course, a quantitative property, and it may therefore depend on the opinion of the investigator whether surface exclusion is called strong or not.

It appears that surface exclusion is often strong when the resident plasmid and the plasmid trying to enter are incompatible (Datta, 1979). The ordering of plasmids into surface exclusion groups gives therefore often the same result as the ordering into incompatibility groups. The latter classification is, however, more universally applicable and is more universally applied.

When several different plasmids with different surface exclusion systems, are present together in a bacterium, their exclusion systems may interact. Willetts & Maule (1974)

discovered that if two different plasmids occur in one host, sometimes only one and sometimes neither of the surface exclusion systems function. When two compatible plasmids encoding the same type of surface exclusion coexist in one bacterium, the degree of surface exclusion is not affected (Willetts & Maule, 1974).

The genes coding for surface exclusion in F-like plasmids are situated in the transfer gene complex (Achtman, Kennedy & Skurray, 1977; Willetts & Skurray, 1980). This implies that in case the transfer is repressed, surface exclusion is also repressed (Willetts & Skurray, 1980). The surface exclusion genes of several other plasmids are also situated in the transfer region (Alfaro & Willetts, 1972; Barth, 1979).

ANTIBIOTIC RESISTANCE

Since the introduction of antibiotics in medicine, there exists a strong selective advantage for resistance to antibiotics in bacteria confronted with these antibiotics. The origin of many antibiotic-resistant bacterial strains was therefore to be expected. However, it appears that the mechanism of antibiotic-resistance found in nature differs greatly from that induced in the lab (Benveniste & Davies, 1973a). The genes for resistance (R factors) found in nature are mostly situated on plasmids and their mode of action is much more sophisticated than that of chromosomal mutations causing resistance in laboratory populations. These plasmid-borne resistance genes can be transferred between bacteria of the same and sometimes also of different species (Jones & Sneath, 1970; Reanney, 1976). In this way, plasmids cause natural genetic engineering.

The R-factors found in different parts of the world are often very similar both in function and in structure (Datta & Hedges, 1972; Heffron et al., 1975; Barth & Datta, 1977; Farrar, 1981; Tietze, Prager & Tschäpe, 1982). This suggests a single origin of these factors. In many soil bacteria antibiotic resistance is already a favourable property for a very long time, because many soil organisms, including

several bacteria, produce antibiotics. The resistance genes in several soil bacteria show a close resemblance to R-factors on plasmids in gut bacteria and in bacteria causing diseases (Benveniste & Davies, 1973a, b; Polak & Novick, 1982). Therefore, it has been suggested that antibiotic resistance, nowadays common for many bacteria in the human gut, originally evolved in bacteria in the soil, and has been successfully transferred to gut bacteria at the time that conditions changed (Benveniste & Davies, 1973a,b; Jones & Sneath, 1970; Reanney, 1976; Polak & Novick, 1982).

The R-factors themselves are often situated on transposable elements (Tn). These elements can transpose one site to another leaving behind a copy at the original site. Transposition can occur inside a piece of DNA but also between different DNA molecules (Kopecko, 1980; Shapiro, 1980). In this way a plasmid can collect several R-factors laying on transposable elements. This implies that if a particular antibiotic is used, a resistance factor against that antibiotic, situated on a plasmid, may disseminate throughout the bacterial population. That plasmid may take along resistance against several other antibiotics. In this way, the spread of antibiotic resistance is far more effective than simple mutation towards resistance can be.

HORIZONTAL GENE TRANSFER

The fast spread of antibiotic resistance all over the world following the introduction of antibiotics is an example of gene transfer between different (bacterial) species. This kind of gene transfer is called horizontal gene transfer. There is little reason to suppose that the observed spread of R-factors, caused by human interference, is exceptional. The vehicles for this spread, the plasmids, were already present before antibiotics were introduced (Hughes & Datta, 1983), and sudden changes in the environment can also have a natural (i.e. non-human) origin. The importance of horizontal gene transfer for bacterial evolution is not yet clear. It may imply that almost every bac-

terial species can obtain any gene of an arbitrary other species (Reanney, 1976). In that case, a species is not a reproductively isolated entity, and the species concept becomes very ill-defined for bacteria.

GENETIC ENGINEERING

Plasmids are often used as tools in genetic engineering. They are capable to introduce genes into a bacterium and this property is used in bio-engineering. In order to prevent the escape of artificially constructed bacteria into nature, the employed bacteria often have a metabolic deficiency. They can only survive when some nutrient is provided which is scarce in nature. However, if the artificially introduced genes are introduced into a plasmid, and that plasmid is capable of independent transfer or mobilization, these genes may be able to escape from their crippled host and may be transferred to a more healthy bacterium. In that case, escape from the lab may not be impossible. Stewart & Levin (1977) studied the theoretical possibility of the establishment of an unfavourable gene if that gene is situated on a conjugative plasmid. This appears to be possible under rather broad conditions. The conditions under which a mobilizable, non-conjugative plasmid can be maintained are, however, far more restricted (Levin & Stewart, 1980), and escape of such a plasmid from the laboratory is therefore not very likely, although by no means impossible.

SELECTION ACTING ON PLASMIDS

Plasmids have an important impact on the evolution of bacteria. They may propagate throughout the bacterial realm. Bacteria can acquire new genes, which are already completely evolved, by means of plasmid transfer. These genes will sometimes be favourable or even necessary for the bacteria. In this way, plasmids increase the adaptability of bacteria (Jones & Sneath, 1970; Reanney, 1976). Plasmids themselves are of course also subject to evolution. It is doubtful

whether plasmids would ever have come into existence if their sole selective advantage was increasing the bacterial adaptability. In the first place, increasing the adaptability of bacteria is a longterm advantage, whereas the costs of the maintenance of plasmids and their transfer are immediate. Besides, the flexibility of the bacterial gene content will only increase after conjugative plasmids have developed. Moreover, plasmids make it possible for bacteria to acquire new genes, but the acquired genes will often be useless and burdensome for the bacteria as energy and nutrients for transcription and translation are needed (Zünd & Lebek, 1980; Godwin & Slater, 1979; Helling et al., 1981).

Conjugative plasmids can spread infectiously over a bacterial population. This may enable them to compensate for their negative effects on the fitness of their bacterial bearer (Stewart & Levin, 1977). In that case they can invade a bacterial population by infection and stay in existence. To see how non-conjugative plasmids maintain themselves is more difficult. Some of these plasmids can be mobilized by conjugative plasmids. Levin & Stewart (1980) have calculated that these mobilizable plasmids can be maintained under rather restricted conditions, even if they are slightly unfavourable for their bacterial host. Non-conjugative, non-mobilizable plasmids cannot spread infectiously. They do not influence horizontal gene transfer. How they can maintain themselves is a still unsolved problem.

In this thesis, the attention will be restricted to conjugative plasmids. For some properties it will be investigated how selection affects them.

Chapter 2 & 3. One of the most important properties of conjugative plasmids is clearly their ability to induce transfer. To enable transfer several transfer products have to be synthesized (Willetts & Wilkins, 1984). Plasmid-bearing bacteria, able to transfer, have pili (Bradley, 1980a, 1981). These pili make the bacterium susceptible to infection by pilus-specific bacteriophages (Brinton, Gemski & Carnahan, 1964; Caro & Schnös, 1966; Bradley, 1976, 1980a). The ability to transfer will burden the energy budget and

increase the nutrient consumption of a cell. The growth rate of bacteria with transferable plasmids will, therefore, in most cases be lower than the growth rate of bacteria with non-conjugative plasmids, and each increase in transfer rate will probably decrease the bacterial growth rate. As long as transfer can only occur after an accidental collision between the plasmid-bearing cell and another bacterium, there is a maximum transfer rate: each collision results in transfer. To exceed this maximum transfer rate, a new mechanism has to be developed, for instance an increased mobility of the host bacterium, a directed motion towards other bacteria or a virus-like infection mechanism. Such a mechanism will probably be, in one way or another, disadvantageous for a bacterium, and decrease the growth rate. The question whether there is an optimal transfer rate if an increase in transfer rate decreases the growth rate of the bacterial host will be answered in Chapter 2 for plasmids in a chemostat. In Chapter 3 it is shown that the answer is qualitatively identical if the host population is periodically transferred to a fresh food supply. It is demonstrated in Chapters 2 and 3 that two incompatible plasmids excluding each other completely can sometimes coexist, it is shown, however, that three such plasmids cannot coexist.

Chapter 4. Plasmids tend to exclude other, incompatible plasmids from their host. Generally there is no entry barrier against compatible plasmids (Datta, 1979; Finger & Krishnapillai, 1980; Winans & Walker, 1985). Sometimes, however, a compatible plasmid is excluded, while an incompatible plasmid can enter (Alfaro & Willetts, 1972; Hedges & Datta, 1973). In order to exclude another plasmid, a plasmid must contain exclusion genes (Achtman, Kennedy & Skurray, 1977; Barth, 1979), and synthesize the proteins responsible for exclusion (Kennedy et al., 1977). These exclusion proteins are situated somewhere in the cell membrane (Kennedy et al., 1977; Hartskeerl, Tommassen & Hoekstra, 1985), and change the properties of the membrane. This will influence the bacterial fitness. Producing extra proteins creates probably extra costs. If the change in the cell membrane is so

advantageous for the bacterium that the costs are more than compensated for, bacteria would probably have arisen, encoding for these proteins themselves. Therefore, the possession of surface exclusion genes probably is in some way disadvantageous for a bacterium, and will reduce the overall growth rate. Then why do plasmids exclude other incompatible plasmids? This question is investigated in Chapter 4.

Chapter 5. Ozeki, Stocker & Smith (1962) discovered that a newly infected bacterium is a much more efficient plasmid donor than a host infected many bacterial generations ago. Since the ability to induce transfer is harmful for the bacterial host, an inefficient donor will have a higher fitness than a very efficient one. Several investigators (Stocker, Smith & Ozeki, 1963; Broda, 1979b; Campbell, 1981) have suggested that the ability to suppress transfer in "old" hosts is of advantage for a plasmid. They reason that transfer from a donor will occur more often if there is a surplus of recipients, for in that case the probability of an accidental collision with a recipient is higher. Therefore, a newly infected host will occur more often in an environment with plenty of recipients. And in such an environment, it pays to have a high transfer rate, because there are many potential victims to infect. On the other hand, if recipients are scarce, transfer will occur only rarely, and most plasmid-bearing bacteria will have been infected many generations ago. If these long ago infected bacteria are inefficient donors, the bacterial fitness is higher. And even if the transfer rate per donor and per recipient would be high, the number of transfers would be small, since the concentration of potential recipients is low. This seems to be a plausible reasoning. A colony of bacteria bearing repressing plasmids will grow faster than a colony bearing non-regulating, permanently derepressed, plasmids. Therefore, if most of the bacteria in the colonies are plasmid-bearing, the number of plasmid copies of the regulating type will increase faster, since the colony with the regulating plasmids grows faster: The colony of regulating plasmids has an advantage over the colony of non-regulating, permanently derepressed plasmids,

as long as they are isolated from each other, and therefore this reasoning is based on group selection. The theory of group selection is mostly involved to explain the existence of characteristics which are (slightly) unfavourable for an individual, but which are in some way or another beneficial for the population. For example, group selection arguments have been used to explain why individuals restrict the number of their offspring in case of overcrowding. This is clearly disadvantageous for the individual, since it gets less descendants, but it may increase the survival probability of the population (Eshel, 1972; Boorman & Levitt, 1973). According to these arguments, unlimited population growth, resulting in depletion, and maybe destruction, of their habitat, is prevented by self imposed reproduction restrictions of the individuals. In the case of regulating plasmids, the advantage of regulation for the group is different: Populations of regulating plasmids can grow faster than populations of non-regulating, permanently derepressed plasmids. However, the faster growth in number of a group of regulating plasmids does not imply that such a group is secured against the invasion of a non-regulating plasmid. What will be the fate of a non-regulating mutant plasmid, which is permanently derepressed in a population of regulating plasmids? Only in the case that such a mutant plasmid is not able to invade and take over the plasmid population, transfer regulation can be an evolutionarily stable strategy. (For a survey of the theory of evolutionarily stable strategies (ESS) see Maynard Smith, 1982). This question will be investigated in Chapter 5.

Population Dynamics of Compatible Plasmids, Chapter 6. In Chapters 2, 3, 4 and 5 the result of selection on plasmid populations is investigated by considering the fate of a mutant plasmid in the population. A plasmid and its mutants are, of course, closely related, and will therefore be in most cases incompatible. However, what will happen with competing compatible plasmids?

In many natural bacterial populations, more than one plasmid type coexist, in most cases belonging to different

incompatibility groups (Christiansen et al., 1973; Datta et al., 1979; Richards & Datta, 1982; Lee et al., 1984; Hedges, Smith & Brazil, 1985). In Chapter 6 the restrictions on coexistence of several compatible plasmids are investigated. The hypothesis that several species can only coexist if they use their environment in some way or another differently (the one niche - one species hypothesis, Gause, 1934; Gilbert et al., 1952) suggests that several plasmids will only be able to coexist if they employ their environment (which is the population of their bacterial hosts) in different ways, for instance if one possesses a high transfer rate, and another a higher growth rate of its bacterial bearer. Hedges, Smith & Brazil (1985) discovered three compatible plasmids in some bacterial strains, and wondered how these three plasmids could coexist considering the one niche - one species hypothesis. They suggested that the coexistence of the three plasmids is only a transient stage. However, Hutchinson remarked in 1957 that the one niche - one species principle will probably not hold in case competition is almost entirely intraspecific. It is therefore an interesting problem whether competition between compatible plasmids is mostly inter- or intra-specific. In the latter case competition between compatible plasmids might be an example contradicting the one niche - one species principle, provided of course that one is prepared to extend the species concept to plasmids. This question is investigated in Chapter 6.

Some unsolved problems, Chapter 7. In this thesis some questions concerning the population genetics and population dynamics of plasmids are investigated. However, it is of course not possible to deal with the whole scope of plasmid evolution and population dynamics even restricting oneself to mathematical modelling. Some interesting, still unsolved problems are therefore shortly introduced in Chapter 7.

J. theor. Biol. (1984) **110**, 411-423

A Mathematical Model for the Co-existence of Incompatible, Conjugative Plasmids in Individual Bacteria of a Bacterial Population

NELLY VAN DER HOEVEN

Department of Genetics, Centre of Biological Sciences, University of Groningen, PO Box 14, 9750 *AA Haren, The Netherlands*

(*Received* 16 *December* 1983, *and in revised form* 23 *March* 1984)

A model is formulated to examine the possibility of coexistence of two or more plasmids of the same surface exclusion group in a bacterial chemostat culture. It appears that two plasmids are able to coexist. If two plasmids can coexist they will follow different survival strategies, one with a high conjugational transfer rate and a low fitness of its host, and the other with a low transfer rate and a high host fitness. Coexistence of three plasmids of the same surface exclusion group is impossible.

Introduction

Plasmids are pieces of extrachromosomal circular DNA. They occur frequently in bacteria. Although plasmids are autonomous some of them are capable of recombination with, and incorporation into, the chromosome of their bacterial host. Other naturally occurring plasmids are known only in an independent state. These plasmids encode a mechanism for their own replication and an (almost) even distribution of their copies over the daughter cells at cell division. Some of these independent plasmids are capable of infectious transmissions to a bacterium without such a plasmid in case of cell to cell contact between the plasmid-bearing (donor) bacterium and a plasmid-free (recipient) bacterium. This transmission is called conjugation. A mathematical model describing the conditions for the maintenance of such plasmids was formulated by Stewart & Levin (1977).

The cell membrane of bacteria containing a conjugational plasmid is often changed in such a way that plasmids of the same type are no longer able to infect these bacteria. Related plasmids are also excluded. This phenomenon is called cell surface exclusion (Willets & Maule, 1974). The combination of incompatibility and surface exclusion is called super infection immunity.

Plasmids can be ordered into incompatibility groups (inc. groups). An inc. group is a group of related plasmids which use in some way or another

the same replication and/or segregation mechanism. This implies that, if a bacterium contains two different plasmid types of the same inc. group no distinction is made between those types when choosing a plasmid copy for replication. When such a bacterium divides both daughter cells will obtain about the same number of plasmid copies, but the distribution of the two different types over the daughter cells will be at random. The rate at which bacteria, containing both types of plasmids, are lost in a population has been calculated by Novick & Hoppensteadt (1978) and Cullum & Broda (1979) among others.

On the other hand, plasmids can also be ordered into surface exclusion groups. This ordering is in many instances the same, but unfortunately not always. Besides, this ordering has the disadvantage that it is possible for two plasmids to exist such that one excludes the other but the other does not exclude the first (Willets & Maule, 1974).

Although surface exclusion is never absolute, it can be quite strong. Finger & Krishnapillai (1980) found that the entry frequency in a recipient which exhibits surface exclusion is 1000 to 100 000 times smaller than that in a non-excluding recipient.

Clearly, two plasmids exhibiting super infection immunity cannot stably co-exist in the same bacteria. If they start in different bacteria, they will never be able to enter a bacterium containing the other plasmid, and if they start in the same bacteria, they will segregate because they are incompatible unless there is strong selection for bacteria carrying both (Cullum & Broda, 1979). However, another question is: can there be stable co-existence of the two types in a bacterial culture? To solve this question a mathematical model will be formulated. It will appear that two plasmids of the same surface exclusion group can sometimes co-exist in one bacterial population. The obvious next question, can three types of plasmids co-exist if two can, will also be solved. The answer to this question provides a prediction about the types of plasmids expected to exist.

Model for Two Competing Plasmids of the Same Surface Exclusion Group

Stewart & Levin (1977) have shown that a plasmid can be maintained in a continuous culture, even if the fitness of the plasmid-bearing bacteria is less than that of the plasmid-free bacteria. However, is it possible for two plasmids, belonging to the same surface exclusion group to co-exist in a chemostat? To answer this question the following model is formulated to describe the behaviour of a two plasmid-one bacterium complex in a continuous culture.

BASIC ASSUMPTIONS

In formulating the model I have made, in addition to the usual chemostat assumptions (see the next section) also the following suppositions.

It is assumed that there is only one limiting resource in the chemostat. The growth rates of the bacteria without plasmids, of bacteria with plasmid species P_1 and with plasmid species P_2 are proportional to the same function of the limiting resource concentration s, $f(s) \cdot f(s)$ is an increasing function of s. The proportionality parameters are measures of fitness of the three different types of bacteria. They are respectively w_0, w_1 and w_2.

The consumption of the limiting resource is proportional to the increase of the bacterial concentration. Per cell division a quantity e of the limiting resource is needed. Plasmid-bearing bacteria lose their plasmid with constant rate τ, independent of the plasmid type.

The conjugational transfer rate is proportional to the chance of an accidental collision between a plasmid-bearing and a plasmid free bacterium. In other words, a simple mass-action model is assumed. The conjugational proportionality parameter differs for the two plasmid types, γ_1 for P_1 and γ_2 for P_2. The surface exclusion is assumed to be absolute. All symbols are listed in Table 1.

TABLE 1

List of parameters used in the model

b_0, b_1, b_2	Concentration of plasmid-free, P_1-bearing and P_2-bearing bacteria.
s	Concentration of the limiting resource in the chemostat.
k	Concentration of the limiting resource in the inflow.
ρ	Turnover rate of the chemostat.
γ_1, γ_2	Conjugational transfer parameter of P_1 and P_2.
τ	Rate of loss of plasmids from plasmid-bearing bacteria.
w_0, w_1, w_2	Fitnesses of plasmid free, P_1-bearing and P_2-bearing bacteria.
$f(s)w_i$	Growth rate of bacteria b_i.
e	Quantity of resource needed for one cell division.
$h(b_0+b_1+b_2) = w_0 f(k - e(b_0+b_1+b_2))$	
$v_1 = w_1/w_0$	
$v_2 = w_2/w_0$	

THE MATHEMATICAL MODEL

The inflow of the chemostat consists of a constant nutrient solution with concentration k. In the chemostat the inflow is thoroughly mixed with the chemostat content which can be considered homogeneous. The rate of removal of the contents is equal to the inflow rate ρ. Therefore the rate of

concentration changes in the chemostat is

$$\frac{ds}{dt} = \rho(k - s) - ef(s)(w_0 b_0 + w_1 b_1 + w_2 b_2) \tag{1a}$$

$$\frac{db_0}{dt} = w_0 f(s) b_0 - \rho b_0 - \gamma_1 b_0 b_1 - \gamma_2 b_0 b_2 + \tau b_1 + \tau b_2 \tag{1b}$$

$$\frac{db_1}{dt} = w_1 f(s) b_1 - \rho b_1 + \gamma_1 b_0 b_1 - \tau b_1 \tag{1c}$$

$$\frac{db_2}{dt} = w_2 f(s) b_2 - \rho b_2 + \gamma_2 b_0 b_2 - \tau b_2. \tag{1d}$$

It is obvious that an input-output equilibrium will rapidly be attained. At this equilibrium $s + e(b_0 + b_1 + b_2) = k$. Therefore one can replace $f(s)$ by a function h of $(b_0 + b_1 + b_2)$ such that

$$h(b_0 + b_1 + b_2) = w_0 f(k - e(b_0 + b_1 + b_2)). \tag{2}$$

Since $f(s)$ is an increasing function of s, $h(b)$ is decreasing. The fitness of the plasmid containing bacteria can be taken relatively to the fitness of the plasmid-free bacteria. The relative fitness w_i / w_0 is called v_i.

Rescaling system (1) yields

$$\frac{db_0}{dt} = h(b_0 + b_1 + b_2) b_0 - \rho b_0 - \gamma_1 b_0 b_1 - \gamma_2 b_0 b_2 + \tau b_1 + \tau b_2 \tag{3a}$$

$$\frac{db_1}{dt} = v_1 h(b_0 + b_1 + b_2) b_1 - \rho b_1 + \gamma_1 b_0 b_1 - \tau b_1 \tag{3b}$$

$$\frac{db_2}{dt} = v_2 h(b_0 + b_1 + b_2) b_2 - \rho b_2 + \gamma_2 b_0 b_2 - \tau b_2. \tag{3c}$$

In order for plasmids P_1 and P_2 to be able to coexist in a chemostat culture, system (2) has to have a stable internal equilibrium, i.e. a stable equilibrium at which $\hat{b}_0 > 0$, $\hat{b}_1 > 0$ and $\hat{b}_2 > 0$.

THE INTERNAL EQUILIBRIUM

At equilibrium the rates of concentration changes are zero. $db_1/dt = 0$ implies that

$$\hat{b}_1 = 0 \tag{4a}$$

or

$$\hat{h} v_1 - \rho - \tau + \gamma_1 \hat{b}_0 = 0, \tag{4b}$$

$db_2/dt = 0$ implies that

$$\hat{b}_2 = 0 \tag{5a}$$

or

$$\hat{h}v_2 - \rho - \tau + \gamma_2\hat{b}_0 = 0 \tag{5b}$$

and $db_0/dt = 0$ if

$$(\hat{h} - \rho)\hat{b}_0 + \hat{b}_1(\tau - \gamma_1\hat{b}_0) + \hat{b}_2(\tau - \gamma_2\hat{b}_0) = 0. \tag{6}$$

In case both plasmids coexist $b_1 > 0$ as well as $b_2 > 0$.

Therefore at internal equilibrium equations (4b) and (5b) hold. Thus

$$\hat{h} = (\tau + \rho)(\gamma_2 - \gamma_1)/(\gamma_2 v_1 - \gamma_1 v_2) \tag{7}$$

and

$$\hat{b}_0 = (\tau + \rho)(v_1 - v_2)/(\gamma_2 v_1 - \gamma_1 v_2). \tag{8}$$

Without loss of generality we take $v_1 > v_2$.

Since $\hat{h}$ as well as b_0 have to be positive, γ_2 must be greater than γ_1. Combining equations (6), (7) and (8) results in

$$\hat{b}_2 = \hat{b}_1\left(\frac{\tau v_1(\gamma_2 - \gamma_1) - \rho\gamma_1(v_1 - v_2)}{\rho\gamma_2(v_1 - v_2) - \tau v_2(\gamma_2 - \gamma_1)}\right) + \frac{(\tau + \rho)(v_1 - v_2)}{(\gamma_2 v_1 - \gamma_1 v_2)} \cdot \frac{(\tau + \rho)(\gamma_2 - \gamma_1) - \rho(\gamma_2 v_1 - \gamma_1 v_2)}{(\rho\gamma_2(v_1 - v_2) - \tau v_2(\gamma_2 - \gamma_1)}. \tag{9}$$

If the fitness of the plasmid containing bacteria is lower than the fitness of the plasmid-free bacteria, i.e. $v_2 < v_1 < 1$ a necessary condition for coexistence, since $\hat{h} \in (\rho, \rho/v_2)$ is

$$\gamma_1/\gamma_2 < \frac{\tau + \rho - v_1\rho}{\tau + \rho - v_2\rho} \tag{10a}$$

and

$$\gamma_1/\gamma_2 > \frac{\tau + \rho - \rho v_1/v_2}{\tau}. \tag{10b}$$

If conditions (10a) and (10b) are satisfied, the existence will still depend on the function h. Provided that h is a decreasing function, it can be proved that the equilibrium, if existing, is stable (see Appendix).

If one of the types of plasmid-bearing bacteria has a higher fitness than the plasmid-free bacteria, and the other type a lower fitness, i.e. $v_2 < 1 < v_1$

a necessary condition for existence, since $\hat{h} \in (\rho/v_1, \rho/v_2)$, is

$$\gamma_1/\gamma_2 < \frac{\tau v_1/v_2}{(\rho+\tau)v_1/v_2 - \rho} \tag{11}$$

while inequality (10b) also has to hold.

If $1 < v_2 < v_1$ inequality (11) has to hold for equilibrium and also, if $(\rho+\tau) - \rho v_1 > 0$

$$\gamma_1/\gamma_2 > \frac{(\tau+\rho) - \rho v_1}{(\tau+\rho) - \rho v_2} \tag{12}$$

since $\hat{h} \in (\rho/v_1, \rho)$.

Unfortunately, in the last two cases, the condition that h decreases is not sufficient to secure the existence of only one equilibrium at which $b_0 > 0$, $b_1 > 0$ and $b_2 = 0$ in case $v_1 > 1$.

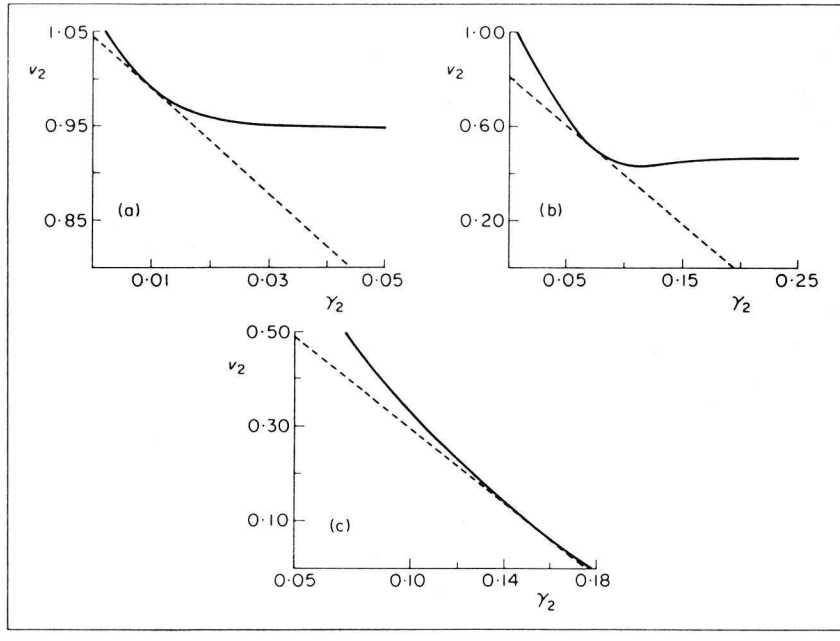


FIG. 1. Possibility of coexistence of two plasmids. Transfer rate γ and hosts fitness v of plasmid P_1 fixed. (a) $\gamma_1 = 0{\cdot}01$, $v_1 = 0{\cdot}99$. (b) $\gamma_1 = 0{\cdot}075$, $v_1 = 0{\cdot}5$. (c) $\gamma_1 = 0{\cdot}15$, $v_1 = 0{\cdot}1$. A second plasmid P_2 can coexist with P_1 if (γ_2, v_2) is between the broken and the solid line. If (γ_2, v_2) is above the solid line then P_2 will expell P_1. If (γ_2, v_2) is under the broken line then P_1 will expell P_2. $\rho = 0{\cdot}1$, $\tau = 0{\cdot}005$, $c = 1{\cdot}125$.

For a given function h the area in which P_1 and P_2 can coexist can be computed. A reasonable choice is the hyperbolic relation between nutrient supply and the growth rate, i.e. $f(s) = \alpha s/(\beta + s)$ (Monod, 1949). Scaling the time in units of generation time of plasmid-free bacteria and k/e at unity h becomes

$$h(b) = \frac{1-b}{c-b}. \tag{13}$$

In Fig. 1 the values of v_2 and γ_2 for which P_2 can coexist with P_1 with given v_1 and γ_1 are shown.

THE FATE OF A THIRD PLASMID

To investigate what will happen when a third plasmid P_3 tries to invade a stable two plasmid-one bacterium equilibrium I will first extend system (3) with the dynamics of the third plasmid. Let the fitness of the P_3-bearing bacteria be v_3 and the conjugational transfer rate parameter of P_3 be γ_3.

The extended form of system (3) becomes

$$\frac{db_0}{dt} = h(b_0 + b_1 + b_2 + b_3)b_0 - \rho b_0 - \gamma_1 b_0 b_1 - \gamma_2 b_0 b_2 - \gamma_3 b_0 b_3 + \tau b_1 + \tau b_2 + \tau b_3 \tag{14a}$$

$$\frac{db_1}{dt} = v_1 h(b_0 + b_1 + b_2 + b_3)b_1 - \rho b_1 + \gamma_1 b_0 b_1 - \tau b_1 \tag{14b}$$

$$\frac{db_2}{dt} = v_2 h(b_0 + b_1 + b_2 + b_3)b_2 - \rho b_2 + \gamma_2 b_0 b_2 - \tau b_2 \tag{14c}$$

$$\frac{db_3}{dt} = v_3 f(b_0 + b_1 + b_2 + b_3)b_3 - \rho b_3 + \gamma_3 b_0 b_3 - \tau b_3. \tag{14d}$$

For coexistence of P_1, P_2 and P_3 equations (4b) and (5b) have to hold just as in case of coexistence of only P_1 and P_2.

Besides, in equilibrium $db_3/dt = 0$ and, when P_3 is not excluded,

$$\hat{h}v_3 - \rho - \tau + \gamma_3 \hat{b}_0 = 0. \tag{15}$$

It is only possible for all three equations (4b), (5b) and (15) to hold together if

$$v_3 = -\gamma_3 \frac{(v_1 - v_2)}{\gamma_2 - \gamma_1} + \frac{(\gamma_2 v_1 - \gamma_1 v_2)}{\gamma_2 - \gamma_1} \tag{16}$$

which is very unlikely to be exactly true.

Therefore, if P_3 can penetrate into an equilibrium of P_1 and P_2 at least one of the plasmids P_1 and P_2 will be eliminated, and P_3 can invade if

$$\hat{h}v_3 - \rho - \tau + \gamma_3\hat{b}_0 > 0 \tag{17}$$

or if

$$v_3 > -\gamma_3\frac{(v_1 - v_2)}{(\gamma_2 - \gamma_1)} + \frac{(\gamma_2 v_2 - \gamma_1 v_2)}{(\gamma_2 - \gamma_1)}. \tag{18}$$

This implies that for every combination (γ_3, v_3) above the line connecting (γ_1, v_1) and (γ_2, v_2) in the (γ, v) plane P_3 can invade an equilibrium of P_1 and P_2 (Fig. 2).

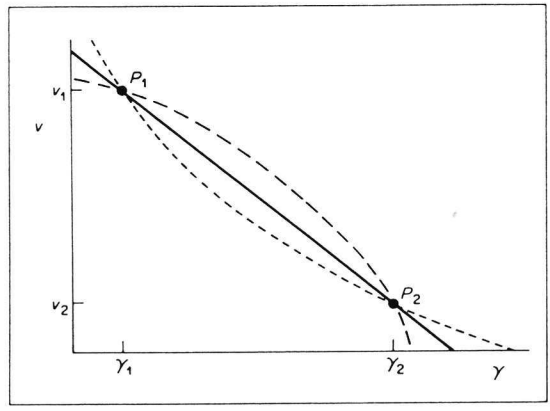


FIG. 2. Possibility for a third plasmid to invade a two plasmid equilibrium. A third plasmid P_3 with transfer rate γ_3 and hosts fitness v_3 can invade an equilibrium of P_1 and P_2 if (γ_3, v_3) above the solid line P_1P_2. If $v = g(\gamma)$ is convex (— —) then a plasmid P_3 with transfer rate γ_3 and host fitness $v_3 = g(\gamma_3)$ can penetrate if (γ_3, v_3) is above the solid line P_1P_2, so if $\gamma_1 < \gamma_3 < \gamma_2$. On the other hand if $v = g(\gamma)$ is concave (· · · · · ·) then a plasmid P_3 has a combination of γ_3 and $v_3 = g(\gamma_3)$ above the solid line P_1P_2, if $\gamma_3 < \gamma_1$ or $\gamma_3 > \gamma_2$.

COMPETITION BETWEEN MANY DIFFERENT PLASMIDS

Suppose that many different plasmids may occur. Each plasmid $\tilde{P}_i$ has given conjugational transferrate $\tilde{\gamma}_i$ and the fitness of a $\tilde{P}_i$ bearing bacterium is $\tilde{v}_i$. Which plasmids will finally survive? Obviously all plasmids $\tilde{P}_i$ with $(\tilde{\gamma}_i, \tilde{v}_i)$ will be eliminated if there exists at least one plasmid $\tilde{P}_j$ with $\tilde{\gamma}_j > \tilde{\gamma}_i$ and $\tilde{v}_j > \tilde{v}_i$. Let $\{P\}$ be the subset of $\{\tilde{P}\}$, the set of all plasmids, such that iff $\tilde{P}_j \in \{\tilde{P}\}$ and for every $\tilde{P}_i \in \{\tilde{P}/\tilde{P}_j\}$, $\tilde{v}_i < \tilde{v}_j$ or $\tilde{\gamma}_i < \tilde{\gamma}_j$ then $\tilde{P}_j \in \{P\}$. In other words there exists no plasmid $\tilde{P}_i \in \{\tilde{P}\}$ so that it has both a higher conjuga-

tional transferrate and a higher hosts fitness than a plasmid $P_j \in \{P\}$. Only plasmids $P_j \in \{P\}$ are candidates for final survival, and therefore I will restrict my attention to plasmids of $\{P\}$.

One can describe the relation between γ_i and v_i for all $P_i \in \{P\}$ by a decreasing function $g: \gamma \to v$, since if $\gamma_i > \gamma_j$ then $v_i < v_j$ (see Fig. 3). Suppose g is convex, i.e. $d^2g/d\gamma^2 < 0$ (Fig. 2). Then, if an equilibrium of two plasmids exists, a third plasmid with host fitness v_3 and conjugational transferrate γ_3 somewhere in between the hosts fitnesses and the transferrates of the two already established plasmids will always be able to invade and expell at least one of the other plasmids. On the other hand, if $\gamma_3 < \min(\gamma_1, \gamma_2)$ on $\gamma_3 > \max(\gamma_1, \gamma_2)$, the third plasmid will not be able to penetrate.

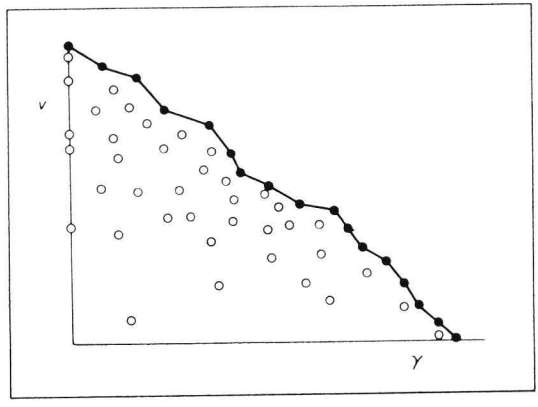


FIG. 3. Collection of plasmids all with a different combination of γ and v. ○, plasmids for which at least one other plasmid exists with higher transfer rate and higher host fitness ($\{\tilde{P}/\{P\}\}$); ●, plasmids for which no other plasmid exists which has both a higher transfer rate and a higher fitness ($\{P\}$). The solid line is an arbitrary decreasing function $g: \gamma \to v$ through all (γ, v) of plasmids ●.

After a new equilibrium is reached with the intermediate plasmid and one of the originals, another plasmid in between these two can invade. This can continue until only one plasmid survives. This finally surviving plasmid will have γ and v as near as possible to $\check{\gamma}$ and $\check{v}$ such that $\check{v} = g(\check{\gamma})$ and the tangent to g at $\check{\gamma}$ coincides with

$$v = \frac{\rho + \tau - \gamma \hat{b}_0(\check{\gamma})}{\hat{h}(\check{\gamma})}$$

in which $\hat{b}_0(\gamma)$ and $\hat{h}(\gamma)$ are the equilibrium values of b_0 and h for an equilibrium with only one type of plasmid with transferrate γ and host fitness $g(\gamma)$.

In other words, $\check{\gamma}$ can be computed by solving

$$\hat{b}_0(\check{\gamma})/\hat{h}(\check{\gamma}) = -\left.\frac{dg}{d\gamma}\right|_{\check{\gamma}}. \tag{19}$$

However since g is completely unknown, and h only approximately the possibility to compute $\check{\gamma}$ exists for the time being only theoretically.

If, on the other hand, g is concave, i.e. $d^2g/d\gamma^2 > 0$ (see Fig. 2) a third plasmid will be able to invade in a two plasmid equilibrium if and only if its conjugational transferrate γ_3 is outside the interval between the transferrates of the two established plasmids. In other words, a third plasmid can invade if $\gamma_3 < \min(\gamma_1, \gamma_2)$ or $\gamma_3 > \max(\gamma_1, \gamma_2)$ and will be excluded if $\min(\gamma_1, \gamma_2) < \gamma_3 < \max(\gamma_1, \gamma_2)$. The invading plasmid P_3 will expell the plasmid with conjugational transferrate somewhere in between its own and that of the other plasmid. This process can of course be repeated until only plasmids with minimal and with maximal transferrate survive.

Discussion

In the previous section it is shown that two plasmids belonging to the same Surface Exclusion group can co-exist in a chemostat. However three plasmids can never be maintained together. To attain these results I have made several assumptions. One would like to know how realistic those assumptions are and if the conclusions are robust to change in them.

The possibility for two and the impossibility for three plasmids to co-exist is based on the fact that equations (4b) and (5b) form a system of two linear equations with two unknowns (b_0 and h) and are therefore solvable, while for co-existence of three plasmids a third equation (15) is added to the system without adding a third unknown. Therefore this system of three equations has almost nowhere in the parameter space a solution. Considering this it is clear that the conclusions are independent of the assumption of equal loss rate τ for all plasmid types.

The assumption that the nutrient need per bacterium (e) does not depend on the presence of a plasmid is reasonable as long as the limiting resource is not needed solely or mostly to build DNA or to construct a plasmid-coded product. However, the assumption itself is redundant because the nutrient need e does not appear in equations (1c) and (1d).

It seems reasonable to assume that the dependence of the growth rate of the different bacteria on the limiting resource has roughly the same configuration. The assumption of proportionality is maybe too strong but offers the possibility to define fitness parameters. If one drops the proportionality one has to be content with "fitness functions" $f_i(s)$ instead of the fitness para-

meters w_i. However, it can be shown that it is sufficient to assume that

$$\frac{1}{\gamma_i}\frac{df_i}{ds} \neq \frac{1}{\gamma_j}\frac{df_j}{ds}$$

for the conclusions to hold. On the other hand, if there is more than one limiting resource and the efficiency of the resource utilization depends on the plasmid types which are carried, it might be possible that more than two plasmid types can co-exist. This would be an example of the one niche–one species theory, while this model shows that in a chemostat with only one niche for the bacteria at most two related plasmids can co-exist.

The assumption that the conjugational transfer rate is proportional to the chance of accidental collision between a donor and a recipient bacterium was checked by Levin, Stewart & Rice (1979) and found reasonably accurate. Collins & Broda (1975), on the other hand, state that the transfer rate per donor and per recipient decreases as the bacterial concentration increases. It can be shown that the conclusions do not change as long as the transfer rate is for all plasmid types P_i proportional to the concentration of P_i-bearing bacteria times a function Γ of all bacterial concentrations, which is the same for all plasmid types. But even if the transfer rate is only approximately proportional to such a function Γ, the subset of the parameter space for which P_1, P_2 and P_3 can co-exist, will be very small and can be safely neglected. Of course, if the surface exclusion is not absolute an extra equation should be added to describe the dynamics of bacteria carrying two different plasmids. However, since surface exclusion is often quite strong (Finger & Krishnapillai, 1980) this type of bacteria will only occur in a non-neglectable quantity if its fitness is higher than that of the other bacterial types. Consequently, the conclusions that sometimes two, but never three plasmids of the same surface exclusion group can co-exist, is rather robust.

Experiments in which incompatibility properties are tested are mainly executed under exponential growth conditions and in absence of conjugational transfer. Under such conditions all plasmid types originally present will stay in the population, because host death is scarce, but their ratio will change. However, under limiting growth condition, selection pressure will be expected to lead to the extinction of all but one or two plasmid types. If two plasmid types survive, one of them will have low or zero conjugational transfer rate. So selection pressure can explain the existence of non-conjugative plasmids, even if they reduce their hosts' fitness.

The relation between transfer rate and hosts' fitness is little studied. Such studies are complicated by the fact that the host growth rate and the transfer

rate depend on the type of bacteria and on the environment. Godwin & Slater (1979) observed the arisal of new plasmid types while studying the maintenance of a plasmid in a continuous culture. In most of their experiments the plasmid population after several weeks continuous culture consists almost entirely out of one or two types. Which types survive depends on the limiting resource.

It is also conceivable that plasmids will arise which combine both survival strategies (low fitness, high transfer rate and high fitness, low transfer rate). This could be realized by sometimes suppressing the ability to transfer. This phenomenon is described by Stocker, Smith & Ozeki (1963) among others. They found that plasmid-bearing bacteria, which have acquired their plasmids only a few generations ago are much more efficient donors than those which carry that plasmid already for many generations. The evolution of this phenomenon will be further investigated using an extension of the present model.

The manuscript was typed by Mrs J. Poelstra-Hiddinga. The figures were prepared by Mr H. Mulder. This investigation was supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

REFERENCES

COLLINS, J. F. & BRODA, P. (1975) *Nature* **258,** 722.
CULLUM, J. & BRODA, P. (1979). *Genet. Res.* **33,** 61.
FINGER, J. & KRISHNAPILLAI, V. (1980). *Plasmid* **3,** 332.
GODWIN, D. & SLATER, J. H. (1979). *J. gen. Microbiol.* **111,** 201.
LEVIN, B. R., STEWART, F. M. & RICE, V. A. (1979). *Plasmid* **2,** 247.
MONOD, J. (1949). *A. Rev. Microbiol.* **3,** 371.
NOVICK, R. P. & HOPPENSTEADT, F. C. (1978). *Plasmid* **1,** 421.
STEWART F. M. & LEVIN, B. R. (1977). *Genetics* **87,** 209.
STOCKER, B. A. D., SMITH, S. M. & OZEKI, H. (1963). *J. gen. Microbiol.* **30,** 201.
WILLETS N. & MAULE, J. (1974). *Genet. Res.* **24,** 81.

APPENDIX

The equilibrium of system (2) with positive $\hat{b}_0$, $\hat{b}_1$, and $\hat{b}_2$ is locally asymptotically stable if all eigenvalues of the jacobian matrix at equilibrium have negative real parts. The first derivative of system (2) at internal equilibrium (the jacobian matrix) is

$$\begin{pmatrix} -\alpha\hat{b}_0 - \tau\dfrac{\hat{b}_1+\hat{b}_2}{\hat{b}_0} & -\alpha\hat{b}_0 - \gamma_1\hat{b}_0 + \tau & -\alpha\hat{b}_0 - \gamma_2\hat{b}_0 + \tau \\ -\alpha v_1\hat{b}_1 + \gamma_1\hat{b}_1 & -\alpha v_1\hat{b}_1 & -\alpha v_1\hat{b}_1 \\ -\alpha v_2\hat{b}_2 + \gamma_2\hat{b}_2 & -\alpha v_2\hat{b}_2 & -\alpha v_2\hat{b}_2 \end{pmatrix}$$

in which

$$\alpha = -\left.\frac{\mathrm{d}h}{\mathrm{d}(b_0+b_1+b_2)}\right|_{(b_0+b_1+b_2)=(\hat{b}_0+\hat{b}_1+\hat{b}_2)} > 0,$$

since h is a decreasing function. Therefore, the eigenvalue equation is

$$\begin{aligned}0 &= \lambda^3 + \lambda^2\{\alpha(\hat{b}_0 + v_1\hat{b}_1 + v_2\hat{b}_2) + \tau(\hat{b}_1 + b_2)/\hat{b}_0\} \\ &\quad + \lambda\left\{\begin{array}{c}\alpha(\tau[(\hat{b}_1+\hat{b}_2)/\hat{b}_0](v_1\hat{b}_1+v_2\hat{b}_2) + \gamma_1\hat{b}_1\hat{b}_0 + \gamma_2\hat{b}_2\hat{b}_0 \\ +(\tau-\gamma_1\hat{b}_0)v_1\hat{b}_1 + (\tau-\gamma_2\hat{b}_0)v_2\hat{b}_2) \\ +\gamma_1\hat{b}_1(\gamma_1\hat{b}_0-\tau) + \gamma_2\hat{b}_2(\gamma_2\hat{b}_0-\tau)\end{array}\right\} \\ &\quad + \alpha\hat{b}_0\hat{b}_1\hat{b}_2(\gamma_2-\gamma_1)(\gamma_2 v_1 - \gamma_1 v_2) \\ &\equiv \lambda^3 + (\alpha p_1 + p_2)\lambda^2 + (\alpha q_1 + q_2)\lambda + r\alpha\end{aligned}$$

All eigenvalues have negative real parts if

$$\alpha p_1 + p_2 > 0, \qquad \alpha q_1 + q_2 > 0, \qquad \alpha r > 0 \quad \text{and} \quad (\alpha p_1 + p_2)(\alpha q_1 + q_2) - \alpha r > 0.$$

We have p_1 and $p_2 > 0$ since $\hat{b}_0$, $\hat{b}_1$ and $\hat{b}_2$ are positive at the internal equilibrium; $r > 0$, since $\gamma_2 > \gamma_1$ and $v_1 > v_2$; and $q_1 > 0$, since if $v_2 < v_1 < 1$, then $\gamma_i\hat{b}_0\hat{b}_i(1-v_i) > 0$ $(i = 1, 2)$, and if $v_2 < 1 < v_1$ then $\tau - \gamma_1\hat{b}_0 > 0$ and $\gamma_2\hat{b}_0\hat{b}_2(1-v_2) > 0$, while if $1 < v_2 < V_1$, then $\tau - \gamma_1\hat{b}_0 > 0$ and $-\gamma_2\hat{b}_0 + \tau + \tau(b_1+b_2)/\hat{b}_0 = \hat{h}v_2 - \rho + \tau(\hat{b}_1+\hat{b}_2)/\hat{b}_0 = \hat{h}(v_2-1) + \gamma_1\hat{b}_1 + \gamma_2\hat{b}_2 > 0$.

However, q_2 is not always positive.

$$\begin{aligned}q_2 &= \gamma_1\hat{b}_1(\gamma_1\hat{b}_0 - \tau) + \gamma_2\hat{b}_2(\gamma_2\hat{b}_0 - \tau) \\ &= \gamma_1\hat{b}_0(\hat{h} - \rho) + (\gamma_2 - \gamma_1)\hat{b}_2(\rho - \hat{h}v_2).\end{aligned}$$

If $v_2 < v_1 < 1$ then $h - \rho > 0$ and $\rho - hv_2 > 0 \Rightarrow q_2 > 0$. Finally we have to show that $(\alpha p_1 + p_2)(\alpha q_1 + q_2) - \alpha r > 0$; $(\alpha p_1 + p_2)(\alpha q_1 + q_2) - \alpha r = \alpha^2 p_1 q_1 + \alpha(p_2 q_1 + p_1 q_2 - r) + p_2 q_2$. $p_1 q_1 > 0$ since $p_1 > 0$ and $q_1 > 0$. It can be proved after tedious calculation that $p_2 q_1 + p_1 q_2 - r > 0$ if $q_2 > 0$. So, if $q_2 > 0$ the internal equilibrium is stable. If $q_2 < 0$ the condition for stability of the equilibrium is

$$\alpha > \frac{r - p_1 q_2 - p_2 q_1 + \sqrt{(p_1 q_1 + p_1 q_2 - r)^2 - 4p_1 p_2 q_1 q_2}}{2p_1 q_1}.$$

CHAPTER 3:

COEXISTENCE OF INCOMPATIBLE PLASMIDS IN A BACTERIAL POPULATION LIVING UNDER A FEAST AND FAMINE REGIME

ABSTRACT

A model is formulated to examine the possibility of (co)existence of plasmids of the same incompatibility and surface exclusion group in a bacterial population living under a feast and famine regime. The condition is given under which a growth rate decreasing plasmid can invade a bacterial population. It appears that in case only one plasmid type is present, the frequency of plasmid bearers will tend to a stable equilibrium if the food supply at each growth site gets exhausted and both plasmid-free and plasmid-bearing bacteria need an equal quantity of food per cell division. Otherwise the frequency of plasmid-bearers might oscillate. Two plasmids will sometimes be able to coexist, but only if they follow different survival strategies, one with a high conjugational transfer rate and a lower fitness of its host, and the other with a low transfer rate, and a higher host fitness. Coexistence of three plasmids of the same surface exclusion group is impossible.

INTRODUCTION

Plasmids are pieces of extrachromosomal circular DNA. They occur frequently in bacteria. Most plasmids are represented with more than one copy in bacterial cells. The number of copies per cell depends both on the plasmid type and on the bacterial species, and is called the copy number. Plasmid replication is often regulated by the plasmid itself. Plasmids encode also a mechanism to ensure an (almost) even distribution of their copies over the daughter cells of their host at cell division. Sometimes the correct segregation of the plasmids over the daughter cells fails, and one of the new born cells is plasmid-free. This seems to occur only rarely. Nordström, Molin & Aagaard-Hansen (1980) and Nordström & Aagaard-Hansen (1984) estimated the loss rate to

be less than 10^{-4} and 3.10^{-6} respectively per cell division.

Related plasmids often use the same mechanism for regulating their replication and segregation. When a bacterium, carrying copies of two related plasmids, divides, each daughter cell may contain only one of the two plasmid types with non-zero probability. In the long run, the descendants of such a bacterium will consist of bacteria carrying either the first or the second of the two plasmid types, but never both. Such related plasmids are called incompatible and the phenomenon, that the descendants of a bacterium bearing two incompatible plasmids will split up in bacteria carrying only one type, is called incompatibility segregation. Plasmids are ordered according to this property into incompatibility groups. Novick & Hoppensteadt (1978), Ishii, Hashimoto-Gotoh & Matsubara (1978) and Cullum & Broda (1979) have studied theoretical models for the rate of incompatibility segregation.

Some plasmids are capable of infectious transmission from their host bacterium (the donor) to a plasmid-free bacterium (the recipient) in case of cell to cell contact between the potential donor and recipient. This transmission is called conjugation. The transfer rate often depends on the generation time of the donor with a limit of one transfer per generation (Cullum, Collins & Broda, 1978a, b).

Plasmids often prevent the entrance by conjugation of a second, related, plasmid into their bacterial host. This phenomenon is called Surface Exclusion or Entrance Exclusion. In most cases, incompatible plasmids exclude each other (Finger & Krishnapillai, 1980). Although surface exclusion will never be absolute, it can be quite strong. Willetts & Maule (1974) found that the entrance frequency can be reduced with a factor of about 100, and Finger & Krishnapillai (1980) found even reduction with a factor of 10^3 to 10^5. In a previous paper (van der Hoeven, in press: Chapter 4) I have investigated whether it is advantageous for a plasmid to exclude other, incompatible plasmids from its host. This appeared to be so for low copy number plasmids.

Plasmids often influence the growth rate of their bacter-

ial bearers in one way or another. Some plasmids make their host resistant against antibiotics, which is of course of great advantage in the presence of antibiotics. Without antibiotics, however, resistance becomes a drawback. In most cases, plasmids appear to be slightly growth rate reducing.

Stewart & Levin (1977) showed that even growth rate reducing plasmids can sometimes maintain themselves by conjugation. They both investigated the case in which the bacterial population lives in a chemostat and in a periodically changing environment in which the bacteria consume their food supply after which they are transferred to a fresh supply (a feast and famine regime). In a previous paper (van der Hoeven, 1984: Chapter 2) I showed under which conditions a second plasmid can invade a plasmid-bearing bacterial population in a chemostat, when both plasmids are incompatible and exclude each other completely. I also showed that under chemostatic growth conditions two incompatible plasmids can coexist, but three cannot. If an increase in conjugational transfer rate causes a reduction of the growth rate of the bacterial host, selection would lead to an optimal transfer rate, or to the coexistence of two plasmids, one with a very high transfer rate, and the other non-conjugative. However, only part of the natural environments of plasmids, such as animal guts, resembles chemostat conditions. What will happen with plasmids, if their bacterial hosts live under feast and famine conditions? Will two plasmids be able to coexist under that regime? And if two plasmids can coexist, can three also? To solve these questions a mathematical model will be formulated.

BASIC ASSUMPTIONS

The growth of the bacterial population is assumed to be food limited and the food supply gets exhausted. After a fixed time T, or when all food is consumed, a fixed number of bacteria is transferred to a fresh food supply. At the new site the initial nutrient concentration is s_0 (s is the nutrient concentration). The bacterial population consists

of plasmid-free bacteria (concentration b) and bacteria carrying one plasmid species (p_i denotes the concentration of bacteria carrying plasmid P_i). The whole bacterial population is assumed to be homogeneously mixed. The growth rates of all types of bacteria are assumed to be proportional to the same function $f(s)$ of the nutrient concentration. $f(s)$ is an increasing function, and $f(0) = 0$. The proportionality parameters are denoted by w_i and can be considered as the fitness of bacteria carrying plasmid P_i (the function $f(s)$ is chosen such that the proportionality parameter for plasmid-free bacteria is 1). A fixed amount of food is needed for each cell division, e for plasmid-free bacteria and e_i for bacteria carrying plasmid P_i.

The plasmids are able to induce conjugational transfer. The probability of transfer of plasmid P_i is proportional to the probability of an accidental collision between a P_i bearing and a plasmid-free bacterium, and therefore proportional to the product of the concentrations b and p_i. The transfer rate is also assumed to be proportional to the growth rate of the bacteria. The proportionality parameter is called the transfer rate of plasmid P_i and denoted by γ_i. It is assumed that plasmids cannot enter a bacterium already containing another plasmid (complete surface exclusion).

Plasmid P_i bearing bacteria can loose their plasmids at cell division. Therefore, the loss rate is proportional to the growth rate. The proportionality parameter is denoted by τ_i. (All symbols are listed in table I).

MODEL FOR ONE PLASMID SPECIES

Stewart & Levin (1977) formulated a model for the case of only one plasmid. Under a feast and famine regime, the bacterial population will, during its stay at one site, consume its food supply and grow according to the following equations:

$$\frac{ds}{dt} = -f(s)(eb+w_1e_1p_1) \qquad (1a)$$

Table I

List of parameters

P_i: The i^{th} plasmid

B : Total bacterial concentration

b : Concentration of plasmid-free bacteria

p_i: Concentration of bacteria carrying plasmid P_i

s : Concentration of the growth rate limiting resource

$f(s)$: Growth rate of plasmid-free bacteria as a function of the limiting resource

$w_i f(s)$: Growth rate of P_i bearing bacteria

γ_i: Conjugational transfer rate parameter of plasmid P_i

τ_i: Loss rate parameter of plasmid P_i

e, e_i: Quantity of resource needed for one cell division of respectively plasmid-free, and plasmid P_i bearing bacteria

$x = \Sigma p_i/(b+\Sigma p_i)$

$y = p_2/(p_1+p_2)$

$z = p_3/(p_2+p_3)$

$B_t(n)$, $b_t(n)$, $s_t(n)$, $x_t(n)$, $y_t(n)$, $z_t(n)$: Respectively B, b, s, x, y, z in site n at time t.

$$\frac{db}{dt} = f(s)(b-\gamma_1 b p_1+\tau_1 p_1) \qquad (1b)$$

$$\frac{dp_1}{dt} = f(s)(w_1 p_1+\gamma_1 b p_1-\tau_1 p_1) \qquad (1c)$$

After a fixed time T, or after exhausting the food (equivalent to $T \to \infty$), a fixed number of bacteria is transferred to a new food supply with food concentration s_0. This will lead to an initial concentration of bacteria at the new site of B_0 (B is the total bacterial concentration). The fraction of P_1 bearing bacteria ($x = p_1/(p_1+b)$) in the transferred bacteria is the same as the end frequency at the former food site. So x_0 at site n ($x_0(n)$) is x_T at site $(n-1)$ ($x_T(n-1)$). During the stay at a site,

$$\frac{dx}{dt} = xf(s)\{(w_1-1)(1-x)+\gamma_1 b-\tau_1\} \qquad (2)$$

A plasmid will be able to penetrate in a plasmid-free population if $x_0(n+1) = x_T(n) > x_0(n)$ for small values of x, therefore if

$$\int_0^T \frac{dx}{dt}\,dt > 0 \Rightarrow \int_0^T \frac{1}{x}\frac{dx}{dt}\,dt > 0.$$

So, a plasmid will be able to penetrate, if in absence of P_1

$$\int_0^T f(s)(w_1-1+\gamma_1 b-\tau_1)dt > 0 \qquad (3)$$

In absence of P_1, $\frac{ds}{dt} = -ef(s)b$ and $eB_0 + s_0 = eb + s \Rightarrow b = B_0 + (s_0-s)/e$. Therefore, inequality (3) implies that

$$\int_{s_0}^{s_T} \frac{f(s)(w_1-1+\gamma_1(b_0+(s_0-s_1)/e)-\tau_1)}{-ef(s)(B_0+(s_0-s)/e)}\,ds > 0$$

$$\Rightarrow \int_{s_T}^{s_0} \frac{(w_1-1-\tau_1)}{eB_0+s_0-s}\,ds + \int_{s_T}^{s_0} \frac{\gamma_1}{e}\,ds > 0$$

$$\Rightarrow (w_1-1-\tau_1)\ln(B_T/B_0) + \gamma_1(B_T-B_0) > 0 \qquad (4)$$

(see also Stewart & Levin (1977) for another derivation of this result).

Successive transfer of the bacteria leads to the sequence $x_0(1), x_0(2), \ldots, x_0(n)\ldots$. Since both B_0 and s_0 are fixed, the whole course of x, B and s at a site is determined by x_0, therefore the sequence is the solution of a first order recursive relation. In case both plasmid-free and plasmid-bearing bacteria need an equal amount of food per cell division ($e=e_1$) and the food supply at each site gets exhausted, the sequence $\{x_0(n)\}$ will converge to a stable equilibrium. (Under these conditions, the system is at each moment com-

pletely determined by x and B, $\frac{dx}{dB} = x\{((w_1-1)+\gamma_1 B)(1-x)-\tau_1\}/\{(1-x+w_1 x).B\}$ and $\frac{dB}{dB} = 1$. Since B_0 and B_T will be the same at each site, and the solution curves cannot intersect, the sequence $\{x_0(n)\}$ will either be increasing or decreasing). At that equilibrium

$$\int_0^T \frac{dx}{dt}\,dt = 0 \Rightarrow \int_0^T f(s).\{(w_1-1)(1-x)+\gamma_1 b-\tau_1\}dt = 0 \qquad (5)$$

However, if either the food supply does not get exhausted, or the quantity of resource needed per cell division depends on the plasmid content, the sequence $\{x_0(n)\}$ will not necessarily converge to a stable equilibrium. Fig. 1 shows examples both of sequences, converging to an equilibrium, and of non-converging sequences. However, even when the infinite sequence $\{x_0(n)\}$ does not converge to an equilibrium, it will have one or more accumulation points since $x_0(n) \in [0,1]$.

MODEL FOR TWO PLASMID SPECIES

Can a second plasmid P_2 invade a population, already containing a plasmid P_1, and can the two plasmids coexist? To answer these questions, the model given in the previous section, will be extended to a two-plasmid model. The growth dynamics at one site now become

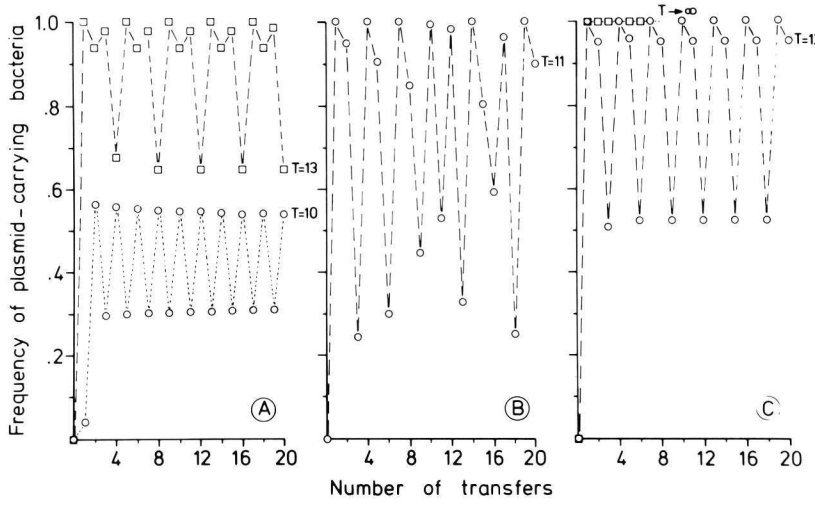
$$\frac{ds}{dt} = -f(s)(eb+w_1 e_1 p_1+w_2 e_2 p_2) \qquad (6a)$$

$$\frac{db}{dt} = f(s)(b-\gamma_1 b p_1-\gamma_2 b p_2+\tau_1 p_1+\tau_2 p_2) \qquad (6b)$$

$$\frac{dp_1}{dt} = f(s)(w_1 p_1+\gamma_1 b p_1-\tau_1 p_1) \qquad (6c)$$

$$\frac{dp_2}{dt} = f(s)(w_2 p_2+\gamma_2 b p_2-\tau_2 p_2) \qquad (6d)$$

The dynamics at each site are completely determined by the initial frequency of plasmid-bearing bacteria at that site



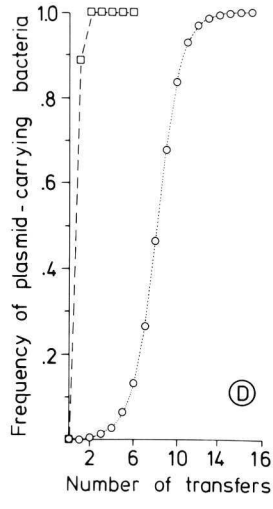


Fig. 1. Invasion of a plasmid-free bacterial population. The initial frequency of plasmid-carrying bacteria is 10^{-3}. The growth function $f(s) = s/(2+s)$, $e = e_1 = 10^{-6}$. At the beginning of each period the concentration of nutrients $s_0 = 100$ and of bacteria $B_0 = 1000$. a, b, c) $w_1 = 0.5$; $\gamma_1 = 5.10^{-7}$; $\tau = 10^{-4}$. a) □: $T = 13$ (4-cycle); o: $T = 10$ (2-cycle). b) o: $T = 11$. c) □: $T \to \infty$ (stable equilibrium); o: $T = 12$ (3-cycle). d) $w_1 = 0.9$; $\tau = 10^{-6}$; $T \to \infty$; o: $\gamma = 2.10^{-8}$; □: $\gamma = 10^{-7}$.

and the initial frequency of P_2 in the plasmid population. These initial frequencies are identical to the end frequencies at the previous site. Let x be the frequency of plasmid-bearing bacteria ($x = (p_1+p_2)/(b+p_1+p_2)$) and y the frequency of P_2 bearing bacteria in the plasmid-bearing bacterial population ($y = p_2/(p_1+p_2)$), then, at a particular

site the dynamics of x and y are

$$\frac{dx}{dt} = xf(s).\{(1-x)(w_1(1-y)+w_2y)+\gamma_1b(1-y)+\gamma_2by-\tau_1(1-y)-\tau_2y\} \tag{7a}$$

and $$\frac{dy}{dt} = y(1-y)f(s).\{(w_2-\tau_2)-(w_1-\tau_1)+(\gamma_2-\gamma_1)b\} \tag{7b}$$

P_2 will be able to penetrate if $y_0(n)$ increases for small values of y_0. In the case that the population without P_2 is at a transfer equilibrium ($x_0(n) = \hat{x}_0$), the situation is easy to analyse. y increases if

$$y_0(n+1) > y_0(n) \Rightarrow \int_0^T f(s)((w_2-\tau_2)-(w_1-\tau_1)+(\gamma_2-\gamma_1)b)dt > 0 \tag{8}$$

which implies that P_2 will certainly be able to invade if both $(w_2-\tau_2) > (w_1-\tau_1)$ and $\gamma_2 > \gamma_1$. In this case y_0 will always increase and P_1 will finally become expelled. If either $(w_2-\tau_2) > (w_1-\tau_1)$ and $\gamma_1 > \gamma_2$ or vice versa, P_2 may or may not be able to invade, depending on the relative magnitude of the two terms in inequality (8). If no transfer equilibrium exists, the same conclusion holds, since for small values of y

$$\frac{dy}{dt} \tilde{>} yf(s)\{(w_2-\tau_2)-(w_1-\tau_1)+(\gamma_2-\gamma_1)b\} \tag{9}$$

so y can only increase if either $(w_2-\tau_2) > (w_1-\tau_1)$ or $\gamma_2 > \gamma_1$. Only in case $(w_2-\tau_2) > (w_1-\tau_1)$ and $\gamma_2 < \gamma_1$ or vice versa, coexistence of P_1 and P_2 may occur.

If two plasmids coexist, the whole course of B, s, x and y at each site is completely determined by x_0 and y_0. The sequence $\{x_0(n),y_0(n)\}$ gives therefore a complete description of the fate of both plasmids. In Fig. 2 some sequences of initial frequences of coexisting plasmids are shown. Sometimes a stable equilibrium of initial frequencies for coexisting plasmids will be reached, in other cases a limit cycle will be attained. The infinite sequence $\{x_0(n),y_0(n)\}$ will have one or more accumulation points. If P_1 and P_2 coexist y

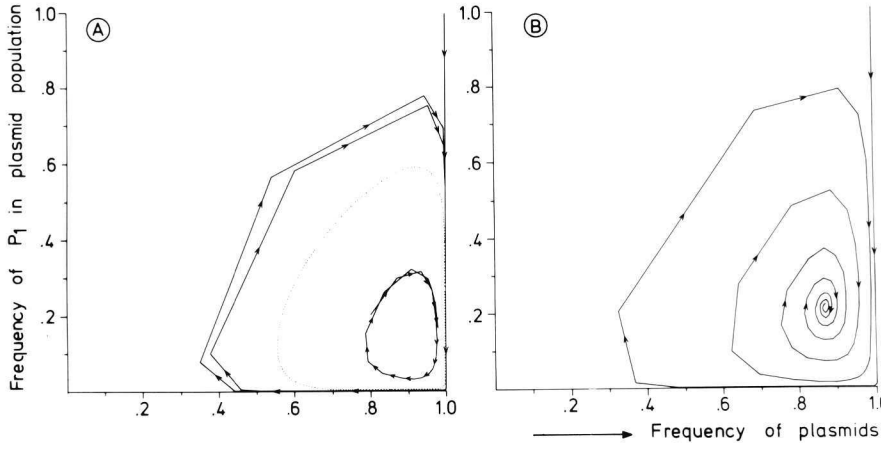


Fig. 2. Coexistence of two incompatible plasmids. $w_1 = 0.9$; $\gamma_1 = 5.10^{-8}$; $\tau_1 = 10^{-6}$; $w_2 = 0.95$; $\gamma_2 = 10^{-9}$; $\tau_2 = 10^{-6}$. (As in Fig. 1: $f(s) = s/(2+s)$; $e = e_1 = 10^{-6}$; $s_0 = 100$; $B_0 = 1000$). a) $T \to \infty$. The dotted line is the stable limit cycle. b) $T = 12$. A stable equilibrium will be attained.

cannot become arbitrarily close to 0 or 1, so there exists an $\varepsilon > 0$ such that $y_t(n) \in [\varepsilon, 1-\varepsilon]$ for each n ($> m_0$). Therefore, there exists for each $\delta > 0$ a subsequence $\{x_0(n_i), y_0(n_i)\}$ such that

$$|y_0(n_{i+1}) - y_0(n_i)| < \delta\varepsilon(1-\varepsilon)$$

$$\Rightarrow \left| \sum_{j=n_i}^{n_{i+1}-1} \int_0^T \frac{dy(j)}{dt}\, dt \right| < \delta\varepsilon(1-\varepsilon)$$

$$\Rightarrow \left| \sum_{j=n_i}^{n_{i+1}-1} \int_0^T \frac{1}{y(j)(1-y(j))} \frac{dy(j)}{dt}\, dt \right| < \delta$$

$$\Rightarrow \left|((w_2-\tau_2)-(w_1-\tau_1)).\sum_{j=n_i}^{n_{i+1}-1}\int_0^T f(s(j))dt - (\gamma_1-\gamma_2).\sum_{j=n_i}^{n_{i+1}-1}\int_0^T f(s(j))b(j)dt\right| < \delta \qquad (10)$$

Since δ can be chosen arbitrarily small, a subsequence $\{(x_0(n_i),y_0(n_i))\}$ can be chosen such that

$((w_2-\tau_2)-(w_1-\tau_1))\sum_{j=n_i}^{n_{i+1}-1}\int_0^T f(s(j))dt$ is arbitrarily close to $(\gamma_1-\gamma_2).\sum_{j=n_i}^{n_{i+1}-1}\int_0^T b(j)f(s(j))dt$. This result will be used in the next section to determine whether a third plasmid will be able to invade.

MODEL FOR THREE PLASMID SPECIES

In the last section it is shown that two plasmids may coexist, provided that one has a higher transfer rate (for instance P_1:$\gamma_1>\gamma_2$) and the other has a higher net growth rate ($w_2-\tau_2>w_1-\tau_1$). Can three plasmids also coexist? In a previous paper (van der Hoeven, 1984: Chapter 2) I have shown that three plasmids cannot coexist in a chemostat culture.

To answer these questions, the model is extended to three plasmid species. At each site the growth dynamics are given by the equations

$$\frac{ds}{dt} = -f(s)(eb+w_1e_1p_1+w_2e_2p_2+w_2e_3p_3) \qquad (11a)$$

$$\frac{db}{dt} = f(s)(b-\gamma_1bp_1-\gamma_2bp_2-\gamma_3bp_3+\tau_1p_1+\tau_2p_2+\tau_3p_3) \qquad (11b)$$

$$\frac{dp_i}{dt} = f(s)(w_ip_i+\gamma_ibp_i-\tau_ip_i) \quad \text{for } i = 1, 2, 3 \qquad (11c)$$

Let x be the frequency of plasmid-bearers in the bacterial population ($x=(p_1+p_2+p_3)/(b+p_1+p_2+p_3)$), y the frequency of

P_2 carriers among P_1 and P_2 carriers ($y=p_2/(p_1+p_2)$) and z the frequency of P_3 carriers among P_2 and P_3 carriers ($z = p_3/(p_2+p_3)$). The initial frequencies (x_0, y_0, z_0) at one site are identical to the end frequencies at the last site. These initial frequencies completely determine the dynamics at a site (provided that $p_2 \neq 0$: in that case the variable $p_3/(p_2+p_3)$ should be replaced by $p_3/(p_1+p_3)$). At a site, the dynamics of x, y and z are given by

$$\frac{dx}{dt} = x.f(s). \begin{pmatrix} (1-x)(w_1(1-y)(1-z)+w_2y(1-z)+w_3yz) \\ +\gamma_1 b(1-y)(1-z)+\gamma_2 by(1-z)+\gamma_3 byz \\ -(\tau_1(1-y)(1-z)+\tau_2y(1-z)+\tau_3yz) \end{pmatrix} /(1-z+yz) \tag{12a}$$

$$\frac{dy}{dt} = y(1-y)f(s)((w_2-\tau_2)-(w_1-\tau_1)+(\gamma_2-\gamma_1)b) \tag{12b}$$

and $$\frac{dz}{dt} = z(1-z).f(s)((w_3-\tau_3)-(w_2-\tau_2)+(\gamma_3-\gamma_2)b) \tag{12c}$$

P_3 will be able to penetrate if z_0 increases for small values of z. Since, however, x_0 and y_0 may differ at different sites, it is possible that $z_0(n) > z_0(n-1)$, while $z_0(n+1) < z_0(n)$. $z_0 = 0$ will be unstable, if the sequence $\{z_0(n)\}$ becomes larger than some value $\varepsilon > 0$ from some value m of n, i.e. if there is an $\varepsilon > 0$ such that if $z_0(0) = \delta > 0$ then $z_0(n) > \varepsilon$ for all $n \geq m$. For small values of z

$$\frac{dz}{dt} \approx z.f(s).\{(w_3-\tau_3)-(w_2-\tau_2)+(\gamma_3-\gamma_2)b\} \tag{13}$$

If P_1 and P_2 coexist, there will be some $\varepsilon > 0$ such that $y_t(n) \in [\varepsilon, 1-\varepsilon]$, i.e. neither the fraction of P_1, nor the fraction of P_2 in the total plasmid population decreases beneath a certain threshold ε. In the absence of P_3, the sequence $\{(x_0(n), y_0(n))\}$ has for each $\delta > 0$ at least one subsequence $\{(x_0(n_i), y_0(n_i))\}$ such that $|y_0(n_i)-y_0(n_{i+1})| < \delta\varepsilon(1-\varepsilon)$, which implies that

$$\left[\sum_{j=n_i}^{n_{i+1}-1} \int_0^T f(s(j))b(j)dt\right] - \tilde{\delta} <$$

$$\frac{(w_2-\tau_2)-(w_1-\tau_1)}{(\gamma_1-\gamma_2)} \sum_{j=n_i}^{n_{i+1}-1} \int_0^T f(s(j))dt <$$

$$\left[\sum_{j=n_i}^{n_{i+1}-1} \int_0^T f(s(j))b(j)dt\right] + \tilde{\delta}, \qquad (14)$$

where $\tilde{\delta} = |\delta/(\gamma_1-\gamma_2)|$.
In that case $z(n_{i+1}) > z(n_i)$ if

$$\sum_{j=n_i}^{n_{i+1}-1} \int_0^T \frac{dz(j)}{dt}\, dt > 0 \Rightarrow \sum_{j=n_i}^{n_{i+1}-1} \int_0^T \frac{1}{z(j)} \frac{dz(j)}{dt}\, dt > 0 \Rightarrow$$

$$\Rightarrow \quad ((w_3-\tau_3)-(w_2-\tau_2)).\sum_{j=n_i}^{n_{i+1}-1} \int_0^T f(s(j))dt +$$

$$+ (\gamma_3-\gamma_2).\sum_{j=n_i}^{n_{i+1}-1} \int_0^T f(s(j))b(j)dt > 0; \text{ therefore always if}$$

$$\{(w_3-\tau_3)-(w_2-\tau_2)+\frac{(\gamma_3-\gamma_2)}{(\gamma_1-\gamma_2)}.((w_2-\tau_2)-(w_1-\tau_1))\} .$$

$$.\left(\sum_{j=n_i}^{n_{i+1}-1} \int_0^T f(s(j))dt\right) - \delta/|\gamma_1-\gamma_2| > 0 \qquad (15)$$

$$\text{If } \gamma_3 > (w_3-\tau_3).\frac{\gamma_2-\gamma_1}{(w_2-\tau_2)-(w_1-\tau_1)} - \frac{(w_1-\tau_1)(\gamma_2-\gamma_1)}{(w_2-\tau_2)(w_1-\tau_1)} \qquad (16)$$

it is always possible to find a $\delta > 0$ such that inequality (15) holds. The same conditions hold for each accumulation point. Therefore, if inequality (16) holds, the sequence $\{z_0(n)\}$ has one or more increasing subsequences. Since only

a finite number of elements of the sequence $\{(x_0(n), y_0(n))\}$ does not belong to a converging subsequence, and some minimal value $k > 0$ will exist, such that $z(n+1) > k.z(n)$ for all n, the sequence $\{z_0(n)\}$ will always become larger than some value ε from some value of n if inequality (16) holds.

Therefore, if inequality (16) holds, a third plasmid P_3 can invade a population already containing the plasmids P_1 and P_2. This is the same condition as that for a third plasmid to invade a bacterial population in a chemostat which contains already two plasmids (van der Hoeven, 1984: Chapter 2). However, it is impossible that each plasmid can invade a population containing the other two plasmids.

Can three plasmids coexist, although they cannot all three invade a population already containg the other two? If they can coexist, both y_t and z_t should remain in the interval $(0,1)$, therefore some value $\varepsilon > 0$ exists, such that $y_t \in [\varepsilon, 1-\varepsilon]$ and $z_t \in [\varepsilon, 1-\varepsilon]$ for all t. The sequence $\{(x_0(n), y_0(n), z_0(n))\}$ has at least one accumulation point $(\tilde{x}_0, \tilde{y}_0, \tilde{z}_0)$. Since $(x_0, y_0, z_0) \in V = \{[0,1] \times [\varepsilon, 1-\varepsilon] \times [\varepsilon, 1-\varepsilon]\}$, the accumulation point $(\tilde{x}_0, \tilde{y}_0, \tilde{z}_0) \in \bar{V}$, and there exist, for each $\delta > 0$, an n_1 and n_2 such that

$$d((x_0(n_i), y_0(n_i), z_0(n_i)), (\tilde{x}_0, \tilde{y}_0, \tilde{z}_0)) < \tfrac{1}{2}\delta\varepsilon(1-\varepsilon) \text{ for } i = 1, 2,$$

so

$$|y_0(n_1) - y_0(n_2)| < \delta\varepsilon(1-\varepsilon) \tag{17a}$$

and $$|z_0(n_1) - z_0(n_2)| < \delta\varepsilon(1-\varepsilon) \tag{17b}$$

The first inequality leads to

$$\left|((w_2-\tau_2)-(w_1-\tau_1)) \cdot \sum_{j=n_1}^{n_2-1} \int_0^T f(s(j))\,dt - \right.$$

$$\left. - (\gamma_1-\gamma_2) \cdot \sum_{j=n_1}^{n_2-1} \int_0^T f(s(j))b(j)\,dt \right| < \delta \tag{18a}$$

and the second to

$$\left|((w_3-\tau_3)-(w_2-\tau_2)).\sum_{j=n_1}^{n_2-1}\int_0^T f(s(j))dt - \right.$$

$$\left. - (\gamma_2-\gamma_3).\sum_{j=n_1}^{n_2-1}\int_0^T f(s(j))b(j)dt\right| < \delta \qquad (18b)$$

Since δ can be chosen arbitrarily small, both conditions can only hold if

$$\frac{\gamma_1 - \gamma_2}{(w_2-\tau_2)-(w_1-\tau_1)} = \frac{\gamma_3 - \gamma_2}{(w_2-\tau_2)-(w_3-\tau_3)} \qquad (19)$$

This condition is very unlikely to be fulfilled. (The parameters should be in a subset of the parameter space with measure 0). Therefore, it is impossible that the sequence $\{(x_0(n),y_0(n),z_0(n))\}$ has an accumulation point in $\bar{V} = \{[0,1]\,x[\,\varepsilon,1-\varepsilon]\,x[\,\varepsilon,1-\varepsilon]\}$. Since $\varepsilon > 0$ can be taken arbitrarily small and the sequence has to have at least one accumulation point, it follows that at the accumulation point either $y = 0$ (no P_2) or $y = 1$ (no P_1), or $z = 0$ (no P_3) or $z = 1$ (no P_2), which implies that at least one plasmid gets expelled. This proves that three plasmids cannot coexist in a bacterial population subjected to a feast and famine regime.

DISCUSSION

It is shown in this paper that a plasmid can be maintained in a bacterial population living under a feast and famine regime, even if it reduces the growth rate of its bacterial host. This result was already obtained by Stewart & Levin (1977). If plasmid-bearing bacteria need an equal amount of resource per cell division as plasmid-free bacteria and the resource is exhausted, a stable initial plasmid frequency will be reached. In other cases the frequency of plasmid-bearers may oscillate. Two incompatible plasmids can coexist, provided that one has a higher transfer rate and

the other a higher intrinsic growth rate (hosts fitness minus loss rate). When two plasmids coexist, they do not have to occur in the same ratio at each bacterial transfer. A great number of transfers may be needed before it becomes clear which plasmid will win, or whether they can coexist. Three plasmids can, however, never coexist. When a bacterial population contains initially three plasmids, at least one of them will be lost.

The qualitative results of this study strongly resemble the conclusions, reached in an earlier paper (van der Hoeven, 1984: Chapter 2) for a bacterial population in a chemostat. By sequential transfer as well as in a chemostat two plasmids can coexist, provided they pursue different strategies (a higher transfer rate versus a higher intrinsic growth rate). Under both conditions it is impossible for three plasmids to coexist in the bacterial population. An important difference is, however, that with sequential transfer in many cases no stable equilibria exist, while in a chemostat stable equilibrium concentrations will be reached. The similarity of the qualitative behaviour of competing incompatible plasmids in a bacterial continuous culture and in a bacterial sequentially transferred population will not be preserved when plasmids, which can surpress their ability to conjugate, are considered (Chapter 5).

In the model it is assumed that incompatible plasmids exclude each other completely. When that assumption is relaxed, extra equations should be added to describe the dynamics of bacteria carrying two or more plasmids. Moreover, since the plasmids are incompatible, an extra equation has to be added for each ratio of two plasmids occupying the same host. The bacteria carrying two incompatible plasmids will disappear from the population but new ones will arise continuously by conjugation. In a previous paper (van der Hoeven, in press: Chapter 4) I have shown that, at least for low copy number plasmids, plasmids inducing surface exclusion, will have a selective advantage. For high copy number plasmids the dilution in frequency of the just entered plasmid by all the plasmid copies, that are already present, has

nearly the same effect as surface exclusion. Experimentally, surface exclusion has been found to be often quite strong (Finger & Krishnapillai, 1980).

The model studied in this paper is based on the widespread use of sequential transfer of bacterial populations in laboratories. It can, however, also be considered as a model for "seasonal" growth, in which only a fraction of the bacteria survives to the next "season" with an ample food supply. In that case, however, neither the initial food supply, nor the initial bacterial concentration will be constant. The time spent at each food site may also differ. Will the qualitative result be influenced by a fluctuating environment? In that case, the dynamical behaviour of the different fractions x, y and z at site n are completely determined by the initial frequencies x_0, y_0 and z_0, the initial bacterial density B_0, the initial nutrient concentration s_0 and the duration of the period $T(n)$. As long as B_0, s_0 and $T(n)$ are elements of closed and bounded sets, i.e. as long as they have an upper limit, the same conclusions can be drawn, based again on the argument that the sequence $\{(x_0, y_0, z_0, B_0, s_0, T)\}$ has to have at least one accumulation point.

It appears that the conclusion that two incompatible, excluding plasmids can coexist, but three cannot, has a broad validity.

CHAPTER 4:

EVOLUTION OF BACTERIAL SURFACE EXCLUSION AGAINST INCOMPATIBLE PLASMIDS†

ABSTRACT

Many conjugative transferable plasmids exhibit surface exclusion against plasmids of the same incompatibility group. A mathematical model is developed to calculate under which conditions surface exclusion against incompatible plasmids can evolve. It appears that plasmids inducing surface exclusion can evolve and even replace non excluding plasmids if the copy number is low and the transfer rate high provided that the cost of surface exclusion is small. They can more easily expel the non excluding plasmids if the possession of a plasmid is not very harmful for a bacterium and if the rate at which plasmids are lost is small.

I. INTRODUCTION

Plasmids are pieces of extrachromosomal, circular DNA. They occur abundantly in most bacterial species (Bukhari, Shapiro & Adhya, 1977). Most plasmids encode a mechanism for their own replication, independent of the replication mechanism of the bacterial chromosome. The replication rate is probably fixed, and not influenced by the number of plasmids present. This implies that during each cell cycle the same number of plasmid replications will occur (Gustafsson et al., 1978; Pritchard & Grover, 1981). At cell division the plasmids are distributed evenly over the daughter cells (Pritchard & Grover, 1981). The combination of these two mechanisms will result in a fixed number of plasmids at the start of each cell cycle. This number is called the copy number of the plasmid.

Related plasmids often use the same mechanism to regulate

†Journal of Theoretical Biology (in press)

the number of replications. When two related plasmids occur in the same bacterial host, the number of replications is the same as when only one of these plasmids is present. At each replication, the plasmid which will be duplicated, is chosen at random among all available plasmid copies. Related plasmids also often use the same mechanism to ensure that both daughter cells obtain the same number of plasmid copies at bacterial cell division. In that case, however, no distinction will be made between plasmid copies of the different types, so that both daughter cells will not necessarily contain the same number of each plasmid type. As a result of the inability to distinguish related plasmids at replication and segregation, the descendants of a bacterium containing two related plasmids will eventually consist of bacteria with either the first or the second plasmid type, but never with both. Plasmids are ordered according to this property into incompatibility groups (Datta, 1979). Novick & Hoppensteadt (1978), Ishii, Hashimoto-Gotoh & Matsubara (1978) and Cullum & Broda (1979) have developed a model to predict the rate at which bacteria containing two different plasmids of the same incompatibility group are lost in a population.

Some plasmids are capable of infectious transmission to other bacteria in case of cell to cell contact between their host and another bacterium. This process is called conjugation. Since only one strand of the plasmid DNA is transferred, the number of copies in the original host is kept constant.

Many conjugationally transferable plasmids change the cell membrane of their host in such a way that plasmids of the same type cannot enter their host. This phenomenon is called cell surface exclusion. In many cases not only plasmids of the same type are excluded, but also related plasmids (Willetts and Maule, 1974; Finger and Krishnapillai, 1980). As a result, plasmids belonging to the same incompatibility group often exclude each other. However, in contrast with incompatibility, surface exclusion is not always reciprocal.

Surface exclusion is produced by a change in the cell

membrane of the bacterial host. This alteration will probably decrease bacterial fitness, for if it were advantageous, bacteria would be expected to encode this property on their own chromosome. However, among conjugationally transferable plasmids surface exclusion is a common property, which suggests that it confers some advantage to a plasmid. This advantage could stem from the fact that surface exclusion frees a plasmid from competition at segregation with related plasmids. In this paper, a mathematical model is constructed to analyse the evolutionary dynamics of surface exclusion. The model predicts that, even if the avoidance of the loss of bacterial hosts as a result of incompatibility segregation is the only advantage of surface exclusion, surface exclusion will be selected under broad conditions as long as the decrease in fitness it causes is small, the copy number low and the transfer rate sufficiently high.

II. DISCRETE MODEL

A. Basic assumptions

Suppose there are two types of plasmids, one type (P^+) which induces its host to prevent the entrance of related plasmids, while the other type (P^-) does not induce surface exclusion.

The presence of surface exclusion is supposed to lower the fitness of the bacterial host.

The plasmids are assumed to be closely related, belonging to the same incompatibility group.

Since incompatibility segregation occurs only at cell division, it is a discrete process. Therefore a model with discrete bacterial generations will be constructed. However, since most bacterial populations are not synchronized, and because a continuous time model is mathematically more tractable, a continuous version of the model will also be analysed.

During each bacterial generation the following events can occur (and in this order):

1. conjugation

2. plasmid replication
3. bacterial mortality
4. plasmid loss
5. bacterial division combined with plasmid segragation.

Both plasmid types have the same copy number N. For simplicity it is assumed that plasmid replication continues until twice the copy number is attained irrespective of the number of plasmids at the beginning of the replication phase.

A bacterium will exhibit surface exclusion against both plasmid types if it contains at least one P^+ plasmid.

Finally, the population size is assumed to be constant; therefore half of the bacterial population will die before cell division.

1. Conjugation

Each cell cycle is assumed to start with a period during which conjugation can occur after an accidental collision between a plasmid-bearing bacterium and a potential recipient. Both plasmid-free bacteria and bacteria carrying only P^- plasmids are potential recipients.

The probability per unit of time of such a collision is proportional to the concentrations of both the potential donor cells and the potential recipients. Therefore, the transfer rate is assumed to be proportional to the product of these concentrations. The proportionality parameter is called $\tilde{\gamma}$. Levin, Stewart & Rice (1979) have shown that this assumption is fairly accurate.

It is assumed that, if a donor bacterium contains both P^- and P^+ plasmids, each plasmid copy has an equal chance to be transferred. However, a plasmid, which has just been transferred into a new host will not be transferred again during that generation.

It is assumed that each potential recipient can receive at most one plasmid by means of conjugation in one transfer period. This assumption has several advantages. First, if each bacterium receives at most one plasmid, no bacterium will contain more than twice the copy number N after a

transfer period. In the second place, one does not have to make awkward assumptions about the immediate effect of a P^+ plasmid on the surface exclusion of its new host. Besides, if the transfer period is short, the probability of acquiring more than one plasmid will be small anyhow.

On the other hand, I have assumed that the ability of a bacterium to serve as a donor during one transfer period is unrestricted. However, as long as the transfer period is short, multiple conjugations will be extremely rare.

These assumptions will lead to the following dynamics of the potential recipients (concentration ξ), the potential donors (concentration ϕ) and the bacteria which have received a plasmid (concentration ω) during the transfer period

$$\frac{d\xi}{dt} = -\tilde{\gamma}\xi\phi \qquad (1a)$$

$$\frac{d\phi}{dt} = 0 \qquad (1b)$$

$$\frac{d\omega}{dt} = \tilde{\gamma}\xi\phi \qquad (1c)$$

in which $\tilde{\gamma}$ is a measure for the transfer rate. At the beginning of a transfer period ξ is equal to $\xi(0)$, $\phi = \phi(0)$ and $\omega = \omega(0) = 0$. At the end of a transfer period of length T the concentrations are therefore

$$\xi(T) = \xi(0)e^{-\tilde{\gamma}T\phi(0)} \qquad (2a)$$

$$\phi(T) = \phi(0) \qquad (2b)$$

$$\omega(T) = \xi(0)\{1-e^{-\tilde{\gamma}T\phi(0)}\} \qquad (2c)$$

Therefore, the probability that a potential recipient will acquire a plasmid during the transfer period is $[1-e^{-\tilde{\gamma}T\phi(0)}]$.

Let the probability that a new-acquired plasmid is P^- be f^- and that it is P^+ be $f^+ = 1-f^-$. Since both plasmid types have equal probability of being transferred, it follows that

$$f^- = \frac{\text{number of } P^- \text{ plasmids at the start of the transfer period}}{\text{total number of plasmids at the start of the transfer period}}$$

After the transfer period the number of plasmids in a bacterium can be 0, 1, N or $N + 1$. The bacteria carrying 1 or $N + 1$ plasmids have acquired a plasmid by means of conjugation. Bacteria containing $N + 1$ plasmids after the transfer period, have been plasmid-bearing as well as potential recipients at the start of the transfer period, carrying N of the P^- plasmids at that time. If $N = 1$, not all bacteria with one plasmid have obtained that plasmid during the transfer period.

2. Plasmid replication

After the transfer period the plasmids in the bacteria are assumed to replicate. This replication will continue until the cells contain $2N$ plasmids each.

It is assumed that the plasmid replications occur successively and that for each duplication a plasmid is chosen at random among all available plasmids. Newly made plasmids can also duplicate. The same replication model has been used by Novick & Hoppensteadt (1978) and by Cullum & Broda (1979) in calculating the rate at which incompatible plasmids will separate. In fact the replication model is a special case of Polya's urn scheme (Feller, 1968).

Thus the probability of a bacterium to end up with $2N - j$ of the P^- and j of the P^+ plasmids when it start with k of the P^- and r of the P^+ plasmids is

$$p(k,r,j) = \begin{cases} \binom{2N-j-1}{k-1}\binom{j-1}{r-1} \Big/ \binom{2N-1}{r+k-1} & \text{if } k \leq 2N-j \\ & \text{and } r \leq j \\ 0 & \text{otherwise.} \end{cases} \qquad (3)$$

When the concentration of bacteria carrying $2N - j$ of the P^- and j of the P^+ plasmids after plasmid replication is denoted as $X^1(2N-j,j)$ and the concentration of bacteria carrying k of the P^- and r of the P^+ plasmids before replication is

denoted as $X(k,r)$ then

$$X^1(2N-j,j) = \sum_{r=0}^{N} p(N-r,r,j)X(N-r,r)+p(N,1,j)X(N,1), \quad (4a)$$

for $0 < j < 2N$,

$$X^1(2N,0) = X(N,0)+X(N+1,0)+X(1,0), \text{ and} \quad (4b)$$

$$X^1(0,2N) = X(0,N)+X(0,1) \quad (4c)$$

3. Bacterial mortality

After plasmid replication, some of the bacteria will die. The probability of dying depends on bacterial fitness, which is determined by the presence or absence of plasmids and of surface exclusion.

To keep the model as simple as possible I have assumed the population size to be constant.

Since all surviving bacteria will divide, the population size will remain constant if half of the bacterial population survives.

Let the relative fitness of bacteria with at least one P^+ plasmid, with only P^- plasmids, and without plasmids be respectively w, $w+s_1$, and $w+s_0$. Then the surviving fraction of these three types will be respectively $\frac{1}{2}[w/V]$, $\frac{1}{2}[(w+s_1)/V]$ and $\frac{1}{2}[(w+s_0)/V]$ in which V is the mean fitness of the population.

4. Plasmid loss

At cell division some bacteria may lose their plasmids. However, to simplify the model I assume plasmid loss to be a separate step just before cell division. The probability that a plasmid-bearing bacterium loses all its plasmids is equal to τ. After losing all its plasmids a bacterium will become a normal plasmid-free bacterium.

5. Bacterial division combined with plasmid segregation

At the end of each cell cycle the bacteria will divide. All plasmid-bearing bacteria contain $2N$ plasmids prior to cell division. At cell division the plasmids are distributed equally over the daughter cells. No discrimination is made between the two types of plasmid. Given these assumptions, the distribution of the two plasmid types over the daughter cells will be hypergeometric (see also Novick & Hoppensteadt, 1978 and Cullum & Broda, 1979).Thus the probability that a particular daughter cell of a bacterium, carrying $(2N-k)$ of the P^- plasmids and k of the P^+ plasmids contains $(N-j)$ of the P^- and j of the P^+ plasmids, is

$$q(k,j) = \binom{k}{j}\binom{2N-k}{N-j}\Big/\binom{2N}{N} \qquad (5)$$

All symbols used in this model are listed in table I.

B. The special case of copy number $N = 1$

In fig. 1 the most simple case (copy number N=1) is shown schematically. In the first period of the cell cycle potential recipients receive a plasmid with probability $1 - \alpha$. $(1-\alpha) = (1-exp(-\tilde{\gamma}T.[conc.\ plasmid\text{-}bearing\ bacteria]))$, as derived in section II-A-1. After the transfer period the plasmid will replicate until each plasmid-bearing bacterium contains 2 plasmids ($2N$).

Half of the bacteria will die. The survival probability of a bacterium, depending on its plasmid content, is defined in section II-A-3.

Some plasmid-bearing bacteria will loose their plasmids and become again normal plasmid-free bacteria. At the end of the cell cycle all bacteria will divide and the plasmids are equally distributed over the daughter cells of their host bacterium.

Let the frequency at the start of the m^{th} cell cycle of P^- bearing bacteria be denoted by $Z_m(0)$ and of P^+ bearing bacteria by $Z_m(1)$.

TABLE I

List of Parameters

P^-, P^+: plasmids without/with surface exclusion.

N : copy number of a plasmid.

$Z_m(i)$: discrete frequency of bacteria carrying $(N-i)$ copies of P^- and i copies of P^+.

y_i : (continuous) a linear combination of $Z(j)$'s,

$$y_0 = \sum_{j=0}^{N} \frac{N-j}{N} Z(j); \quad y_N = \sum_{j=0}^{N} (j/N).Z(j).$$

w : fitness of bacteria bearing at least one P^+ plasmid.

$w+s_1$: fitness of bacteria bearing only P^- plasmids.

$w+s_0$: fitness of plasmid-free bacteria.

V_m : mean fitness during the m^{th} generation.

τ : (discrete) probability of plasmid loss per generation; (continuous) plasmid loss rate per generation time.

$\tilde{\gamma}$: (discrete) transfer rate during the transfer period (in volume x x cell^{-1} x time $^{-1}$).

T : (discrete) lengthe of the transfer period.

γ : (discrete) = $\tilde{\gamma}.T.$total bacterial concentration; (continuous) transfer rate per generation time.

α_m : the probability that a potential recipient will not receive a plasmid during the transfer period of the m^{th} generation.

f_m^-, f_m^+ : frequency of P^-, P^+ plasmids in the m^{th} generation $(f_m^- + f_m^+ = 1)$.

$p(k,r,j)$: the probability that a bacterium which contains before plasmid replication k copies of P^- and r of P^+ will contain after plasmid replication $(2N-j)$ copies of P^- and j of P^+.

$q(k,j)$: the probability that a daughter cell of a bacterium containing $(2N-k)$ copies of P^- and k copies of P^+ will contain $(N-j)$ copies of P^- and j of P^+.

The probability that a potential recipient will not receive a plasmid by conjugation during the m^{th} generation is equal to

$$\alpha_m = e^{-\gamma(Z_m(0)+Z_m(1))} \qquad (6)$$

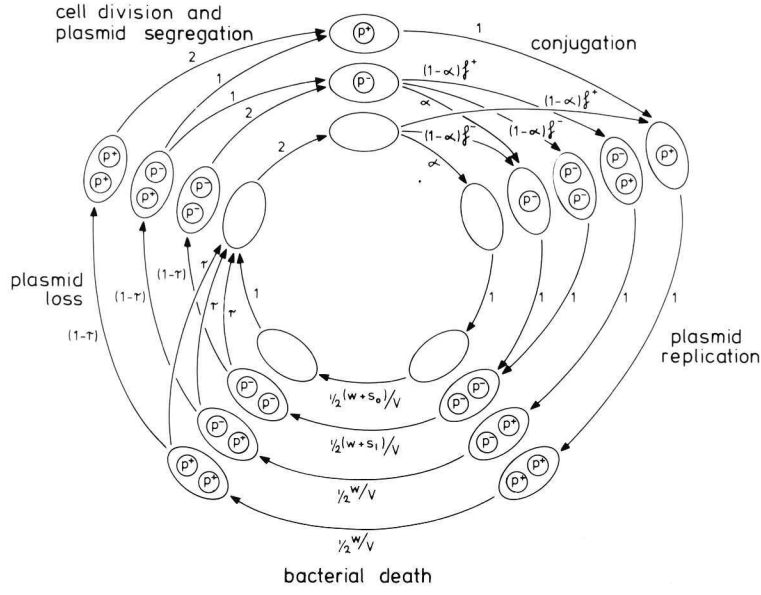


Fig. 1. A diagram of the successive events in a cell cycle of the model. The arrows indicate possible transitions, and at each arrow the expectation of that transition is given.

where $\gamma = \tilde{\gamma}T.[\,total\ bacterial\ concentration\,]$.

Let f_m^- and f_m^+ denote the fractions of all plasmids which are respectively P^- and P^+ at the start of the m^{th} generation:

$$f_m^- = Z_m(0)/(Z_m(0)+Z_m(1)) \tag{7a}$$

$$f_m^+ = Z_m(1)/(Z_m(0)+Z_m(1)) \tag{7b}$$

The mean fitness of the bacterial population in the m^{th} generation is equal to

$$V_m = w+s_0\{1-Z_m(0)-Z_m(1)\}\alpha_m+s_1\{Z_m(0)\alpha_m+f_m^-(1-\alpha_m)(1-Z_m(1))\} \tag{8}$$

So the relation between the frequencies of P^- and P^+ bearing bacteria at the start of two successive generations is

$$Z_{m+1}(0) = (1-\tau)\frac{(s_1+w)}{V_m}.[\,Z_m(0)\alpha_m+(1-Z_m(1))(1-\alpha_m)f_m^-] + \\ + \tfrac{1}{2}(1-\tau)\frac{w}{V_m}.[\,Z_m(0)(1-\alpha_m)f_m^+] \qquad (9a)$$

and

$$Z_{m+1}(1) = \frac{w(1-\tau)}{V_m}.\left[\begin{array}{l} Z_m(1)+(1-Z_m(0)-Z_m(1))(1-\alpha_m)f_m^+ \\ +\tfrac{1}{2}.Z_m(0)(1-\alpha_m)f_m^+ \end{array}\right] \qquad (9b)$$

C. Only one plasmid type present

If only one plasmid type is present - P^+, say - equation (9b) reduces to

$$Z_{m+1}(1) = \frac{w(1-\tau)}{V_m}.[\,Z_m(1)+(1-\alpha_m)(1-Z_m(1))] \qquad (10)$$

with $\alpha_m = e^{-\gamma Z_m(1)}$
and $V_m = w + s_0(1-Z_m(1))\alpha_m$
P^+ can invade a plasmid-free bacterial population if $Z_{m+1}(1) > Z_m(1)$ for small values of $Z_m(1)$, so if

$$\gamma \geq (s_0+\tau w)/w(1-\tau) \qquad (11)$$

If inequality (11) is satisfied the frequency of P^+ bearing bacteria will tend to a stable equilibrium $\hat{Z}(1)$, which is implicitly given by the equation

$$e^{-\gamma\hat{Z}(1)} = w(1-\tau-\hat{Z}(1))/[\,(s_0\hat{Z}(1)+w(1-\tau))(1-\hat{Z}(1))] \qquad (12)$$

with $0 < \hat{Z}(1) < 1-\tau$.
If, however, P^+ cannot invade a plasmid-free population ($\gamma<(s_0+\tau w)/w(1-\tau)$), P^+ might still be able to maintain itself as long as γ exceeds a certain minimum value $\gamma_{min}(1)$, an expression for which is derived in appendix A. In that case equation (12) has two solutions $\hat{Z}_1(1)$ and $\hat{Z}_2(1)$, with

$0 < \hat{Z}_1(1) < \hat{Z}_{min}(1) < \hat{Z}_2(1) < 1-\tau$ ($\hat{Z}_{min}(1)$ is the equilibrium frequency of $Z(1)$ if $\gamma = \gamma_{min}$). If the initial frequency is beneath $\hat{Z}_1(1)$, P^+ will disappear, while if it is above $\hat{Z}_1(1)$ the frequency of P^+ bearing bacteria will tend to the equilibrium frequency $\hat{Z}_2(1)$.

The case in which only P^- plasmids are present is exactly analogous, with w replaced by $w + s_1$ and s_0 by $s_0 - s_1$.

Equation (10) is derived from the special case of $N = 1$. However, since the copy number only influences the competition between incompatible plasmids equation (10) is independent of copy number.

D. Evolution of surface exclusion in the special case $N = 1$

Can P^+ invade an equilibrium with only P^- present, or in other words, can surface exclusion establish itself. The answer is yes; P^+ can penetrate an equilibrium of P^- if

$$e^{-\gamma\hat{Z}(0)}((s_1+\tfrac{1}{2}w)\hat{Z}(0)-s_1) < \tfrac{1}{2}w\hat{Z}(0)-s_1 \qquad (13)$$

where $\hat{Z}(0)$ is the equilibrium frequency of P^- prior to introduction of P^+. (For a derivation of inequality (13), as well as (14) and (15), see appendix B). It follows, that the excluding plasmid can always penetrate in a population with only non-excluding plasmids if $s_1 \leq 0$, that is, if surface exclusion has no negative effect on bacterial fitness. If surface exclusion reduces the fitness of bacteria, a surface exclusion inducing plasmid can still penetrate provided

$$\hat{Z}(0) > \left\{ s_1(w+s_0)+\tfrac{1}{2}w[(s_0-s_1)+\tau(w+s_1)] - \sqrt{\{s_1(w+s_0)+\tfrac{1}{2}w[(s_o-s_1)+\tau(w+s_1)]\}^2-2ws_1(s_0-s_1)(w+s_o)} \right\} /$$

$$\{w(s_0-s_1)\} \equiv \Theta \qquad (14)$$

If $\Theta > Z_{min}(0)$ (the minimum of the stable equilibrium frequencies of P^- in absence of P^+) then surface exclusion will become established if

$$\gamma > \frac{1}{\theta}\{ln[(1-\theta)((s_0-s_1)\theta+(w+s_1)(1-\tau))] - ln[(w+s_1)(1-\tau-\theta)]\} \quad (15)$$

In fig. 2 the relation between s_1 (the difference in fitness induced by surface exclusion) and γ is given for which P^+ can invade an equilibrium with P^-. For some parameter values, it is possible for P^+ to invade a P^- equilibrium although neither P^- nor P^+ can penetrate in a plasmid-free population. In that case the invasion of a P^- equilibrium by a P^+ plasmid may lead to the extinction of both plasmids (fig. 3). So, surface exclusion can become established under broad conditions and it becomes more profitable if the transfer rate is high.

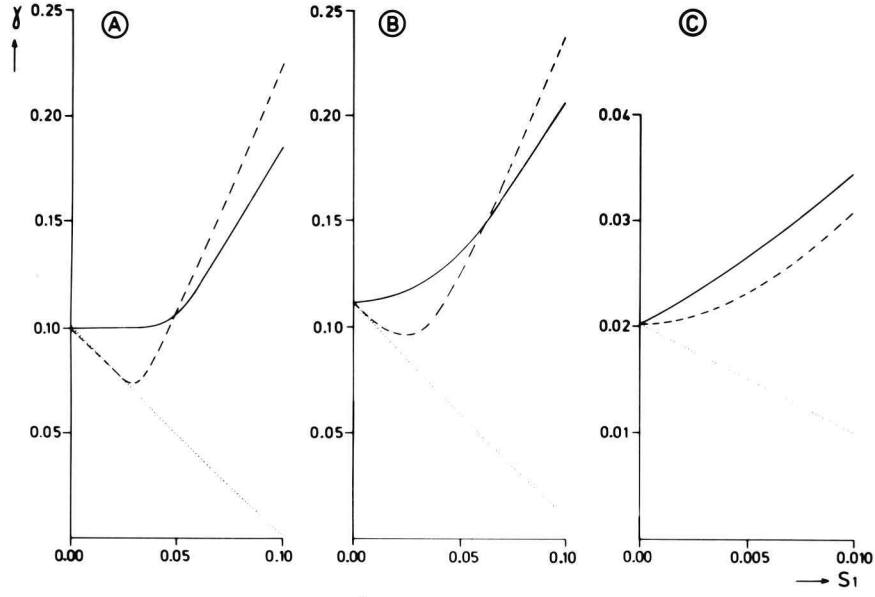


Fig. 2. Competition between P^+ and P^- plasmids, discrete model. For given s_0 and τ (2a: $s_0 = 0.1$, $\tau = 0.001$; 2b: $s_0 = 0.1$, $\tau = 0.01$; 2c: $s_0 = 0.01$, $\tau = 0.01$) a P^+ plasmid can intrude into an equilibrium of P^- plasmids if the combination of the values of s_1 and γ are above the broken line in the (s_1,γ) plane. A P^- plasmid can invade an equilibrium of P^+ plasmids if (s_1,γ) is beneath the solid line. Below the dotted line a P^- plasmid cannot invade in a plasmid-free situation. In all cases the copy number $N = 1$.

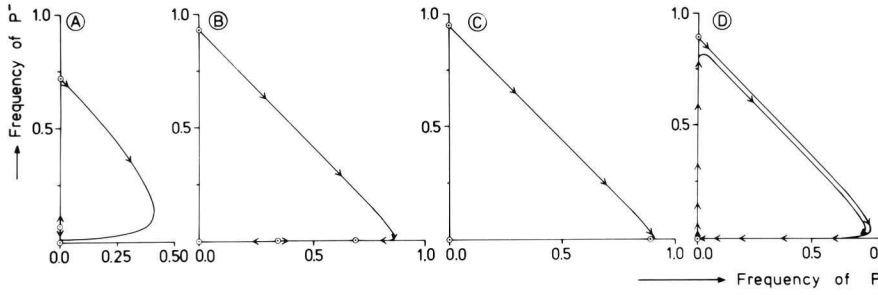


Fig. 3. Some examples of the dynamics of competition between P^+ and P^- plasmids, discrete model. In all figures a P^+ plasmid invades a bacterial population at equilibrium, carrying plasmid P^-. $s_0 = 0.1$; $s_1 = 0.01$ and $\tau = 0.001$. The equilibrium frequencies of (P^+,P^-) are indicated by Q. In all examples a P^+ mutant can invade the equilibrium with P^- plasmids. 3a: $\gamma = 0.09$. The P^+ plasmid expels the P^- plasmid, but as a consequence becomes extinct itself. Neither P^+ nor P^- can invade a plasmid-free bacterial population. 3b: $\gamma = 0.095$. The P^+ plasmid almost expels the P^- plasmid, and as a consequence becomes almost extinct itself. The few remaining P^- plasmids can increase in frequency when P^+ plasmids are rare. This interaction results in a limit cycle. 3c: $\gamma = 0.1$. The P^+ plasmid expels the P^- plasmid and an equilibrium with only P^+ plasmids is reached. However, the P^+ plasmid is not able to invade a plasmid-free population. 3d: $\gamma = 0.105$. The P^+ plasmid expels the P^- plasmid. The P^+ plasmid can now also intrude into a plasmid-free population.

Can the loss of surface exclusion ever be advantageous? Yes, it might, in case

$$e^{-\gamma\hat{Z}(1)}((\tfrac{1}{2}w+2s_1)\hat{Z}(1)-s_1) > (\tfrac{1}{2}w+s_1)\hat{Z}(1)-s_1 \qquad (16)$$

The derivation of the inequality is analogous to that of inequality (13). Inequality (16) can only hold if $s_1 > 0$, that is, if surface exclusion confers a selective disadvantage. Inequality (16) combined with equation (12) implies that P^- can invade an equilibrium with P^+ if

$$\hat{Z}(1) < \eta, \tag{17}$$

where $\eta = \left\{ s_1(w+s_0)+(\tfrac{1}{2}w+s_1)(s_0+w\tau)- \right.$

$\left. -\sqrt{\{s_1(w+s_0)+(\tfrac{1}{2}w+s_1)(s_0+w\tau)\}^2-4s_0s_1(\tfrac{1}{2}w+s_1)(w+s_0)} \right\} /$

$/2s_0(s_1+\tfrac{1}{2}w)$

It follows, that P^- can invade a P^+ equilibrium if

$$\gamma \leq \frac{1}{\eta}\{ln\ (1-\eta)(s_0\eta+w(1-\tau))\ -ln(w(1-\tau-\eta))\} \tag{18}$$

provided that $\eta > \hat{Z}_{min}(1)$. In fig. 2 the relation between s_1 and γ for some values of w_1, s_0 and τ is given for which P^- can invade an equilibrium with P^+. Thus a non-excluding plasmid may be able to invade a bacterial population in which only excluding plasmids are present, and may even expel that plasmid, but only if surface exclusion is sufficiently harmful for a bacterium and if the transfer rate is small.

E. The general discrete model with copy number N

For an arbitrary copy number N the model can be derived in exactly the same way as was done for $N = 1$. The various transition probabilities are given in section II-A. Let $Z_m(i)$ be the frequency of bacteria carrying i of the P^+ and $(N-i)$ of the P^- plasmids in the m^{th} generation. The frequencies of P^- and P^+ plasmids at the start of the m^{th} generation are respectively,

$$f_m^- = \sum_{i=0}^{N}(N-i)Z_m(i) / \sum_{i=0}^{N}N.Z_m(i) \tag{19a}$$

and

$$f_m^+ = \sum_{i=0}^{N} i Z_m(i) / \sum_{i=0}^{N} N . Z_m(i) = 1 - f_m^- \qquad (19b)$$

The probability that a potential recipient will not receive a plasmid by conjugation in the m^{th} generation is given by

$$\alpha_m = e^{-\gamma \sum_{i=0}^{N} Z_m(i)} \qquad (20)$$

The mean fitness of the bacterial population in the m^{th} generation is equal to

$$V_m = w + s_0 (1 - \sum_{i=0}^{N} Z_m(i)) \alpha_m + s_1 \{ Z_m(0) \alpha_m + (1 - \sum_{i=1}^{N} Z_m(i)) (1-\alpha_m) f_m^- \} \qquad (21)$$

Let

$$p(k,r,j) = \begin{cases} \dfrac{\binom{2N-j-1}{k-1}\binom{j-1}{r-1}}{\binom{2N-1}{r+k-1}} & \text{if } k \leq 2N-j \text{ and } r \leq j \\ 0 & \text{otherwise} \end{cases} \qquad (22)$$

and

$$q(k,j) = \binom{k}{j}\binom{2N-k}{N-j} / \binom{2N}{N} \qquad (23)$$

as defined respectively in section A2 and A5.

Then,

$$Z_{m+1}(0) = \frac{w(1-\tau)}{V_m} \sum_{k=1}^{N} q(k,0) . \cdot [\sum_{r=1}^{N-1} \{ p(N-r,r,k) . Z_m(r) \} + p(N,1,k) Z_m(0) (1-\alpha_m) f_m^+] + + \frac{(s_1+w)(1-\tau)}{V_m} . [Z_m(0) \alpha_m + (1 - \sum_{j=1}^{N} Z_m(j)) (1-\alpha_m) f_m^-] \qquad (24a)$$

$$Z_{m+1}(N) = \frac{w(1-\tau)}{V_m} \cdot$$

$$\cdot \left\{ \sum_{k=N}^{2N-1} q(k,N) \cdot \left[\sum_{r=1}^{N-1} (p(N-r,r,k) Z_m(r) + p(N,1,k) Z_m(0)(1-\alpha_m) f_m^+ \right] \right.$$

$$\left. + Z_m(N) + (1 - \sum_{j=0}^{N} Z_m(j))(1-\alpha_m) f_m^+ \right\} \qquad (24b)$$

$$Z_{m+1}(j) = \frac{w(1-\tau)}{V_m} \cdot$$

$$\cdot \sum_{k=j}^{N+j} q(k,j) \cdot \left[\sum_{r=1}^{N-1} (p(N-r,r,k) \cdot Z_m(r)) + p(N,1,k) \cdot Z_m(0)(1-\alpha_m) f_m^+ \right]$$

$$\text{for } j = 1, 2, \ldots, N-1 \qquad (24c)$$

Unfortunately, the model is in its general form rather untractable. However, the continuous equivalent of model (24) is easier to handle.

III. THE CONTINUOUS TIME MODEL

In this section a continuous version of model (24) will be developed. First, we replace the variables $Z(i)$ by new variables y_i, given by

$$y_i = \sum_{j=0}^{N} e_{ij} Z(j) \qquad (25)$$

This linear transformation is chosen such that
$[a_{ij}] = [e_{ij}]^{-1} \Lambda [e_{ij}]$, in which
$[a_{ij}]$ is the $((N+1)x(N+1))$ matrix with elements

$$a_{ij} = \sum_{k=0}^{2N} q(k,i) \cdot p(N-k,k,j),$$

and Λ is a diagonal matrix with diagonal elements $\lambda_0, \lambda_1, \ldots, \lambda_N$. Therefore, if the elements of $[e_{ij}]^{-1}$ are denoted by e_{ij}^{-1}, then $e_{\cdot i}^{-1}$ is an eigenvector of $[a_{ij}]$ with corresponding eigenvalue λ_i. The matrix $[a_{ij}]$ has two identical largest eigenvalues, λ_0 and λ_N, both equal to 1. The variables y_0 and y_N denote respectively the frequency of P^- and P^+ plas-

mids multiplied by the frequency of plasmid bearing bacteria, so

$$y_0 = \sum_{i=0}^{N} \frac{N-i}{N} Z(i) \qquad (26a)$$

and

$$y_N = \sum_{i=0}^{N} \frac{i}{N} Z(i) \qquad (26b)$$

Furthermore, $e_{i0} = e_{iN} = 0$ for $i = 1,2,\ldots,N-1$, therefore $e_{00}^{-1} = 1$ and $e_{0N}^{-1} = 0$, and

$$Z(0) = \sum_{i=0}^{N} e_{0i}^{-1} y_i = y_0 + \sum_{i=1}^{N-1} e_{0i}^{-1} y.$$

Let $\mu_i = ln\gamma_i$, then $\mu_0 = 0$ and $\mu_N = 0$, since $\lambda_0 = \lambda_N = 1$ and $\mu_i < 0$ for $i = 1,2,\ldots,N-1$. Putting

$\delta_k = \sum_{i=1}^{N-1} e_{ki} \sum_{j=i}^{N} q(j,i)p(N,1,j)$ for $k = 1,2,\ldots,N-1$ and denoting the continuous equivalents of the parameters γ, τ, s_0 and s_1 also by γ, τ, s_0 and s_1, the continuous version of model (24) becomes

$$y_0 = -\tau y_0 + (\gamma - s_0) y_0 (1 - y_0 - y_N) + \\ + (y_0 + \sum_{j=1}^{N-1} e_{0j}^{-1} y_j)(s_1(1-y_0) - \gamma y_N/(N+1)), \qquad (27a)$$

$$y_N = -\tau y_N + (\gamma - s_0) y_N (1 - y_0 - y_N) + \\ + (y_0 + \sum_{j=1}^{N-1} e_{0j}^{-1} y_j)(-s_1 y_N + \gamma y_N/(N+1)), \qquad (27b)$$

and

$$y_k = -\tau y_k + \mu_k y_k - s_0(1-y_0-y_N) + (y_0 + \sum_{j=1}^{N-1} e_{0j}^{-1} y_j)(-s_1 y_k + \gamma \delta_k y_N), \qquad (27c)$$

for $k = 1,2,\ldots,N-1$.

If only one of the plasmid types is present $y_i = 0$ for $i = 1,2,\ldots,N-1$. A $P^+(P^-)$ plasmid will increase in frequency when introduced in a plasmid-free bacterial population if $\dot{y}_N > 0$ ($\dot{y}_0 > 0$) for small values of y_N (y_0), therefore if $\gamma > s_0 + \tau$ ($\gamma > s_0 - s_1 + \tau$). In other words a plasmid will increase

in frequency if its transfer rate compensates both the decrease in bacterial fitness caused by the plasmid and the loss rate of the plasmid. In absence of the other plasmid, the frequency of P^+ (P^-) bearing bacteria will tend to a stable equilibrium of $1-\tau/(\gamma-s_0)$ $(1-\tau/(\gamma-s_0+s_1))$, provided P^+ (P^-) can invade a plasmid-free population. If P^+ (P^-) cannot invade a plasmid-free population, no (stable) equilibrium exists with only P^+ (P^-).

Can an excluding plasmid mutant invade a plasmid population consisting entirely of non-excluding plasmids? Yes it will, if the Jacobian matrix of system (27) at the equilibrium point with P^- and without P^+ has at last one positive eigenvalue. It appears that the largest eigenvalue of that Jacobian matrix is

$$-\tau+(\gamma-s_0)(1-\hat{y}_0)+\hat{y}_0(-s_1+\gamma/(N+1)).$$

Therefore, since $\hat{y}_0 = 1-\tau/(\gamma-s_0+s_1)$, an excluding plasmid can invade a bacterial population containing already non-excluding plasmids if

$$\gamma > \left\{ (s_0-s_1)+\tau+(N+1)s_1+ \right. \\ \left. +\sqrt{\left((s_0-s_1)+\tau+(N+1)s_1\right)^2-4(N+1)s_1(s_0-s_1)} \right\} / 2 \qquad (28)$$

or $s_1 \leq 0$. (see fig. 4 and 5)

Many plasmid species have a very small loss rate. Nordström & Aagaard-Hansen (1984), for instance estimated the probability of plasmid loss to be less then 3.10^{-6} per cell and per generation. If τ can be neglected, condition (28) for P^+ to be able to invade a P^- equilibrium reduce to

$$\gamma/s_0 > 1-s_1/s_0 \text{ for } s_1/s_0 \leq 1/(N+2) \qquad (29a)$$

and

$$\gamma/s_0 > (N+1)s_1/s_0 \text{ for } s_1/s_0 > 1/(N+2) \qquad (29b)$$

Inequality (29a) is exactly the condition for P^- to invade a plasmid-free bacterial population. Therefore, if τ is

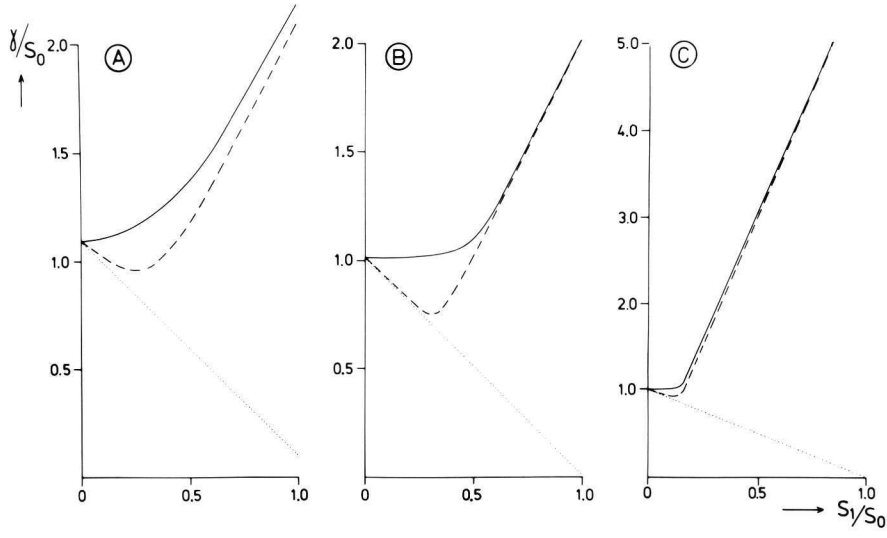


Fig. 4. Competition between P^+ and P^- plasmids, continuous model. For given τ/s_0 and N (4a: $\tau/s_0 = 0.1$, $N = 1$; 4b: $\tau/s_0 = 0.01$, $N = 1$; 4c: $\tau/s_0 = 0.01$, $N = 5$) a P^+ plasmid can intrude into an equilibrium of P^- plasmids if the combination of the values of s_1/s_0 and γ/s_0 is above the broken line in the $(s_1/s_0, \gamma/s_0)$ plane. A P^- plasmid can invade an equilibrium of P^+ plasmids if $(s_1/s_0, \gamma/s_0)$ is beneath the solid line. Below the dotted line a P^- plasmid cannot invade a plasmid-free population.

negligible, the P^+ plasmid can always invade an equilibrium with P^- plasmids if $s_1/s_0 \leq 1/(N+2)$. Since P^+ can only invade a plasmid-free population if $\gamma/s_0 > 1$ ($\tau \approx 0$), it follows from condition (29a) that if $s_1/s_0 < 1/(N+2)$ then P^+ can easier be established in a population with P^- than in a plasmid-free population.

On the other hand, the condition for a P^- mutant to be able to invade a P^+ population, is that the Jacobian matrix of system (27) has at least one positive eigenvalue at equilibrium with P^+ and without P^-. This condition reduces to

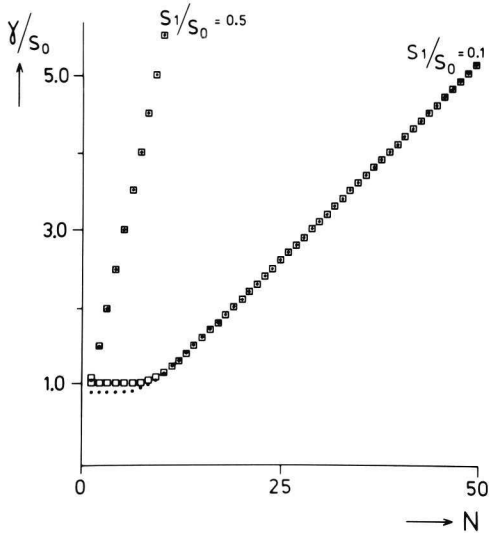


Fig. 5. Competition between P^+ and P^- plasmids, continuous model. For $\tau/s_0 = 0.01$ and both for $s_1/s_0 = 0.1$ and $s_1/s_0 = 0.5$ the minimal value of γ/s_0 for which a P^+ plasmid can invade an equilibrium of P^- plasmids (both plasmids with copy number N) is marked with a ● and the maximal value of γ/s_0 for which a P^- plasmid can invade an equilibrium of P^+ plasmids is marked with a □. P^+ can only invade a plasmid-free population if $\gamma/s_0 > 1.01$ and P^- if $\gamma/s_0 > 0.91$ (s_1/s_0=0.1) or $\gamma/s_0 > 0.51$ (s_1/s_0=0.5).

$$-\tau+(\gamma-s_0)(1-\hat{y}_N)+s_1-\gamma\hat{y}_N/(N+1) > 0 \qquad (30)$$

Since $\hat{y}_N = 1-\tau/(\gamma-s_0)$, it follows from inequality (30) that a non-excluding plasmid can establish itself if

$$\gamma < \frac{s_0+\tau+(N+1)s_1+\sqrt{(s_0+\tau+(N+1)s_1)^2-4(N+1)s_0s_1}}{2} \qquad (31)$$

and $s_1 > 0$ (see fig. 4 and 5)

This implies that if τ is negligible P^- can only invade a P^+ bearing bacterial population if

$$\gamma/s_0 < (N+1)s_1/s_0 \text{ and } s_1/s_0 > 1/(N+1) \qquad (32)$$

If $s_1/s_0 < 1/(N+1)$, P^+ will expel P^- plasmids from the population, provided P^+ can establish itself in a plasmid-free bacterial population (fig. 4). But even if τ is not

negligible, P^+ will expel P^- if surface exclusion is not harmful for the bacterial host. Moreover, the presence of P^- will facilitate the invasion of P^+ if surface exclusion is only slightly disadvantageous, provided $\tau < s_0$.

In general, the selective advantage for a plasmid to induce surface exclusion increases with decreasing fitness loss of the bacterial host due to the exclusion, with increasing transfer rate, and with decreasing copy number.

IV. DISCUSSION

In the previous sections conditions were derived for the evolution of plasmid-induced surface exclusion. How likely are these conditions to be fulfilled? For a number of parameters used in this study, it is possible to obtain realistic estimates based on empirical evidence. The influence of a plasmid on bacterial fitness appears to be very variable. Zünd & Lebek (1980), for instance, found that the generation time of bacteria carrying different plasmids might vary between 29 and 58 min. (mean (and median) 36 min.; s.d. 6.6 min.), compared to a generation time of the plasmid-free bacteria of 30 min. This is equivalent with values of s_0 between -0.03 and 0.93 (mean 0.2; s.d. 0.22).

The plasmid loss rate is in most cases very small. Nordström, Molin & Aagaard-Hansen (1980) and Nordström & Aagaard-Hansen (1984) could detect no loss of respectively R1 and R1-*drd*-19 plasmids in an E. coli strain, which implied a loss rate of less then 10^{-4} and 3.10^{-6} respectively.

The copy number of plasmids can vary greatly (from 1 to over 800 (Projan, Carleton & Novick, 1983)). However, most conjugative plasmids have a relatively low copy number (somewhere between 1 and 10).

The transfer rate appears to be rather small in most experiments. Levin, Stewart & Rice (1979) found the transfer rate per donor and per recipient to be almost independent of the recipient concentration. Their estimates of the transfer rate per donor are in the range of $1.5.10^{-12}$ to $2.0.10^{-9}$ ml/(cell x hour) in an exponentially growing population. This

would lead to values of γ somewhere between 5.10^{-5} and 10^{-1} if the bacterial population density is in the order of 10^{8}/ml. The estimates of Freter, Freter & Brickner (1983) are of about the same magnitude.

Cullum, Collins & Broda (1978a) state that the transfer rate per donor reaches a maximum for high recipient concentrations (above 2.10^{7} cells/ml). According to them, the maximum transfer rate is almost 1 per donor generation (corresponding to values of γ of almost 1). Stocker, Smith & Ozeki (1963) found that donors with newly acquired plasmids are far more efficient. They lose their efficiency after 3 to 7 generations. The above mentioned estimates of the transfer rate are, however, all based on in vitro experiments. Estimating the natural transfer rate is much more difficult. Caugant, Levin & Selander (1981) have looked for evidence of plasmid transfer between human gut bacteria without, however, finding convincing evidence of transfer. On the other hand, Freter, Freter & Brickner (1983) state that the transfer rate per donor and per recipient is about the same in vivo and in vitro, but that transfer cannot be easily detected in vivo, because the bacterial density is much lower.

To my knowledge no research has been done on the cost of surface exclusion for a bacterium. If that property were advantageous for a bacterium, one would expect the evolution of bacterial gene(s) for surface exclusion, which might lead to plasmids developing another transfer mechanism. However, it is not clear how harmful surface exclusion is to bacteria, and to what extent the bacterial fitness loss caused by a plasmid is due to the induction of it.

Not knowing how much of the decrease in bacterial growth rate is caused by surface exclusion makes it difficult to predict when surface exclusion will become established. However, as long as surface exclusion is only responsible for a small part of the fitness decrease, say about 10%, low copy number plasmids, i.e. most conjugative plasmids, will develop surface exclusion against other, incompatible plasmids.

APPENDIX-A

MINIMUM VALUE OF γ FOR WHICH A P^+ PLASMID CAN BE MAINTAINED IN ABSENCE OF P^- PLASMIDS

The relation between the frequency of plasmid-bearing bacteria in generation m and $m + 1$ is given by equation (10). A shorthand notation for it is

$$Z_{m+1}(1) = f(Z_m(1)) \tag{A-1}$$

In the interval $[0,1]$ $f(Z)$ is an increasing function which has at most one point of inflection. At equilibrium $f(\hat{Z}) = \hat{Z}$ the trivial equilibrium $\hat{Z} = 0$ always exists. Since $f(1) = 1 - \tau < 1$, exactly one non-trivial equilibrium value exists in case $\frac{df}{dZ}|_{Z=0} < 1$.

If two different non-trivial equilibrium values exist, one will be stable (the largest) and one instable (the smallest). For given w, s_0 and τ the two equilibrium values will approach each other if γ decreases. They will coincide on the boundary of the area in which there are no and two non-trivial equilibria. In that case $\hat{Z}_{min}$ is a solution of equation (12) and

$$\frac{df}{dZ}|_{\hat{Z}_{min}} = 1 \tag{A-2}$$

which implies that $\hat{Z}_{min}$ is a solution of

$$(w\tau+s_0)(1-\tau)-2s_0(1-\tau)\hat{Z}_{min}+s_0\hat{Z}^2_{min}$$
$$+\frac{(1-\hat{Z}_{min})}{\hat{Z}_{min}}.\{(1-\tau)^2w+(s_0-w)(1-\tau)\hat{Z}_{min}-s_0\hat{Z}^2_{min}\}.$$
$$.ln\{w(1-\tau-\hat{Z}_{min})/[(1-\tau)w+(s_0-(1-\tau)w)\hat{Z}_{min}-s_0\hat{Z}^2_{min}]\} = 0 \tag{A-3}$$

The stable non-trivial equilibrium value of (A-1) will, if existing, always be larger then $\hat{Z}_{min}$.

For given values of w, s_0 and τ the lower bound of γ, γ_{min} for which a stable equilibrium with P^+ exist is

$$\gamma_{min} = \frac{1}{\hat{Z}_{min}} \cdot ln\{(s_0\hat{Z}_{min}+w(1-\tau))(1-\hat{Z}_{min})/w(1-\tau-\hat{Z}_{min})\} \qquad \text{(A-4)}$$

in which $\hat{Z}_{min}$ is the solution of (A-3) with $0 < \hat{Z}_{min} < 1-\tau$.

APPENDIX-B

CAN A P^+ PLASMID INVADE AN EQUILIBRIUM WITH P^- AND VICE VERSA IN CASE COPY NUMBER $N = 1$

The discrete model for $N = 1$ is given by equations (9). A shorthand notation for them is

$$Z_{m+1}(0) = f(Z_m(0), Z_m(1)) \qquad \text{(B-1a)}$$

$$Z_{m+1}(1) = g(Z_m(0), Z_m(1)) \qquad \text{(B-1b)}$$

P^+ can invade an equilibirum with P^- if the matrix

$$\begin{pmatrix} \frac{\partial f}{\partial Z(0)} \Big|_{(\hat{Z}(0),0)} & \frac{\partial f}{\partial Z(1)} \Big|_{(\hat{Z}(0),0)} \\ \frac{\partial g}{\partial Z(0)} \Big|_{(\hat{Z}(0),0)} & \frac{\partial g}{\partial Z(1)} \Big|_{(\hat{Z}(0),0)} \end{pmatrix} \qquad \text{(B-2)}$$

has at least one eigenvalue λ with $Re(\lambda) < -1$ or $Re(\lambda) > 1$ (with corresponding eigenvector $(e_1,e_2)^T$, $e_2 \neq 0$). Since $\frac{\partial g}{\partial Z(0)}\Big|_{(\hat{Z}(0),0)} = 0$, P^+ can invade an equilibrium with P^- if

$\frac{\partial g}{\partial Z(1)} \Big|_{(\hat{Z}(0),0)} > 1$, therefore if

$$\frac{\partial g}{\partial Z(1)}\Big|_{(\hat{Z}(0),0)} = w(1-\tau).\{1+[(1-\hat{Z}(0)+\tfrac{1}{2}\hat{Z}(0)](1-\hat{\alpha})/(\hat{Z}(0)\}/\hat{V} > 1 \qquad \text{(B-3)}$$

with $\hat{\alpha} = e^{-\gamma\hat{Z}(0)}$

In equilibrium $\hat{Z}(0) = f(\hat{Z}(0),0)$, therefore

$$\hat{Z}(0) = (1-\tau)(s_1+w)(\hat{Z}(0)\hat{\alpha}+(1-\hat{\alpha})) \;/\; \hat{V} \qquad \text{(B-4)}$$

(B-3) combined with (B-4) lead to

$$e^{-\gamma\hat{Z}(0)}((s_1+\tfrac{1}{2}w)\hat{Z}(0)-s_1) < \tfrac{1}{2}w\hat{Z}(0)-s_1 \qquad \text{(B-5)}$$

as condition for P^+ to be able to invade a P^- equilibrium. This condition can only be satisfied if

$$\hat{Z}(0) > s_1/(s_1+\tfrac{1}{2}w) \qquad \text{(B-6)}$$

According to equation (12) (replacing w by $w+s_1$, and s_0 by s_0-s_1)

$$e^{-\gamma\hat{Z}(0)} = (w+s_1)(1-\tau-\hat{Z}(0))/\,[\,((s_0-s_1)\hat{Z}(0)+(w+s_1)(1-\tau))(1-\hat{Z}(0))] \qquad \text{(B-7)}$$

Combining condition (B-5) with equation (B-7) leads to the condition

$$\hat{Z}(0) > \left\{ s_1(w+s_0)+\tfrac{1}{2}w[\,(s_0-s_1)+\tau(w+s_1)] - \sqrt{\{s_1(w+s_0)+\tfrac{1}{2}w[\,(s_0-s_1)+\tau(w+s_1)]\}^2-2ws_1(s_0-s_1)(w+s_0)} \right\} / w(s_0-s_1) \qquad \text{(B-8)}$$

$$\equiv \Theta$$

Since $\Theta > s_1/(s_1+\tfrac{1}{2}w)$ condition (B-6) is superfluous. Θ does not depend on γ. For each combination of the parameters w, s_0, s_1 and τ there exists a value $Z_{min}(0)$, given implicitly by equation (A-3) after replacing w by $w+s_1$ and s_0 by s_0-s_1, so that $\hat{Z}(0) > Z_{min}(0)$. Therefore P^+ can always penetrate if $\Theta < Z_{min}(0)$. The, in the one plasmid situation stable, equilibrium $\hat{Z}(0)$ increases if γ increases, therefore $\hat{Z}(0) > \Theta$ if

$$\gamma > \frac{1}{\Theta}\{ln[\,(1-\Theta)((s_0-s_1)\Theta + (w+s_1)(1-\tau))] - ln[\,(w+s_1)(1-\tau-\Theta)]\} \qquad \text{(B-9)}$$

(The right-hand side of this inequality is the solution for γ of equation (B-7) in which $\hat{Z}(0)$ is replaced by Θ). In an analogous way the conditions for P^- to be able to invade a P^+ equilibrium can be derived.

CHAPTER 5:

WHY DO PLASMIDS REPRESS THEIR TRANSFER RATE?

ABSTRACT

A model is presented of the population dynamics of a transfer repressing plasmid, which is derepressed in newly infected hosts. Using this model, it is analysed whether individual selection on plasmids can explain the occurrence of conjugation repression, both in a chemostat and in a periodically transferred bacterial population. It appears that in a chemostat regulation has no advantage, while in a periodically transferred population regulation is in itself advantageous. However, if the growth rate of the bacterial plasmid host diminishes when a plasmid synthesizes regulation products, the dynamical advantage of regulation will not always be able to overcome its costs.

INTRODUCTION

Plasmids are pieces of extrachromosomal circular DNA which occur abundantly in most bacterial species (Bukhari, Shapiro & Adhya, 1977). They are mostly autonomous, regulating their own replication and the distribution of their copies over the daughter cells of their bacterial host.

Many plasmids encode for a mechanism for transferring a strand of their own DNA to another bacterium which has accidentally collided with their host. This process is called conjugation. A competent donor, i.e. a plasmid bearing bacterium which is able to transfer a strand of plasmid DNA, has pili. A pilus is a kind of extracellular filamentous organelle. Pili play a role in the pair formation between donor and recipient (Ou & Anderson, 1970; Tomoeda, Inuzuka & Oates, 1975). They can also serve as attachment sites for some pilus-specific bacteriophages (Caro & Schnös, 1966; Bradley, 1976, 1980). In most cases, the growth rate of a plasmid-bearing bacterium will be reduced by being competent, both because several kinds of transfer products have to be

synthesized, and because a competent donor is more liable to infection by particular bacteriophages.

Ozeki et al. (1962) and Stocker et al. (1963) discovered that a newly infected host is a much more efficient donor than a bacterium infected a long time ago. It has been suggested that this phenomenon is an adaptation which enables a plasmid to spread fast by infection after the first transfer, without hampering its host too much when the plasmid is already spread throughout the bacterial population (Stocker et al., 1963; Broda, 1979; Campbell, 1981). This seems a plausible explanation, although the ability to repress conjugation probably also has some costs.

The genetical mechanism of conjugation repression of F-like plasmids has been extensively investigated (for a review, see Willetts & Skurray, 1980). It is established that the combination of the products of two different plasmid genes, *finO* and *finP*, is necessary for repression. The two products interact in some way and repress together the expression of another gene, *traJ*. The *traJ* gene product positively controls the "transfer operon". This operon contains most of the genes directly involved in plasmid transfer (the *tra* genes, other than *traJ*). The disappearance of *traJ* gene products and of these products directly responsible for transfer is supposed to be caused mainly by dilution in a growing population. The transcription of the *traJ* gene can either be derepressed, enabling the plasmid to synthesize its transfer products, or repressed, in which case it is not possible to transcribe the other transfer genes (at least not after the remainder of the *traJ* gene products has disappeared). Although the repression will occur some time before the plasmid host stops being a competent donor, plasmids in an incompetent donor will be loosely indicated as repressed and in a competent donor as derepressed.

In this paper, I will investigate by mathematical modelling, whether repression of the ability to conjugate is advantageous for a plasmid, both for a chemostat population and for a sequential transferred bacterial population.

MODELS FOR THE POPULATION DYNAMICS OF TRANSFER REPRESSING PLASMIDS

Assumptions

Stewart & Levin (1977), Freter, Freter & Brickner (1983) and van der Hoeven (1984: Chapter 2 and 3) have modelled the population dynamics of derepressed plasmids. Transfer repression will, however, influence these dynamics. The transfer rate depends on the amount of time (ω) elapsed since infection.

The growth rate of the plasmid carrying bacteria will depend among other things on the transfer rate and possibly on the concentrations of the different transfer inducing and repressing products. Since these concentrations and the transfer rate depend on the time since infection (ω), the growth rate will also depend on ω. The most straightforward way to model the population dynamics of a transfer repressing plasmid is therefore to assume that the transfer rate and the growth rate of a plasmid-bearing bacterium are functions of that time ω. The growth rate will also depend on the nutrient concentration s in the bacterial environment. For simplicity it will be assumed that the growth rate of all bacteria (plasmid-free and plasmid-bearing, infected time ω ago) is proportional to the same function of the nutrient concentration, $f(s)$. $f(s)$ will be chosen such that the growth rate of plasmid-free bacteria is $1.f(s)$. The proportionality paramater for plasmid bearing bacteria w depends on ω.

The transfer rate depends on the growth rate of their hosts. This will be modelled by assuming proportionality: The probability of transfer per donor and per recipient per unit of time is $\gamma'(\omega)w(\omega)f(s) = \gamma(\omega)f(s)$. γ will be loosely indicated at the transfer rate.

It is supposed that only one nutrient is growth limiting, and both plasmid-free and plasmid-bearing bacteria need an equal amount of that nutrient per cell division ($\tilde{e}$). After cell division, one of the daughter cells of a plasmid-bearing bacterium might end up without plasmids (with probability τ).

These assumptions will lead to a model with partial differential equations. The disadvantages of such a model are that it is mathematically difficult, not easy to simulate and employs an infinite number of parameters ($\gamma(\omega)$ and $w(\omega)$ for each value of ω). If it is assumed that plasmid-bearing bacteria can only occur in two states, either able to conjugate (derepressed), or not (repressed), and that the transition rates between the two states are independent of the time since infection, a much more simple model can be constructed. It will be assumed that a just infected host is always derepressed. In such a model, transfer rate regulation can be captured in only two parameters, the rate of repression (ϕ_R) and of derepression (ϕ_D). For reference all symbols are listed in table I.

MODEL FOR PLASMIDS IN A CHEMOSTAT

Let the concentration at time t of plasmid-free bacteria be $b(t)$ and of bacteria, carrying a plasmid, infected time ω ago, $p(t,\omega)$. If the bacteria live in a chemostat with constant turnover rate ρ and a fixed nutrient concentration s_0 in the inflow, the model of the dynamics is

$$\frac{ds}{dt} = \rho(s_0-s)-\tilde{e}f(s)\{b+\int_0^\infty w(\omega)p(t,\omega)d\omega\} \tag{1a}$$

$$\frac{db}{dt} = f(s)b-\rho b-\int_0^\infty \gamma(\omega)f(s)bp(t,\omega)d\omega+ \tag{1b}$$

$$+\int_0^\infty w(\omega)\tau f(s)p(t,\omega)d\omega$$

$$\frac{\partial p}{\partial t} = -\frac{\partial p}{\partial \omega}+p(t,\omega)\{f(s)w(\omega)(1-\tau)-\rho\} \tag{1c}$$

with

$$p(t,0) = \int_0^\infty \gamma(\omega)f(s)b(t)\ p(t,\omega)d\omega \tag{1d}$$

TABLE I

LIST OF THE PARAMETERS

b: concentration of plasmid-free bacteria

$p(t,\omega)$: concentration of plasmid-bearing bacteria at time t infected time ω ago

p_c^i, p_r^i: concentration of bacteria carrying the i^{th} regulating plasmid resp. derepressed (conjugative) and repressed

p_n: concentration of bacteria carrying non-regulating plasmids

$\bar{b}$: total bacterial concentration

$w(\omega)$: relative growth rate of plasmid-bearing bacteria infected time ω ago

w_c, w_r, w_n: relative growth rate of bacteria carrying respectively derepressed regulating, repressed regulating and non-regulating plasmids

$w_\varepsilon = w_n - w_c$

s: nutrient concentration

$f(s)$: growth rate of plasmid-free bacteria at nutrient concentration s

$h(\bar{b})$: growth rate of plasmid-free bacteria at total bacterial concentration $\bar{b}$

$\tilde{e}$: quantity of nutrient needed for one cell division

$\gamma'(\omega), \gamma'$: transfer rate per recipient per generation time of the donor resp. per donor infected time ω ago and per competent donor

$\gamma(\omega), \gamma$: transfer rate per recipient per generation time of the recipient resp. per donor infected time ω ago and per competent donor

τ: plasmid loss rate per generation

ϕ_R^i, ϕ_D^i: repression/derepression rate of the i^{th} regulating plasmid

T: length of each period in the sequential transfer model

x = frequency of plasmid-bearers in the bacterial population

$\Theta^i = p_r^i/(p_c^i+p_r^i)$

$y = (p_c^2+p_r^2)/(p_c^1+p_r^1+p_c^2+p_r^2)$

$z = p_n/(p_c+p_r+p_n)$

After a relatively short time an input-output equilibrium will be reached in the chemostat. In that case the amount of nutrient in the inflow is identical to the quantity of nutrient, either free or in a bacterium, in the outflow, in mathematical terms,

$$s+\tilde{e}(b+\int_0^\infty p(t,\omega)d\omega) = s_0 \qquad (2)$$

$b + \int_0^\infty p(t,\omega)d\omega$ is the total bacterial concentration and will be denoted by $\bar{b}$. At that equilibrium the function $f(s)$ can be replaced by a function $h(\bar{b})$, such that

$$h(\bar{b}) = f(s_0-\tilde{e}\bar{b})$$

At the input-output equilibrium the system of differential equations can be reduced to

$$\frac{db}{dt} = h(\bar{b})b-\rho b-h(\bar{b})b\int_0^\infty \gamma(\omega)p(t,\omega)d\omega+\tau h(\bar{b})\int_0^\infty w(\omega)p(t,\omega)d\omega \qquad (3a)$$

$$\frac{\partial p}{\partial t} = -\frac{\partial p}{\partial \omega} + p\{h(\bar{b})w(\omega)(1-\tau)-\rho\} \qquad (3b)$$

with

$$p(t,0) = b(t)h(\bar{b})\int_0^\infty \gamma(\omega)p(t,\omega)d\omega \qquad (3c)$$

This model will be referred to as regulation model I.

A mathematically more simple model can be constructed if it is supposed that the conjugation system in a plasmid-bearing bacterium can either be derepressed or repressed, and (de)repression occurs with a fixed probability per unit of time (or per generation). The conjugation system in a just infected host is derepressed. Let p_c be the concentration of plasmid-bearing bacteria which can induce transfer, and p_r the concentration of repressed plasmid-bearers. If ϕ_R is the repression rate and ϕ_D the derepression rate, the model (at input-output equilibrium) becomes

$$\frac{db}{dt} = h(\bar{b})b-\rho b-\gamma h(\bar{b})bp_c+\tau h(\bar{b})(w_c p_c+w_r p_r) \qquad (4a)$$

$$\frac{dp_c}{dt} = w_c(1-\tau)h(\bar{b})p_c-\rho p_c+\gamma h(\bar{b})bp_c-\phi_R p_c+\phi_D p_r \qquad (4b)$$

$$\frac{dp_r}{dt} = w_r(1-\tau)h(\bar{b})p_r-\rho p_r+\phi_R p_c-\phi_D p_r \qquad (4c)$$

This model will be referred to as regulation model II.

CAN A REGULATING PLASMID INVADE A PLASMID-FREE POPULATION?

In the absence of plasmids the equilibrium value of b is $\hat{b}$ and $h(\hat{b}) = \rho$. In the neighbourhood of that equilibrium the equations of regulation model I can be approximated by

$$\frac{\partial p}{\partial t} = -\frac{\partial p}{\partial \omega} + p\{\rho[w(\omega)(1-\tau)-1]\} \qquad (5a)$$

with

$$p(t,0) = \hat{b}\rho \int_0^\infty \gamma(\omega)p(t,\omega)d\omega \qquad (5b)$$

A solution of this equation is

$$p(t,\omega) = p(0,0)e^{rt}e^{\int_0^\omega \{\rho[(1-\tau)w(x)-1]-r\}dx} \qquad (6a)$$

with r the solution of

$$\rho\hat{b}\int_0^\infty \gamma(\omega)e^{\int_0^\omega \{\rho[(1-\tau)w(x)-1]-r\}dx} d\omega = 1 \qquad (6b)$$

In case $r > 0$ the concentration of p will increase and the plasmid will be able to invade. Three cases will be considered:

1) $w(\omega) = w$ and $\gamma(\omega) = \gamma$ } for all $\omega \geq 0$.

Then

$$\rho\hat{b}\int_0^\infty \gamma(\omega)e^{\int_0^\omega \{\rho[(1-\tau)w(x)-1]-r\}dx}\,d\omega = \frac{-\rho\hat{b}\gamma}{\rho[(1-\tau)w-1]-r} = 1 \tag{7}$$

$$\Rightarrow \quad r = \rho\{(1-\tau)w-1+\gamma\hat{b}\} \tag{8}$$

Therefore, a non-regulating plasmid can invade if

$$\gamma\hat{b} > 1 - w(1-\tau) \tag{9}$$

2) $w(\omega) = w_1$ and $\gamma(\omega) = \gamma_1$ for $\omega < a$
$w(\omega) = w_2$ and $\gamma(\omega) = \gamma_2$ for $a \leq \omega < \infty$
(with $\gamma_1 > \gamma_2$ and $w_1 < w_2$).
In the case r is the solution of

$$\rho\hat{b}.\left(\gamma_1\left[\frac{e^{\{\rho[(1-\tau)w_1-1]-r\}a}-1}{\rho[(1-\tau)w_1-1]-r}\right]-\gamma_2.\left[\frac{e^{\{\rho[(1-\tau)w_1-1]-r\}a}}{\rho[(1-\tau)w_2-1]-r}\right]\right) = 1 \tag{10}$$

For which value of a the fastest invasion rate is obtained? In other words, what is for an invading plasmid the optimal time for switching from fast conjugating to repression of conjugation? Denote the left hand side of equation (10) with $g(r,a)$. r is maximal if $dr/da = 0$ or if $a = 0$ or $a \to \infty$. For the first of these conditions holds

$$\frac{dr}{da} = 0 \Leftrightarrow \frac{\partial g}{\partial a} = 0,$$

and

$$\frac{\partial g}{\partial a} = \rho\hat{b}.\left(\gamma_1-\gamma_2\,\frac{\{\rho[(1-\tau)w_1-1]-r\}}{\{\rho[(1-\tau)w_2-1]-r\}}\,.e^{\{\rho[(1-\tau)w_1-1]-r\}a}\right) = 0 \tag{11}$$

$$\text{if } r = -\rho + (1-\tau)\rho(\gamma_1 w_2-\gamma_2 w_1)/(\gamma_1-\gamma_2). \tag{12}$$

This condition is independent of a. If equation (12) holds $g(r,a) = \hat{b}.(\gamma_1-\gamma_2)/\{(1-\tau)(w_2-w_1)\}$. Since $g(r,a) = 1$, this equation can only hold if $\hat{b} = (1-\tau)(w_2-w_1)/(\gamma_1-\gamma_2)$, in which case all values of a give the same invasion rate. In all

other cases the invasion rate is either maximal if $a = 0$ (in case $\hat{b} > (1-\tau)(w_2-w_1)/(\gamma_1-\gamma_2)$) or if $a \to \infty$ (in case $\hat{b} < (1-\tau)(w_2-w_1)/(\gamma_1-\gamma_2)$). Therefore, in all but some very exceptional cases, a plasmid which either does not repress its conjugation or which immediately represses conjugation can invade more rapidly in a plasmid-free bacterial population than a regulating plasmid.

3) w and γ are stepfunctions: $w(\omega) = w_i$ and $\gamma(\omega) = \gamma_i$ for $\sum_{j=1}^{i-1} a_j \leq \omega < \sum_{j=1}^{i} a_j$ for $i = 1,2,\ldots,n$, and $\sum_{j=1}^{n} a_j \to \infty$.
In this case, r is the solution of

$$\rho\hat{b} \cdot \sum_{j=1}^{n} \gamma_j e^{\sum_{i=1}^{j-1} \{\rho[(1-\tau)w_i - 1] - r\} a_i} \left(\frac{e^{\{\rho[(1-\tau)w_j - 1] - r\} a_{j-1}}}{\{\rho[(1-\tau)w_j - 1] - r\}} \right) = 1 \qquad (13)$$

Again, the invasion rate is maximal if the optimal combination of γ and w is reached as fast as possible and is never abandoned.

Regulation model II gives the same quantitative result. A regulating plasmid can invade a plasmid-free population if the jacobian matrix of system (4) at the plasmid-free equilibrium has at least one positive eigenvalue, i.e. if the matrix

$$\begin{pmatrix} \rho[(1-\tau)w_c - 1 + \gamma\hat{b}] - \phi_R & \phi_D \\ \phi_R & \rho[(1-\tau)w_r - 1] - \phi_D \end{pmatrix} \qquad (14)$$

has at least one positive eigenvalue. A necessary condition is that either $w_c(1-\tau) - 1 + \gamma\hat{b} > 0$ (an always derepressed plasmid can invade) or $(1-\tau)w_r - 1 > 0$ (an always repressed plasmid can invade).

The largest eigenvalue of matrix (14) is maximal if either $\phi_R = 0$ (if $\gamma\hat{b} > (1-\tau)(w_r-w_c)$), or $\phi_D = 0$ (if $\gamma\hat{b} < (1-\tau)(w_r-w_c)$). Therefore, regulating the conjugation rate will not increase the invasion rate of a plasmid in a

chemostat.

Since regulation is not supposed to be particularly advantageous in a plasmid-free population, this conclusion is not really surprising. However, in an almost entirely plasmid infected bacterial population, regulation is supposed to be advantageous because it will increase the mean fitness of the bacterial population. In the next sections it will be investigated whether regulation is really advantageous for the maintenance of a plasmid in a chemostat.

EQUILIBRIUM WITH ONE REGULATING PLASMID

What is the fate of a regulating plasmid once it has invaded a bacterial population in a chemostat? Some elaborate calculations show that, provided that $h(\bar{b})$ is a decreasing function of $\bar{b}$ there exists one stable non-trivial equilibrium. This equilibrium will be attained. At equilibrium

$$w_r(1-\tau)\hat{h} - \rho = \phi_D - \phi_R \hat{p}_c/\hat{p}_r \qquad (15a)$$

and

$$w_c(1-\tau)\hat{h} - \rho + \gamma\hat{h}\hat{b} = \phi_R - \phi_D \hat{p}_r/\hat{p}_c \qquad (15b)$$

In fig. 1 the equilibrium frequencies of plasmid bearing bacteria and repressed plasmids as function of ϕ_R are shown.

COMPETITION BETWEEN TWO REGULATING PLASMIDS (MODEL II)

Can a second regulating plasmid invade a bacterial population, already containing a plasmid if both plasmids only differ in their regulation parameter? Model II for the dynamics of a two plasmid competition becomes

$$\frac{db}{dt} = h(\bar{b})b-\rho b-\gamma h(\bar{b})b(p_c^1+p_c^2)+\tau h(\bar{b})\{w_c p_c^1+w_r p_r^1+w_c p_c^2+w_r p_r^2\} \qquad (16a)$$

$$\frac{dp_c^i}{dt} = w_c(1-\tau)h(\bar{b})p_c^i - \rho p_c^i + \gamma h(\bar{b})bp_c^i - \phi_R^i p_c^i + \phi_D^i p_r^i \qquad (16b)$$

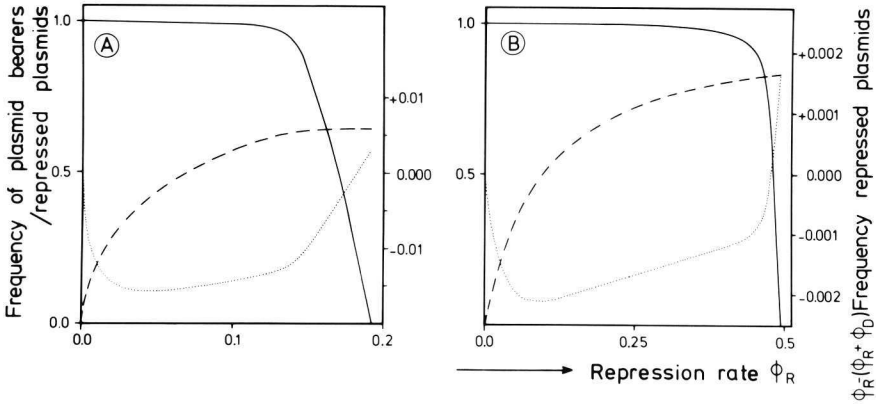


Fig. 1. Equilibrium frequency of transfer regulating plasmids in a bacterial population in a chemostat (drawn line) and frequency of repressed plasmids in the plasmid population (broken line) as a function of the repression rate ϕ_R. The dotted line gives $(\phi_R+\phi_D) - \phi_D$ *(freq. of repressed plasmids)* (scaled at the left side of the figure). If this term is negative a regulating plasmid with a higher repression rate and/or a lower derepression rate can invade and vice versa. Derepression rate $\phi_D = 0.1$ a) $w_c = 0.5$; $w_r = 0.95$; $\gamma = 0.6$; $\tau = 10^{-4}$. b) $w_c = 0.9$; $w_r = 0.98$; $\gamma = 0.2$; $\tau = 10^{-4}$. In both figures the growth function $h(\bar{b}) = (1-\bar{b})/(1.125-\bar{b})$ and the turnover rate of the chemostat $\rho = 0.1$.

$$\frac{dp_r^i}{dt} = w_r(1-\tau)h(\bar{b})p_r^i - \rho p_r^i + \phi_R p_c^i - \phi_D p_r^i \qquad (16c)$$

for $i = 1, 2$.
The second plasmid can invade an equilibrium of the first if the matrix

$$\begin{pmatrix} \hat{h}.(w_c(1-\tau)+\gamma\hat{b})-\rho-\phi_R^2 & \phi_D^2 \\ \phi_R^2 & \hat{h}w_r(1-\tau)-\rho-\phi_D^2 \end{pmatrix} \qquad (17)$$

has at least one positive eigenvalue. Since at the

equilibrium with only the first regulating plasmid

$$\hat{h}.(w_c(1-\tau)+\gamma\hat{b})-\rho = \phi_R^1 - \phi_D^1\hat{p}_r^1/\hat{p}_c^1 \qquad (18a)$$

and

$$\hat{h}w_r(1-\tau) - \rho = \phi_D^1 - \phi_R^1\hat{p}_c^1/\hat{p}_r^1 \qquad (18b)$$

the second plasmid can invade in case $\phi_R^1\hat{p}_c^1 - \phi_D^1\hat{p}_r^1 < 0$ if it represses conjugation stronger and/or has a lower derepression rate, and in case $\phi_R^1\hat{p}_c^1 - \phi_D^1\hat{p}_r^1 > 0$ if it represses conjugation less and/or has higher derepression rate. In fig. 1 the relation between ϕ_R and $\phi_R^1 p_c^1 - \phi_D^1 p_r^1$ is shown. A plasmid with both repression and derepression rate sufficiently close to zero can always invade.

If the second plasmid can invade it will either expel the first plasmid, or they can coexist. They can only coexist in case the first plasmid is also able to invade a bacterial population already carrying the second. If the two plasmids coexist, their concentrations will tend to an equilibrium at which

$$\phi_R^i/\phi_D^i = p_r^i/p_c^i, \qquad (19)$$

therefore, $w_r(1-\tau)\hat{h} - \rho = 0$ (20a)

and $(w_c(1-\tau)+\gamma\hat{b})\hat{h} - \rho = 0$ (20b)

If two regulating plasmids coexist, the total frequency of respectively derepressed and repressed plasmids is the same as in the case of two coexisting non-regulating plasmids, one able to transfer (parameters w_c and γ), and the other unable to transfer (parameters w_r and 0). Two regulating plasmids with identical w_c, w_r and γ can only coexist if the corresponding non-regulating plasmids can coexist. Otherwise, if of the non-regulating plasmids the conjugative would expel the non-conjugative, selection will favor a regulating plasmid with repression rate as small as possible and derepression rate going to infinity. Conversely, if, of the non-regulating plasmids, the non-conjugative would expel the

conjugative plasmid, selection will favor a regulating plasmid which represses conjugation as effective as possible. The second situation can only occur when bacteria carrying conjugation repressed plasmids have a higher growth rate than plasmid-free bacteria.

Therefore, selection on regulation rates will lead to a situation in which either a regulating plasmid will prevail which behaves as a non-regulating plasmid, or a non-regulating plasmid with the same growth parameters is neutral. However, a regulating plasmid has extra genes to make conjugation repressors. It seems likely that the production of these gene products will reduce the growth rate of the bacterial host. A non-regulating plasmid will then be able to invade a population of regulating plasmids, if that population is stable against invasion by other regulating plasmids. In competition with such a non-regulating plasmid the regulating plasmid will only be able to survive if its host grows fast enough in the repressed state, and regulating plasmids with high repression rate and slow derepression rate will be favored.

Concluding, one can state that in the constant environment of a chemostat, regulation is not an advantageous property for a plasmid. The model leads to the prediction that plasmids, which are cultivated during a long period in a chemostat will lose their ability to repress conjugation.

PLASMIDS IN A PERIODICALLY TRANSFERRED BACTERIAL POPULATION, THE MODEL

If the bacterial host lives under a "feast and famine" regime, i.e. the bacteria exhaust their food, after which they are transferred to a fresh food supply, the growth dynamics at each food site are

$$\frac{ds}{dt} = -f(s)\tilde{e}(b+w_c p_c+w_r p_r) \qquad (21a)$$

$$\frac{db}{dt} = f(s)b - \gamma f(s)bp_c + \tau f(s)(w_c p_c + w_r p_r) \qquad (21b)$$

$$\frac{dp_c}{dt} = w_c(1-\tau)f(s)p_c + \gamma f(s)bp_c - \phi_R p_c + \phi_D p_r \qquad (21c)$$

$$\frac{dp_r}{dt} = w_r(1-\tau)f(s)p_r + \phi_R p_c - \phi_D p_r \qquad (21d)$$

When the repression and derepression rates are fixed per unit of time, ϕ_R and ϕ_D are constants. When they depend on the growth rate, ϕ_R and ϕ_D are proportional to $f(s)$ ($\phi_R = \phi'_R f(s)$; $\phi_D = \phi'_D f(s)$). Since both plasmid-free and plasmid-bearing bacteria use an equal amount of nutrients, s is at each moment determined by the initial nutrient concentration s_0, the initial total bacterial concentration $\bar{b}_0$ and the total bacterial concentration at that moment, $\bar{b}_t$: $s_t = s_0 - (\bar{b}_t - b_0)\tilde{e}$. Therefore, the first equation of system (22) can be eliminated and the function $f(s)$ can be replaced by a function $h(\bar{b})$.

After a fixed time T or after exhausting the food ($T \to \infty$) the bacteria are transferred to a new food-site with initial nutrient concentration s_0. The frequency of bacteria carrying a plasmid ($x = (p_c + p_r)/(b + p_c + p_r)$) and of repressed bacteria ($\Theta = p_r/(p_c + p_r)$) at the start of the growth at the new site are the same as at the end of the growth at the previous site. The changes of x and Θ at a site are

$$\frac{dx}{dt} = h(\bar{b})x.\left(\begin{array}{l}(1-x).[(w_c - w_r)(1-\Theta) + (w_r - 1) + \gamma\bar{b}(1-\Theta)] \\ - \tau(w_c(1-\Theta) + w_r\Theta)\end{array}\right) \qquad (22a)$$

and

$$\frac{d\Theta}{dt} = \Theta(1-\Theta)h(\bar{b}).\{(1-\tau)(w_r - w_c) - \gamma\bar{b}(1-x)\} + \phi_R(1-\Theta) - \phi_D\Theta \qquad (22b)$$

The dynamics of plasmid bearers and repressed plasmids are completely determined by the sequence of initial frequencies of plasmid bearers (x_0) and of conjugation repressed plasmids (Θ_0) at successive sites.

CAN A REGULATING PLASMID INVADE A PLASMID-FREE POPULATION?

A regulating plasmid is able to invade a plasmid-free bacterial population if its initial frequency x_0 increases for small values of x_0. For small values of x

$$\frac{dx}{dt} \approx x.h(\bar{b})\{(w_c-w_r)(1-\theta)+(w_r-1)+\gamma\bar{b}(1-\theta)-\tau(w_c(1-\theta)+w_r\theta)\} \quad (23a)$$

and

$$\frac{d\theta}{dt} \approx \theta(1-\theta)h(\bar{b})\{(1-\tau)(w_r-w_c)-\gamma\bar{b}\} + \phi_R(1-\theta) - \phi_D\theta \quad (23b)$$

(and $\bar{b} \approx b$).

As long as x is small, the dynamics of θ are independent of x, and θ_t will converge to a function $\hat{\theta}_t$ with $\hat{\theta}_0 = \hat{\theta}_T$. The plasmid can invade if

$x_0(n+1) = x_T(n) > x_0(n)$ when $\theta_t = \hat{\theta}_t$, therefore if

$$\int_0^T \frac{dx}{dt}\,dt > 0 \Rightarrow \int_0^T \frac{1}{x}\frac{dx}{dt}\,dt > 0$$

$$\Rightarrow \int_0^T h(\bar{b}).\{(1-\hat{\theta}).[(w_c-w_r)(1-\tau)+\gamma\bar{b}] + w_r(1-\tau)-1\}dt > 0 \quad (24)$$

Since $\hat{\theta}_0 = \hat{\theta}_T$,

$$\int_0^T h(\bar{b})(1-\hat{\theta}).[(w_c-w_r)(1-\tau)+\gamma\bar{b}]\,dt = \int_0^T \phi_R+\phi_D-\frac{\phi_R}{\hat{\theta}}\,dt \quad (25)$$

(if $\hat{\theta}_t \neq 0$ and $\hat{\theta}_t \neq 1$), and since

$\int_0^T (w_r(1-\tau)-1)h(\bar{b})dt$ does not depend on the regulation parameters, the invasion rate is maximal if ϕ_R and ϕ_D are chosen so that

$$\int_0^T \phi_R + \phi_D - \phi_R/\hat{\theta}\,dt \text{ is maximal.}$$

The maximum is not always reached for ($\phi_R \to 0$ and $\phi_D \to \infty$) or for ($\phi_R \to \infty$ and $\phi_D \to 0$), as in a chemostat. In fig. 2 the dependence of the invasion rate of a plasmid on the regulation parameter is shown.

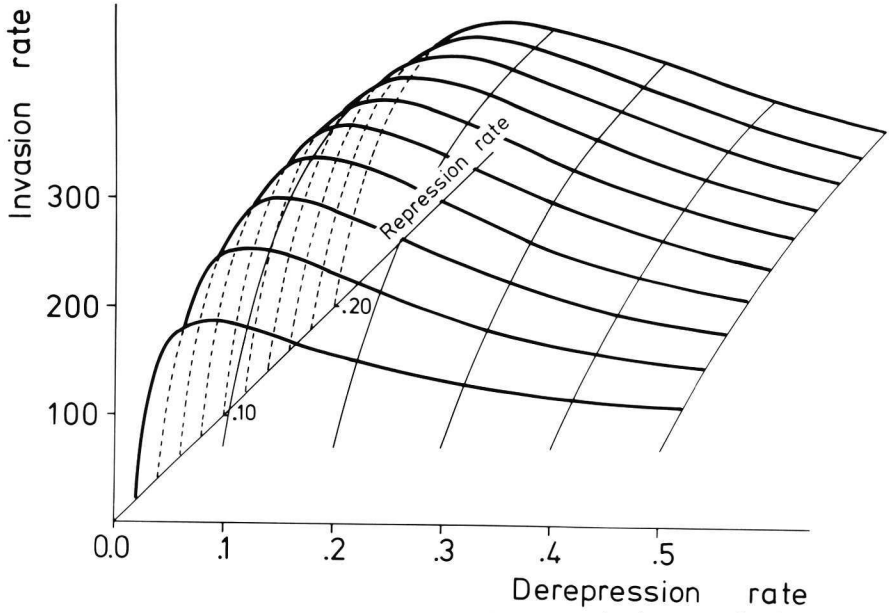


Fig. 2. The invasion rate of a regulating plasmid in a periodically transferred bacterial population (plasmid frequency at the new site/frequency at the previous site for low plasmid frequencies). $w_c = 0.5$; $w_r = 0.99$; $\gamma = 10^{-7}$; $\tau = 10^{-6}$. Initial bacterial concentration $b_0 = 10^3$. Initial nutrient concentration $s_0 = 10^2$. Nutrient/bact. $\tilde{e} = 10^{-6}$. Growth function $f(s) = s/(2+s)$. The invasion rate is maximal if $\phi_R = 0.14$ and $\phi_D = 0.13$.

Inequality (24) can be rewritten as

$$\int_0^T \{(w_c-1)+\gamma\bar{b}-\tau w_c\}h(\bar{b})dt + \int_0^T \Theta[(w_r-w_c)(1-\tau)-\gamma\bar{b}]\,h(\bar{b})dt > 0 \qquad (26)$$

If neither non-regulating, in parameters equivalent plasmids can invade ($w_r \leq 1-\tau$; $\int_0^T \{(w_c-1)+\gamma\bar{b}-\tau w_c\}h(\bar{b}) \leq 0$), the regulating

plasmid can sometimes invade, in case

$$\int_0^T \Theta[(w_r - w_c)(1-\tau) - \gamma\bar{b}]\, h(\bar{b})\, dt$$

is sufficiently larger than 0. In other words, the maximum invasion rate is not always reached when the regulating plasmid behaves as a non-regulating plasmid, and sometimes a regulating plasmid will be able to invade whereas a non-regulating plasmid cannot establish itself.

CAN A MUTANT PLASMID ENTER THE PLASMID POPULATION?

System (21) can easily be extended for a second regulating plasmid. Let the second plasmid only differ from the first in regulation parameters, then the model of the growth dynamics at one site becomes

$$\frac{db}{dt} = h(\bar{b})b - \gamma h(\bar{b})b(p_c^1 + p_c^2) + \tau h(\bar{b})(w_c p_c^1 + w_r p_r^1 + w_c p_c^2 + w_r p_r^2) \tag{27a}$$

$$\frac{dp_c^i}{dt} = w_c^i(1-\tau)h(\bar{b})p_c^i + \gamma h(\bar{b})bp_c^i - \phi_R^i p_c^i + \phi_D^i p_r^i \tag{27b}$$

$$\frac{dp_r^i}{dt} = w_r^i(1-\tau)h(\bar{b})p_r^i + \phi_R^i p_c^i - \phi_D^i p_r^i \tag{27c}$$

for $i = 1, 2$.

Denoting the relative frequency of plasmids by $x = (p_c^1 + p_r^1 + p_c^2 + p_r^2)/(b + p_c^1 + p_r^1 + p_c^2 + p_r^2)$, the relative frequency of the second plasmid by $y = (p_c^2 + p_r^2)/(p_c^1 + p_r^1 + p_c^2 + p_r^2)$, and the relative frequencies of the repressed plasmid of the first/second types by $\Theta^i = p_r^i/(p_c^i + p_r^i)$; $(i=1,2)$, the frequency changes at a site are given by

$$\frac{dx}{dt} = h(\bar{b})x\cdot\left(\begin{array}{l}(1-x)\left(\begin{array}{l}(w_c - w_r)((1-y)(1-\Theta^1) + y(1-\Theta^2)) + w_r - 1\\ +\ \gamma\bar{b}((1-y)(1-\Theta^1) + y(1-\Theta^2))\end{array}\right)\\ -\tau[w_c((1-y)(1-\Theta^1) + y(1-\Theta^2)) + w_r((1-y)\Theta^1 + y\Theta^2)]\end{array}\right) \tag{28a}$$

$$\frac{dy}{dt} = y(1-y).h(\bar{b})(\Theta^1-\Theta^2)[(1-\tau)(w_c-w_r)+\gamma\bar{b}(1-x)] \qquad (28b)$$

$$\frac{d\Theta^i}{dt} = \Theta^i(1-\Theta^i)h(\bar{b}).[(1-\tau)(w_r-w_c)-\gamma\bar{b}(1-x)]+\phi_R^i(1-\Theta^i)-\phi_D^i\Theta^i \qquad (28c)$$

for $i = 1,2$.

For very low frequencies of the second plasmid, the change in frequency of repressed plasmids of the second type is dictated by the dynamics of the first plasmid. The frequency Θ_t^2 will tend to an equilibrium function $\hat{\Theta}_t^2$. The second plasmid will be able to invade a population containing the first plasmid, if y increases when sufficiently small, i.e. if

$$\int_0^T h(\bar{b}_t)(\Theta_t^1-\hat{\Theta}_t^2)[(1-\tau)(w_c-w_r)+\gamma(1-x_t)\bar{b}_t]\,dt > 0 \qquad (29)$$

Unfortunately, this equation can only be solved numerically. It appears by simulation that two regulating plasmids with identical growth parameters, but different regulation parameters can sometimes coexist. At least under some conditions, a combination of regulation parameters ϕ_R and ϕ_D exists such that no second regulating plasmid can invade. In that case regulation gives a plasmid an advantage over non-regulating plasmids.

A plasmid, which cannot repress the transcription of its transfer products can do without the genes coding for the repressors (in F-like plasmids the gens *fin*O and *fin*P). A plasmid, missing these genes, and therefore not synthesizing their gene products, will probably reduce the growth rate of its host less than a regulating plasmid in the derepressed phase. Let the growth rate of the bacteria carrying a non-regulating plasmid be $w_n = w_c+w_\varepsilon$ and denote the concentration of these bacteria by p_n, then the competition between a regulating and a non-regulating plasmid leads at each site to the following growth dynamics.

$$\frac{db}{dt} = h(\bar{b})b-\gamma h(\bar{b})b(p_c+p_n(w_n/w_c))+\tau h(\bar{b}).(w_cp_c+w_rp_r+w_np_n) \qquad (30a)$$

$$\frac{dp_c}{dt} = w_c(1-\tau)h(\bar{b})p_c+\gamma h(\bar{b})bp_c-\phi_R p_c+\phi_D p_r \qquad (30b)$$

$$\frac{dp_r}{dt} = w_r(1-\tau)h(\bar{b})p_r + \phi_R p_c - \phi_D p_r \qquad (30c)$$

$$\frac{dp_n}{dt} = w_n(1-\tau)h(\bar{b})p_n + \gamma(w_n/w_c)h(\bar{b})bp_n \qquad (30d)$$

(The transfer rate is supposed to be proportional to the growth rate). The dynamics at each site are completely determined by the initial frequency of plasmid bearers $x = (p_c+p_r+p_n)/(b+p_c+p_r+p_n)$; the relative frequency of non-regulating plasmids $z = p_n/(p_c+p_r+p_n)$, and the relative frequency of repressed plasmids in the regulating plasmid population $\Theta = p_r/(p_c+p_r)$. These initial frequencies are the same as the end frequencies at the previous site. At each site the changes of the frequencies are given by

$$\frac{dx}{dt} = h(\bar{b})x\Big((1-x)\{[w_c-1+w_\varepsilon z+(1-z)\Theta(w_r-w_c)]+ + \gamma\bar{b}[z(1+(w_\varepsilon/w_c))+(1-z)(1-\Theta)]\} - \tau\{(w_r-w_c)(1-z)\Theta+w_c+w_\varepsilon z\}\Big) \qquad (31a)$$

$$\frac{d\Theta}{dt} = \Theta(1-\Theta)h(\bar{b})\{(1-\tau)(w_r-w_c)-\gamma\bar{b}(1-x)\} + \phi_R(1-\Theta)-\phi_D\Theta \qquad (31b)$$

$$\frac{dz}{dt} = z(1-z)h(\bar{b}).\{(1-\tau)w_\varepsilon+\gamma(w_\varepsilon/w_c)(1-x)\bar{b}+ + \Theta[(w_c-w_r)(1-\tau)+\gamma(1-x)\bar{b}]\} \qquad (31c)$$

A non-regulating plasmid will be able to invade a bacterial population containing a regulating plasmid if

$$\int_0^T h(\bar{b})\{(1-\tau)w_\varepsilon+\gamma(w_\varepsilon/w_c)(1-x)\bar{b}+\Theta[(w_c-w_r)(1-\tau)+\gamma(1-x)\bar{b}]\,dt$$

$$> 0 \qquad (32)$$

in absence of the non-regulating plasmid. In table II some numerical examples are given of the minimal value of w_ε for

TABLE II

The optimal repression and derepression rates of a plasmid in a periodically transferred bacterial population. At each site the growth function is $f(s) = s/(2+s)$ and the food supply becomes depleted. A non-regulating plasmid can invade in a population of regulating plasmids with optimal regulation rates if the growth rate of its bearers is more than $w_\varepsilon\ (min)$ higher than the growth rate of bearers of derepressed regulating plasmids.

w_c	w_r	γ	τ	$s_0/\tilde{e}$	b_0	$\phi_R(opt)$	$\phi_D(opt)$	$w_\varepsilon(min)$
0.9	0.99	5.10^{-8}	10^{-6}	10^8	10^3	0.359	0.00923	0.0154
0.9	0.98	5.10^{-8}	10^{-6}	10^8	10^3	0.335	0.0175	0.0121
0.9	0.98	2.10^{-8}	10^{-6}	10^8	10^3	0.298	0.0619	0.00971
0.9	0.98	5.10^{-8}	10^{-6}	5.10^7	10^3	0.325	0.0414	0.00998
0.9	0.98	5.10^{-8}	10^{-6}	10^8	10^3	0.359	0.00923	0.0154
0.9	0.98	5.10^{-8}	10^{-3}	10^8	10^3	0.328	0.0229	0.0117
0.9	0.98	5.10^{-8}	10^{-2}	10^8	10^3	0.272	0.0686	0.00869
0.9	0.98	5.10^{-8}	10^{-3}	10^8	10^3	0.328	0.0229	0.0117
0.9	0.98	5.10^{-8}	10^{-3}	10^8	10^4	0.395	0.0229	0.00889
0.9	0.98	5.10^{-8}	10^{-3}	10^8	10^5	0.501	0.0231	0.00580
0.9	0.98	5.10^{-8}	10^{-3}	10^8	10^6	0.716	0.0247	0.00274
0.5	0.99	5.10^{-7}	10^{-6}	10^8	10^3	0.339	0.000712	0.163
0.5	0.95	5.10^{-7}	10^{-6}	10^8	10^3	0.321	0.00317	0.147
0.5	0.95	10^{-7}	10^{-3}	10^8	10^3	0.302	0.0209	0.138
0.5	0.95	10^{-7}	10^{-3}	10^8	10^4	0.367	0.0212	0.112
0.5	0.95	10^{-7}	10^{-3}	10^8	10^5	0.475	0.0214	0.0811
0.5	0.95	10^{-7}	10^{-3}	10^8	10^6	0.690	0.0214	0.0438

which a non-regulating plasmid is able to invade a bacterial population bearing a regulating plasmid in case no second regulating plasmid can invade. It appears that under this growth condition the ability to regulate the conjugation rate is in itself advantageous for a plasmid. However, since the ability to regulate will probably not be acquired with-

out some costs, it depends on the magnitude of these costs, i.e. how severely the growth rate of the bacterial host is reduced by regulation, whether regulation will overall be profitable. If the growth rate reduction caused by regulation is considerably less than the reduction caused by being a competent donor, a regulating donor with optimal regulation rates will in most cases be able to prevent the invasion of a non-regulating plasmid. However, these optimal regulation rates will not be easily reached. Deviations can easily lead to a situation in which the non-regulating plasmid can invade, even if non-regulating does not increase the bacterial growth rate ($w_\varepsilon = 0$).

DISCUSSION

It is shown in this paper that the ability to regulate the conjugation rate is disadvantageous in the very constant environment of a chemostat. In a growing bacterial population, which is periodically transferred, repression may be advantageous.

Some aspects of the model used require some additional discussion. First, the model does not take into account that competent donors are easy victims to bacteriophage infection. However, when a virus, which can only infect bacteria carrying derepressed plasmids, is introduced into the chemostat model, selection on regulation parameters will lead to the same result ($\phi_R p_c - \phi_D p_r = 0$ and a non-regulating plasmid is neutral if the growth rate of its host is the same as that of bacteria carrying derepressed plasmids). However, the introduction of a virus may destabilize the population because of the time-delay between virus infection and the release of new viruses at cell lysis.

Furthermore, it is assumed that a plasmid cannot enter a bacterium already carrying another plasmid. This is a reasonable assumption, since related plasmids exclude each other in most cases very effectively from their host (Finger & Krishnapillai, 1980). Since a regulating plasmid is assumed to arise by mutation from a non-regulating plasmid, the

competing plasmids are closely related.

The fact that there seems to be a maximum conjugation rate per donor is not taken into account. When that maximum is reached, the addition of extra recipients will not increase the conjugation rate per donor (Cullum, Collins & Broda, 1978a). This will lead to a lower transfer rate in case a surplus of recipients is present. The alleged advantage of transfer regulation is that there will be many newly infected, and therefore competent, donors in case of an abundance of recipients, leading to an overall high transfer rate, and only few competent donors in case recipients are scarce, leading to a higher growth rate but a lower overall transfer rate. However, if the transfer rate per recipient decreases when the number of recipients increases, the effect of transfer regulation is lessened.

Model II in this paper assumes that the transfer ability of a plasmid can either be on or off. Occasionally a plasmid, repressing transfer, can become derepressed. Of course, it might also be that plasmids have initially a high transfer rate and fall back after some generations in their host on a much lower transfer rate. This assumption leads to exactly the same results, the low transfer rate, achieved after some bacterial generations, is the mean transfer rate after several generations according to model II. Freter, Freter & Brickner (1983) have estimated the transfer rates for two plasmids, R1, a transfer repressing plasmid, and R1*drd*-19, a non-regulating plasmid, both in the original host and in transconjugants. For R1 the estimated transfer rate of the newly infected donors is in the order of 10^5 to 10^8 higher than that of the original hosts, corresponding to a ratio between repression and derepression rate of about 10^5:1 to 10^8:1. This ratio departs considerably from the ratios of the optimum regulation rates in the numerical examples of table II. They also estimated the transfer rate in the newly infected hosts and a number of hours after the start of their experiment, using their model "SWITCH", comparable with model I in this paper. In that case the transfer rate of plasmid R1 in the newly infected host is about 10^1 to 10^4 higher than

the transfer rate after the switch, corresponding to a ratio between repression and derepression rate of 10:1 to 10^4:1.

The estimates of Freter, Freter & Brickner (1983) of the transfer rate of newly infected hosts is in the order of 10^{-7} to 10^{-9} ml/(cells x hours). The values of γ chosen in the numerical examples are in reasonal accordance with these experimental data, provided that the bacterial concentration is scaled correspondingly in cells/ml.

Nordström, Molin & Aagaard-Hansen (1980) and Nordström & Aagaard-Hansen (1984) estimated the loss rate of plasmids and arrived at values in the order of 10^{-4} to 10^{-6} per cell per generation. However, under less favorable growth conditions the loss rate may be much higher.

Zünd & Lebek (1984) have investigated the effect of the presence of a plasmid on the growth rate of the plasmid bearing bacteria. The generation time of bacteria bearing plasmids varied in their experiments between 29 and 58 min (mean 36 min) compared with a generation time of plasmid-free bacteria of 30 min. As far as I know, no systematic research is done to establish the difference in effect on the bacterial growth rate of being a competent donor and an incompetent one.

The effect of the ability to repress conjugation on the growth rate of its host, has, to my knowledge, never been investigated. Finnegan & Willetts (1971, 1972) and Grindley et al. (1973) have shown that at least two genes are needed, *finO* and *finP*, to repress conjugation in an F-like plasmid. It seems likely that the presence of these genes and their transcription and translation is at the expense of the bacterial host, i.e. of its growth rate. The knowledge of transfer regulation of other plasmid types is less detailed. However, also in those cases presumably extra gene products are involved. Therefore, one would expect the growth rate of bacteria bearing derepressed regulating plasmids to be less than that of bacteria bearing non-regulating mutants of that plasmid.

Stewart & Levin (1977) have investigated whether a conjugative plasmid can maintain itself although it decreases the

growth rate of its host. They concluded that under a rather broad range of parameters the plasmid can survive. In vivo, the conjugation rate might, however, be too small to satisfy even these broad conditions. One of the reasons why the transfer rate in a natural population is often that small, is that most plasmids are long-time residents in their host and therefore transfer repressed. However, the model in this paper suggests that a high transfer rate of derepressed plasmids might be a sufficient condition for a plasmid to be able to invade a plasmid-free bacterial population. Only estimating the transfer rate in a long time ago infected bacterial population will, therefore, lead to a too optimistic idea about the improbability of the successful spread of artificially constructed plasmids.

We have seen that the question whether transfer regulation is favourable for a plasmid depends on the growth condition of the bacterial host population. In a chemostat regulation will never be favoured. Regulating plasmids are, therefore, not optimally adapted for living in a chemostat. Prolonged cultivation of regulating plasmids in a chemostat will either lead to their extinction or to the loss of their ability to repress conjugation. For plasmids in a serial transferred bacterial population, the situation is different. In a very stable long time serial transfer the repression and derepression rate will evolve towards an optimum if the regulation has no severe effect on the growth rate of the bacterial host. The advantage of regulation will, of course, be greater if the difference between the growth rates of bacteria bearing repressed and derepressed plasmids increases. The advantage also increases if the difference between initial and end bacterial concentration increases, i.e. if the conditions fluctuate more at each site. Especially if the difference is small, the advantage of regulation is small, and it might be doubted if it could abolish the cost of regulation, i.e. the cost of having extra genes and synthesizing extra gene products.

The advantage of transfer repression, which several investigators said to be obvious (Ozeki et al., 1962; Broda, 1979;

Campbell, 1981, among others) is at the level of individual selection on plasmids dubious. The advantage which these authors probably had in mind, is based on group selection: If several colonies of bacteria exist and larger colonies have a higher probability of contributing to the foundation of new colonies, it might pay for a plasmid to live in a fast growing colony, instead of maximizing its frequency in the colony. Since in many laboratory situations no "inter-colony" competition exists selection will there be against transfer regulation.

CHAPTER 6:

A MODEL FOR THE COEXISTENCE OF MULTIPLE SPECIES OF PLASMIDS IN CONTINUOUS CULTURE POPULATIONS OF BACTERIA

ABSTRACT

A model is formulated to describe the dynamics of coexistence of several compatible plasmids in a bacterial continuous-flow culture. The model leads to some surprising conclusions. It appears not only that compatible plasmids can easily coexist, but that the presence of one plasmid may facilitate the establishment of a second plasmid. Sometimes two plasmids can coexist in a population although neither of them can be maintained separately and although the two plasmids together reduce the fitness of their host more than each of them separately. Two plasmids, of which one is in every respect inferior (lower transfer rate, lower fitness of the bacterial host, higher rate of loss), can coexist, and sometimes the inferior plasmid can even predominate. It does not seem very profitable for a plasmid to exclude other, compatible, plasmids from its bacterial host. Three plasmids are also able to coexist. It may occur that, although all three plasmids are identical in parameter value, one of them reaches another equilibrium concentration than the other two. Sometimes even the final concentrations of all three plasmids will differ.

INTRODUCTION

Plasmids are pieces of extrachromosomal DNA. They occur abundantly in many bacterial populations (Christiansen et al., 1973; Datta et al., 1979; Lee, Gerding & Cleary, 1984). They are autonomous replicons that are stably inherited in their extrachromosomal state. Naturally occurring plasmids of prokaryotes are generally dispensable (Novick et al., 1976). Plasmids regulate their own replication and the distribution of their copies among the daughter cells of their bacterial host. Some related plasmid types use an identical mechanism for the regulation of both replication and segragation. This

implies that at replication no distinction is made between the copies of the two plasmid types. No more is discriminated between the two plasmid types at partitioning among the daughter cells. This leads to a gradual disappearance of bacteria carrying two or more related plasmids (Novick & Hoppensteadt, 1978). Because for two related plasmid types stable coexistence in a bacterial clone is impossible, they are called *incompatible*. Plasmids are ordered according to this property into incompatibility groups (Datta, 1979). A list of all known incompatibility groups and the plasmids belonging to them is given in Appendix B of Bukhari, Shapiro & Adhya, 1977.

Unrelated plasmids, belonging to different incompatibility groups, use different mechanisms for duplication and segregation. Therefore, one of them does not necessarily disappear from a bacterial clone as a result of drift caused by random replication and segregation.

Several factors influence the population dynamics of plasmids. If a bacterium bearing a plasmid divides, two new bacteria arise, both containing copies of the plasmid. Therefore, the higher the growth rate of the plasmid-bearing bacteria, the faster the growth of the plasmid population. Many plasmids, however, have a negative effect on the growth rate of their bearers (see for example Zünd & Lebek, 1980; Helling, Kinney & Adams, 1981). If that is the case, selection will lead to a decrease in the frequency of plasmid-bearing bacteria. Sometimes plasmid segregation is imperfect, resulting in a plasmid-free daughter cell. Many plasmids encode a mechanism for transferring a strand of their own DNA to another bacterium with which their host has accidentally collided. This process is called *conjugation*. Conjugation is also a factor influencing the population dynamics of plasmids. There are, therefore, at least three factors with an effect on this population dynamics: plasmid loss, conjugation and effect on bacterial fitness.

Plasmid loss will always decrease the frequency of plasmid bearers, and conjugation will always increase that frequency. Whether selection will enlarge or decrease the fre-

quency of plasmid-bearers depends on whether the presence of a plasmid in a bacterium increases or decreases the bacterial fitness. Stewart & Levin (1977) have calculated under which circumstances a conjugationally transmissable plasmid impairing the fitness of its bearer can be maintained.

It is often more difficult for a plasmid to be transmitted to a bacterium already carrying another plasmid than to a plasmid-free cell, a phenomenon called *cell surface exclusion*. The extent of exclusion depends both on the plasmid already present and on the type of plasmid entering the bacterium. Surface exclusion can be especially strong between incompatible plasmids. According to Finger & Krishnapillai (1980), the entry frequency can be reduced by as much as a factor of 10^5. Between compatible plasmids, however, exclusion is, in most cases, not very strong (Hedges & Datta, 1973; Finger & Krishnapillai, 1980).

In a previous theoretical study I have shown that, in the case of complete surface exclusion, at most two different plasmid species can coexist in a bacterial population (van der Hoeven, 1984: Chapter 2 and 3).

In many bacterial strains, when screened on plasmid content, several compatible plasmids were discovered (Christiansen et al., 1973; Datta et al., 1979; Richards & Datta, 1982; Lee, Gerding & Cleary, 1984). In most studies it is not investigated whether the plasmids coexist in the same bacterial cell or only in different bacteria of the same strain. In laboratory experiments, however, plasmids are readily transferred to bacteria already carrying another plasmid. And, if both plasmids are compatible, the two plasmids will remain together in the descendants of that bacterium (e.g., Finger & Krishnapillai, 1980). Hedges, Smith & Brazil (1985) have discovered plasmids of three different incompatibility groups in some *Aeromonads* populations. They remarked that the one-niche - one-species-hypothesis (Gause, 1934; Gilbert, Reynoldsen & Hobart, 1952) suggests that several plasmids can coexist only if they employ different niches. According to them, the coexistence of different compatible plasmids in one bacterial population implies either that these plasmids use

different niches or that this coexistence is a transient stage. In this chapter this hypothesis is investigated. It is analysed whether different compatible plasmids with comparable population dynamics are theoretically able to coexist. It will be shown that two or three plasmids can easily coexist, even if they exclude each other slightly, and if the growth rate of bacteria bearing both plasmids is considerably lower than that of bacteria bearing only one plasmid. Moreover, two plasmids can coexist if one of them is in every respect less fit than the other.

In a previous study (van der Hoeven, in press: Chapter 4) I have shown that surface exclusion directed against incompatible plasmids will be profitable in case of low copy number and a high transfer rate. The question whether surface exclusion directed against compatible plasmids can be advantageous for a plasmid, although it reduces the growth rate of the bacterial host, is also investigated. The conditions under which this is the case seem to be more restrictive.

THE MODEL

To analyse the dynamics of the competition between different compatible plasmids in a chemostat, a mathematical model is formulated.

Basic assumptions of the model and a survey of the parameters

First a model will be formulated for the competition between two compatible plasmids, P_1 and P_2, in a chemostat. An individual bacterium can be plasmid-free (concentration b_0), carry only plasmid P_1 (concentration b_1), carry only plasmid P_2 (concentration b_2) or carry both P_1 and P_2 (concentration $b_{1,2}$).

The bacteria are supposed to grow in a chemostat with a constant turnover rate ρ. There is only one limiting resource in the chemostat (concentration s) and the growth rate of plasmid-free and plasmid-bearing bacteria is proportional to the same function of the limiting resource, $f(s)$. $f(s)$ is an

increasing function of s, i.e. the bacteria grow faster if the concentration of the limiting resource is higher. The proportionality parameter, indicating the relative growth rate, depends on the plasmid content of the bacteria. The relative growth rate of plasmid-free bacteria is set equal to 1, for bacteria carrying only plasmid P_i it is w_i (i=1,2), and for bacteria carrying both P_1 and P_2 it is $w_{1,2}$.

A quantity e of the limiting resource is supposed to be needed per cell division, independent of the plasmid content of the bacterium. Then, the consumption of the limiting resource is proportional to the increase of the bacterial concentration.

A plasmid-bearing bacterium may lose one of its plasmids. It is assumed that the rate at which bacteria lose their plasmid is constant, only depending on the plasmid type and whether or not the other plasmid is present. The rate of loss of plasmid P_i from bacteria carrying only that plasmid, is τ_i, and from bacteria carrying also the other plasmid type τ_i' (i=1,2).

Plasmids can be infectiously transferred through conjugation from a plasmid-bearing bacterium to another bacterium. The conjugational transfer rate is assumed to be proportional to the probability of an accidental collision between a potential donor bacterium and a recipient. This assumption has been experimentally tested by Levin, Stewart & Rice (1978) and was found to be reasonable. For very high bacterial concentrations this assumption is, however, no longer tenable (Collins & Broda, 1975; Cullum, Collins & Broda, 1978a).

The transfer rate is assumed to depend both on the ability of the plasmid-bearer to donate a plasmid and on the ability of the recipient to receive it. More precisely, it is assumed to be the product of the donor efficiency and the recipient competence. The recipient competence is scaled to 1 for a plasmid-free recipient. A bacterium only carrying plasmid P_i may have a reduced recipient ability for the other plasmid. This ability is indicated by α^i, and called the recipient competence. Its inverse is the degree of

surface exclusion of the plasmid. A bacterium may or may not be able to receive a copy of a plasmid, which it already contains. This will, however, not change the plasmid content of the bacterial cell, and is therefore irrelevant for the model. The transfer rate of plasmid P_i from a donor bacterium only carrying that plasmid to a plasmid-free bacterium is γ_i, and from a donor bacterium carrying also the other plasmid type γ_i' (i=1,2). And, for example, the transfer rate of plasmid P_2 from a donor carrying P_1 and P_2 to a recipient, carrying only P_1 is $\alpha^1\gamma_2'$. The transfer rate to a plasmid-free bacterium will be referred to as "the transfer rate". For reference, all symbols are listed in table I.

The model for two competing compatible plasmids can straightforwardly be extended to a more general model for competition between N different compatible plasmids, only the notation becomes much more complicated.

Model for two plasmid species

In fig. 1 a diagram is given of the interactions between plasmid-free bacteria, bacteria with one plasmid and bacteria containing both plasmids. The assumptions in the previous section, rendered in that diagram, lead to the following model of the dynamics of the different bacterial concentrations in a chemostat with a constant inflow of nutrient solution with concentration s_0.

$$\frac{ds}{dt} = \rho(s_0-s) - ef(s)(b_0+w_1b_1+w_2b_2+w_{1,2}b_{1,2}) \qquad (1a)$$

$$\frac{db_0}{dt} = f(s)b_0-\rho b_0-(\gamma_1b_1+\gamma_2b_2+(\gamma_1'+\gamma_2')b_{1,2})b_0+\tau_1b_1+\tau_2b_2 \qquad (1b)$$

$$\frac{db_1}{dt} = w_1f(s)b_1-\rho b_1-\tau_1b_1+\gamma_1b_0b_1+\gamma_1'b_0b_{1,2}-\alpha^1(\gamma_2b_2+\gamma_2'b_{1,2})b_1 + \tau_2'b_{1,2} \qquad (1c)$$

$$\frac{db_2}{dt} = w_2f(s)b_2-\rho b_2-\tau_2b_2+\gamma_2b_0b_2+\gamma_2'b_0b_{1,2}-\alpha^2(\gamma_1b_1+\gamma_1'b_{1,2})b_2 + \tau_1'b_{1,2} \qquad (1d)$$

TABLE I

LIST OF PARAMETERS USED IN THE TWO PLASMID MODEL

P_i: the i^{th} plasmid

$b_0, b_1, b_2, b_{1,2}$: concentration of bacteria, which are plasmid-free, contain only P_1, contain only P_2 or contain P_1 and P_2 respectively (cells x volume^{-1})

$\bar{b}$: total bacterial concentration (cells x volume^{-1})

$w_1, w_2, w_{1,2}$: growth rate of bacteria bearing respectively only P_1, only P_2, and P_1 and P_2, relative to the growth rate of plasmid-free bacteria

γ_i, γ_i': transfer rate of plasmid P_i from a donor bearing only P_i, respectively bearing also the other plasmid to a plasmid-free recipient (volume x cell^{-1} x time^{-1})

α^i: the efficiency of a bacterium carrying plasmid P_i as recipient for the other plasmid

τ_i, τ_i': the rate of loss of plasmid P_i from a bacterium carrying only P_i, respectively carrying also the other plasmid (time^{-1})

s: concentration of limiting resource in the chemostat (mass x volume^{-1})

s_0: concentration of limiting resource in the inflow of the chemostat (mass x volume^{-1})

$f(s)$: growth rate of plasmid-free bacteria at resource concentration s (time^{-1})

e: quantity of resource needed for one cell division (mass/cell)

$h(\bar{b}) = f(s_0 - e\bar{b})$

ρ: turnover rate of the chemostat (time^{-1})

c: parameter of the function h in the numerical examples

$$h(\bar{b}) = (1-\bar{b})/(c-\bar{b})$$

$$\frac{db_{1,2}}{dt} = w_{1,2} f(s) b_{1,2} - \rho b_{1,2} - (\tau_1' + \tau_2') b_{1,2} + \alpha^1 (\gamma_2 b_2 + \gamma_2' b_{1,2}) b_1 + \alpha^2 (\gamma_1 b_1 + \gamma_1' b_{1,2}) b_2 \qquad (1e)$$

In a chemostat an input-output equilibrium will rapidly be attained. At the equilibrium the amount of free nutrient plus the amount of nutrient converted to bacteria flowing out of the chemostat is equal to the amount of free nutrient

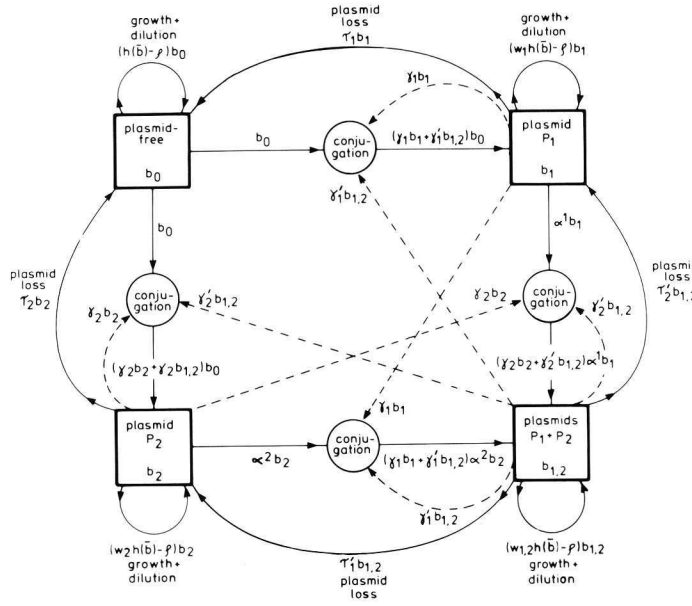


Fig. 1. A diagram of the possible interactions when two compatible plasmids compete. Drawn arrows indicate a possible transition of one bacterial type into another. The transition rates are shown along the arrows. Broken arrows indicate the donation of a plasmid by a bacterial type. The donor bacterium does not change in that case.

in the inflow, i.e. $s + e(b_0+b_1+b_2+b_{1,2}) = s_0$. Therefore the function $f(s)$ can be replaced by a function h of the total bacterial concentration $\bar{b}$, viz. $h(\bar{b}) = f(s_0-e\bar{b})$, and equation (1a) can be eliminated. In all numerical examples a hyperbolic growth function is assumed (Monod, 1949). The variables can be scaled in such a fashion that $h(\bar{b}) = (1-\bar{b})/(c-\bar{b})$.

This model can be considered as an extension of the model of Stewart & Levin (1977) for the dynamics of one plasmid in a chemostat. The general conclusions for the single plasmid model are (Stewart & Levin, 1977):

1) A plasmid-free bacterial population can establish itself in a formerly bacteria-free chemostat, if $f(s_0) > \rho$, i.e. if the bacterial growth rate at low bacterial density is higher

than the dilution rate. In that case a stable equilibrium will be attained with $h(\hat{b}_0) = \rho$, in other words at the equilibrium the bacterial growth rate is identical to the dilution rate.

2) A plasmid, say P_1 can invade a plasmid-free bacterial population if

$$(w_1-1)\rho - \tau_1 + \gamma_1\hat{b}_0 > 0$$

in which $\hat{b}_0$ is the equilibrium concentration of plasmid-free bacteria in absence of plasmids (Stewart & Levin, 1977). This implies that a plasmid can invade although it reduces the growth rate of its bacterial host, as long as its transfer rate is high enough to compensate the lower growth rate with its infecteous spread. If the plasmid can invade, it will be maintained, and a stable equilibrium will be reached with both plasmid-free and plasmid-bearing bacteria.

Results of the analysis of the two plasmid model

Analysis of the two plasmid model leads to the following results.

If the bacterial population containing the first plasmid P_1 is at equilibrium $(\hat{b}_0,\hat{b}_1)$ (and $\hat{h}=h(\hat{b}_0+\hat{b}_1)$) the second plasmid P_2 can invade if either

$$(w_2-w_1)\hat{h} + (\gamma_2-\gamma_1)\hat{b}_0 + (\tau_1-\tau_2) - \alpha^2\gamma_1\hat{b}_1 > 0 \qquad (2a)$$

or

$$\{(w_2-w_1)\hat{h}+(\gamma_2-\gamma_1)\hat{b}_0+(\tau_1-\tau_2)\}\left\{\begin{matrix}(w_{1,2}-w_1)\hat{h}+(\tau_1-(\tau_1'+\tau_2'))\\ -\gamma_1\hat{b}_0+\alpha^1\gamma_2'\hat{b}_1\end{matrix}\right\} \qquad (2b)$$

$$- \alpha^2\gamma_1\hat{b}_1\{(w_{1,2}-w_1)\hat{h}+(\tau_1-\tau_2')+(\gamma_2'-\gamma_1)\hat{b}_0+\alpha^1\gamma_2'\hat{b}_1\}$$
$$- \alpha^1\gamma_2\hat{b}_1(\tau_1'+\gamma_2'\hat{b}_0) < 0$$

provided either $\alpha^1 > 0$ or $\alpha^2 > 0$ (see appendix A). Inequality (2a) can only be true if either the growth rate of a P_2 carrying bacterium is greater than that of a P_1 carrier, or the transfer rate of P_2 is greater than that of P_1, or P_2 is less easily lost from a bacterial cell line, in other words if P_2 is "fitter" than P_1.

Condition (2b) is, however, far less restrictive. If, for instance, both plasmids have equal transfer rate, rate of loss and degree of surface exclusion, while both plasmids have the same effect on the bacterial growth rate ($\gamma_1=\gamma_2\equiv\gamma$; $\gamma_1'=\gamma_2'\equiv\gamma'$; $\tau_1=\tau_2\equiv\tau$; $\tau_1'=\tau_2'\equiv\tau'$; $\alpha^1=\alpha^2\equiv\alpha$; $w_1=w_2\equiv w$) the second plasmid P_2 can invade an equilibrium with the first plasmid P_1 if

$$w_{1,2} > w - (\tau+(2\gamma'-\gamma)\hat{b}_0+\alpha\gamma'\hat{b}_1) \;/\; \hat{h} \qquad (3)$$

provided that the plasmids do not exclude each other completely ($\alpha>0$). This implies that even if the growth rate of a bacterium carrying both plasmids is considerably lower than the growth rate of bacteria carrying only one plasmid, the second plasmid will still be able to invade!

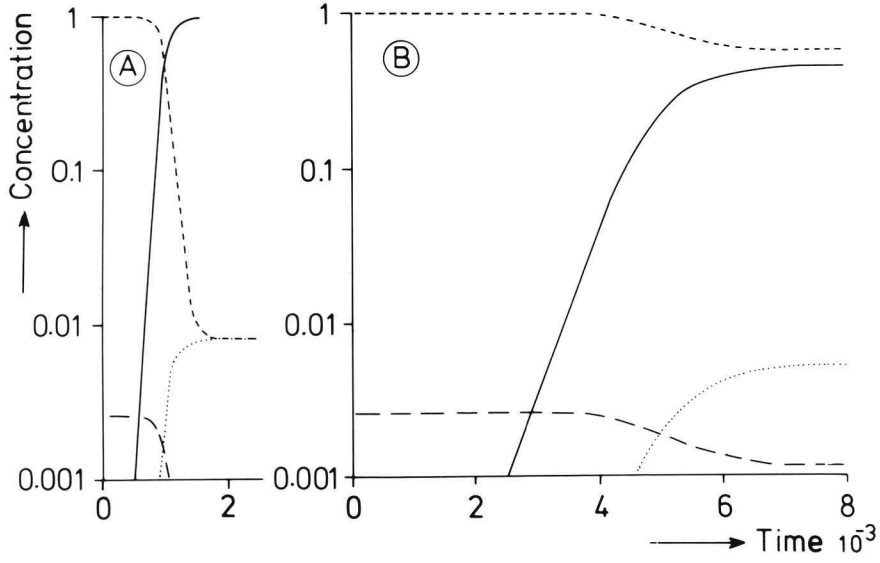


Fig. 2. Dynamics of the invasion of plasmid P_2 in the equilibrium with only plasmid P_1 ($\gamma_1=\gamma_2=\gamma_1'=\gamma_2'=0.05$; $\tau_1=\tau_2=\tau_1'=\tau_2'=10^{-4}$; $\alpha^1=\alpha^2=0.5$; $\rho=0.1$; $c=1.125$). The initial concentration of b_2 is 10^{-6}.

—— —— : b_0
- - - - : b_1
..... : b_2
——— : $b_{1,2}$

1a) $w_1=w_2=0.9$; $w_{1,2}=0.8$
1b) $w_1=w_2=0.9$; $w_{1,2}=0.7$

In fig. 2 some examples of such an invasion are given. In these examples the second plasmid in a bacterium carrying already one plasmid, reduces the fitness even more than the first plasmid does reduce the fitness of a plasmid-free bacterium ($w_{1,2}<w^2$). Computer simulations show that if the second plasmid can invade, the bacterial concentrations will converge to a stable equilibrium value. This process can, however, be quite slow (fig. 2). If the growth rate of bacteria carrying both plasmids ($w_{1,2}$) is not much smaller than the growth rate of bacteria bearing only one plasmid (w), there exists only one stable combination of equilibrium concentrations (fig. 2a). At that equilibrium both plasmids occur in equal densities. However, if $w_{1,2}$ becomes smaller, this equilibrium becomes unstable. Two new equilibrium points appear, one in which the first, and one in which the second plasmid predominates (see Appendix B). In fig. 3 an example of these equilibrium values is shown.

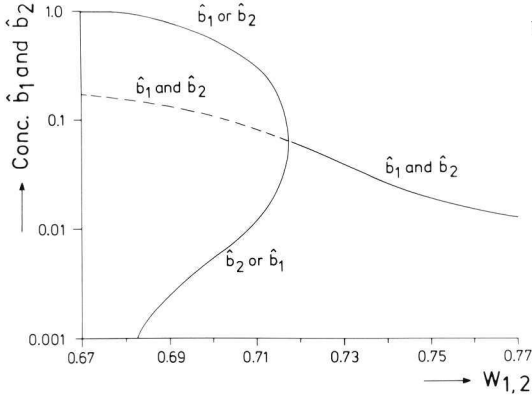


Fig. 3. Equilibrium values of b_1 and b_2 when P_1 and P_2 coexist and are identical in parameters. ————: stable equilibrium; —— ——: instable equilibrium. If $w_{1,2} > 0.718$ only one stable equilibrium exists with $\hat{b}_1 = \hat{b}_2$.

If $w_{1,2} < 0.718$ the stable equilibrium values of b_1 and b_2 differ. Which plasmid attains the highest equilibrium concentration depends on the initial conditions. The equilibrium with $\hat{b}_1 = \hat{b}_2$ is in this case instable. ($w_1=w_2=0.9$; $\gamma_1=\gamma_2=\gamma_1'=\gamma_2'=0.05$; $\tau_1=\tau_2=\tau_1'=\tau_2'=10^{-4}$; $\alpha^1=\alpha^2=0.5$; $\rho=0.1$; $c=1.125$).

For large values of $w_{1,2}$ ($w_{1,2}>0.718$) $b_1 = b_2$ at equilibrium. For smaller values of $w_{1,2}$ the equilibrium with $\hat{b}_1 = \hat{b}_2$

becomes unstable and two equilibria arise, one with $\hat{b}_1 > \hat{b}_2$ and the other vice versa. It depends on the initial conditions which equilibrium will be reached. In this case, if the second plasmid P_2 invades a population at equilibrium containing the first plasmid, P_2 will always remain in a minority position.

The second plasmid may also be able to invade and maintain itself if it is in every respect less fit than the first plasmid, i.e. when P_2 induces a stronger reduction of the growth rate of its host ($w_2<w_1$), when it has a lower transfer rate ($\gamma_2<\gamma_1$), when it is more easily lost ($\tau_2>\tau_1$) and when it has a lower degree of surface exclusion ($\alpha^2>\alpha^1$). Such a less fit plasmid may even maintain itself if the fitness reduction caused by two plasmids together is more than the product of the fitness reductions caused by each of the two plasmids alone ($w_{1,2}<w_1w_2$). This is in sharp contrast with the conditions for coexistence of two incompatible plasmids (van der Hoeven, 1984: Chapter 2). Besides, the more disadvantageous plasmid (smaller w and γ, greater τ) may reach a higher frequency in the bacterial population than the more advantageous plasmid, depending on the initial concentrations (fig. 4).

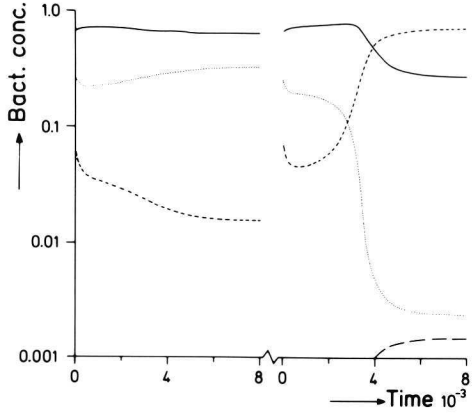


Fig. 4. Dynamics of competition between two plasmids P_1 and P_2 of which one (P_2) is in every respect less "fit" than the other. Two different initial conditions (b_0=0.0007 and $b_{1,2}$==0.68 in both a) and b); a) b_1=0.06; b_2=0.26; b) b_1=0.07; b_2=0.25) lead to different equilibria. — — —: b_0; - - - - -: b_1; .....: b_2; ———: $b_{1,2}$ (w_1=0.905; w_2=0.895; $w_{1,2}$=0.7; $\gamma_1=\gamma_1'$=0.0505; $\gamma_2=\gamma_2'$=0.0495; $\tau_1=\tau_1'=0.95.10^{-4}$; $\tau_2=\tau_2'=1.05.10^{-4}$; α^1=0.495; α^2=0.505; ρ==0.1; c=1.125)

Even if neither P_1 nor P_2 can invade a plasmid-free bacterial population, the combination of P_1 and P_2 may be able to do so if the growth rate of bacteria bearing both plasmids is high, i.e. if

$$(w_{1,2}-1)\rho - (\tau_1'+\tau_2') > 0 \qquad (4)$$

However, even if neither P_1 nor P_2 nor a combination of P_1 and P_2 can invade a plasmid-free bacterial population, there may exist a stable equilibrium with both plasmids present (fig. 5). Simulation shows that such an equilibrium can exist even if a bacterium carrying both plasmids has a lower fitness than a bacterium with only one plasmid, although the fitness of bacteria bearing both plasmids may not be as low as the product of the fitness of bacteria carrying only one of the plasmid types ($w_1 w_2$).

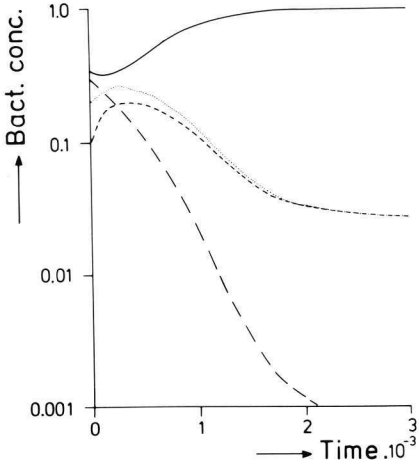


Fig. 5. Dynamics of the competition between two plasmids. A case in which both plasmids can coexist, while neither of them can be maintained separately. —— ——: b_0; - - - -: b_1; .....: b_2; ————: $b_{1,2}$ (w_1= w_2=0.9; $w_{1,2}$=0.89; $\gamma_1=\gamma_2=\gamma_1'=\gamma_2'$= =0.01; $\tau_1=\tau_2=\tau_1'=\tau_2'=10^{-4}$; $\alpha^1=\alpha^2$= =0.5; ρ=0.1; c=1.125).

Surface exclusion

The recipient competence of a bacterium carrying P_2 for plasmid P_1 is given by α^2. If α^2 = 1 plasmid P_2 does not exclude P_1 at all, if α^2 = 0 the surface exclusion of P_2 against P_1 is complete. It can be asked if it is advantageous for a plasmid (P_2) to increase its surface exclusion against

another compatible plasmid (P_1). To increase surface exclusion the cell membrane of the bacterial host has to be changed. This change will probably be disadvantageous for the bacterium, for otherwise bacterial mutants, encoding for that property themselves, will arise.

Strong surface exclusion against compatible plasmids will only evolve if a mutant with a higher degree of surface exclusion can successfully invade a plasmid population. Suppose, for instance, that a mutant of P_2 arises, say P_{2m}, and that P_2 and P_{2m} only differ from each other in their degree of surface exclusion against P_1 (respectively α^2 and α^{2m}), and in their influence on the growth rate of their hosts (respectively w_2 and w_{2m}; $w_{1,2}$ and $w_{1,2m}$). Under what conditions will the mutant P_{2m} be able to invade the original plasmid population of P_2? P_{2m} is a mutant of P_2, therefore, P_2 and P_{2m} will be incompatible. It will be supposed that they are also mutually exclusive. Assume furthermore that the relative growth rate of bacteria bearing two plasmids is the product of the relative growth rates of bacteria bearing either of them alone ($w_{1,2}=w_1w_2$ and $w_{1,2m}=w_1w_{2m}$) and that both the transfer rate and the rate of loss of a plasmid do not depend on the presence of a second plasmid in the bacterium ($\gamma_i=\gamma_i'$; $\tau_i=\tau_i'$), the mutant P_{2m} can invade under the following conditions (see Appendix C for the mathematical derivations).

- In case the mutant has the same degree of surface exclusion ($\alpha^2=\alpha^{2m}$) it can invade if its bacterial host has a higher growth rate ($w_{2m}>w_2$).
- In case the mutant has the same effect on the growth rate of its bacterial host ($w_2=w_{2m}$), it depends on the effect of the other plasmid, P_1, on the bacterial growth rate whether a mutant of P_2 with a higher or with a lower degree of surface exclusion can invade. If P_1 decreases the bacterial growth rate ($w_1<1$) a mutant P_{2m} with a higher degree of surface exclusion ($\alpha^{2m}<\alpha^2$) can invade, and if P_1 increases the growth rate of the bacteria ($w_1>1$) a mutant P_{2m} with a lower degree of surface exclusion can invade ($\alpha^{2m}>\alpha^2$).

- If the mutant differs both in degree of surface exclusion and in the costs for its host, it is most likely that an increase in surface exclusion will cause a decrease in the bacterial growth rate. In that case, and if the other compatible plasmid P_1 increases the bacterial growth rate ($w_1 > 1$), only mutants with a higher growth rate (and a lower degree of surface exclusion) can invade (fig. 6b). If P_1 decreases the bacterial growth rate ($w_1 < 1$) a mutant with a higher degree of surface exclusion may be able to invade, although it reduces the bacterial growth rate. However, this can only occur if the mutant has a considerably higher degree of surface exclusion (fig. 6a). Slight increases in surface exclusion will not be able to compensate for a decrease in growth rate. And even if a mutant with a higher degree of surface exclusion can invade, it will not be able to expel the original plasmid P_2. As soon as the plasmid P_1 disappears from the environment of P_2 and its mutant P_{2m}, the mutant, with the higher degree of surface exclusion, will be selected against.

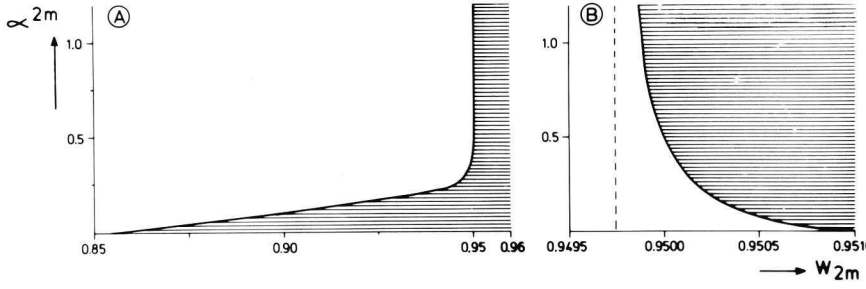


Fig. 6. The combination of growth rate (w_{2m}) and recipient competence (α^{2m}) for which a mutant P_{2m} of P_2 can invade an equilibrium with P_1 and P_2 (hatched area). P_2 and its mutant P_{2m} are incompatible and mutually exclusive. The transfer and rate of loss of P_2 and P_{2m} are identical. The effects on the bacterial growth rate of P_1 and P_2 or P_{2m} are multiplicative, i.e. $w_{1,2} = w_1 w_2$ and $w_{1,2m} = w_1 w_{2m}$ ($\gamma_1 = \gamma_1' = 0.05$; $\gamma_2 = \gamma_2' = 0.02$; $\tau_1 = \tau_2 = \tau_1' = \tau_2' = 10^{-4}$; $\alpha^1 = \alpha^2 = 0.5$; $\rho = 0.1$; $c = 1.125$).

6a: $w_1 = 0.9$; $w_2 = 0.95$; $w_{1,2} = 0.855$

6b: $w_1 = 1.1$; $w_2 = 0.95$; $w_{1,2} = 1.045$.

The general model

The model will now be extended to the general form of competition between N different competing compatible plasmids in a chemostat. In that case, the notation of the model becomes much more cumbersome. A bacterium can contain any combination of the N different plasmids. Therefore, there are 2^N different possible plasmid combinations in a bacterium, ranging from plasmid-free to all N plasmids. The plasmid content of a bacterium can be represented by a vector $\xi = (\xi_1, \xi_2, \ldots \xi_N)$ in which $\xi_i = 1$ if the bacterium carries P_i and $\xi_i = 0$ if the bacterium does not bear P_i; the vector ξ indicates a combination of plasmids. Now the concentration of bacteria carrying plasmid combination ξ can be indicated by b_ξ and the relative growth rate of bacteria carrying that combination by w_ξ, the rate of loss of plasmid P_i by τ_i^ξ, the transfer rate of plasmid P_i by γ_i^ξ and the recipient competence of a bacterium carrying plasmid combination ξ for plasmid P_i by α_i^ξ. It should be noted that a bacterium can neither lose nor donate a plasmid it does not contain, therefore if $\xi_i = 0$, then $\tau_i^\xi = 0$ and $\gamma_i^\xi = 0$. By definition the recipient competence of a bacterium for any plasmid is 1 if the recipient is plasmid-free, so $\alpha_i^\xi = 1$ if ξ is the zero-vector. Since the entrance of a plasmid into a bacterium already containing that plasmid, does not change the plasmid content of the bacterium, and therefore has no influence on the dynamics in the model, surface exclusion can be considered to be absolute against plasmids already present in the bacterium, so $\alpha_i^\xi = 0$ if $\xi_i = 1$. Let Ξ_ξ be the set of the numbers of the plasmid types in combination ξ, and Ω_ξ the set of the numbers of the plasmid types not present in combination ξ (for example: N=5 and ξ=(0,1,1,0,0), then Ξ_ξ={2,3} and Ω_ξ={1,4,5}). If the plasmid combination indicated by vector ξ does not contain plasmid P_i, that combination with the addition of plasmid P_i can be indicated by $\xi + p_i$. On the other hand, if the plasmid combination indicated by vector ξ does contain plasmid P_i, the otherwise identical combination without P_i can be indicated by $\xi - p_i$ (for example, if ξ=(0,1,1,0,0) then $\xi + p_4 =$

$=(0,1,1,1,0)$ and $\xi-p_2=(0,0,1,0,0)$). A general model for the dynamics of N plasmids in a chemostat becomes in this notation

$$\frac{ds}{dt} = \rho(s_0-s) - ef(s)\sum_{\xi} w_\xi b_\xi \tag{5a}$$

$$\frac{db_\xi}{dt} = w_\xi f(s) b_\xi - \rho b_\xi - \sum_i \tau_i^\xi b_\xi - b_\xi \sum_i \alpha_i^\xi \sum_\theta \gamma_i^\theta b_\theta + \sum_{i\varepsilon\Omega_\xi} \tau_i^{(\xi+p_i)} b_{(\xi+p_i)}$$

$$+ \sum_{i\varepsilon\Xi_\xi} \alpha_i^{(\xi-p_i)} b_{(\xi-p_i)} \sum_\theta \gamma_i^\theta b_\theta \tag{5b}$$

Three compatible plasmids

Using the general model the result of competition between three compatible plasmids has been examined by means of computer simulation. It appears that three plasmids can coexist. However, the dynamics of the model become very complicated. Even if the three plasmids do not differ in their parameters, the model gives rise to 23 different dynamical solutions listed in table II. In fig. 7 a diagram is shown of the solutions for the case that two plasmids can coexist and the equilibrium concentrations of both plasmids differ.

The general conclusions which can be derived from the three plasmid model resemble the conclusions of the two plasmid model.

- Three plasmids can coexist, even if one of them has a more negative effect on its host than the others, In fig. 8 an example is given of the dynamics of three competing plasmids, where the growth rate of P_3 bearers is lower than that of P_2 bearers, which in its turn is again smaller than the growth rate of P_1 bearers ($w_{(0,0,1)}<w_{(0,1,0)}<w_{(1,0,0)}$), while the effect of the plasmids on the relative growth rates of their hosts are multiplicative (for instance, $w_{(1,1,0)}=w_{(1,0,0)}w_{(0,1,0)}$ and $w_{(1,1,1)}=w_{(1,0,0)}w_{(0,1,0)}w_{(0,0,1)}$). Even under these conditions coexistence of three plasmids appears possible.
- Sometimes three plasmids are able to coexist, although a

TABLE II

Can P_1 invade a plasmid-free population?	Can $P_2(+P_1)$ invade the thus attained stable equilibrium?	Does a stable equilibrium exist with both P_1 and P_2?	Can $P_3(+P_2(+P_1)$ invade the thus attained stable equilibrium?	Number of equilibria with $[P_1]=[P_2]=[P_3]>0$ stable/instable	Existence of other stable equilibria with $[P_1],[P_2],[P_3]>0$	Existence of other instable equilibria with $[P_1],[P_2],[P_3]>0$
Yes 1	Yes 1.1	Yes $[P_1]=[P_2]$	Yes 1.1.1	1, stable	No	No
			Yes 1.1.2	1, stable	Yes, $[P_1]=[P_2]\succ[P_3]$	Yes, $[P_1]\succ[P_2]=[P_3]$
			No 1.1.3	0	No	Yes, $[P_1]\succ[P_2]=[P_3]$
	Yes 1.2	Yes $[P_1]\succ[P_2]$	Yes 1.2.1	1, stable	No	No
			Yes 1.2.2	1, stable	Yes, $[P_1]\succ[P_2]=[P_3]$	Yes, $[P_1]\succ[P_2]=[P_3]$
			Yes 1.2.3	1, instable	Yes, $[P_1]\succ[P_2]=[P_3]$	Yes, $[P_1]=[P_2]\succ[P_3]$
			Yes 1.2.4	1, instable	Yes, $[P_1]\succ[P_2]=[P_3]$	No
			Yes 1.2.5	1, instable	Yes, $[P_1]\succ[P_2]\succ[P_3]$	Yes, $[P_1]\succ[P_2]=[P_3]$
			No 1.2.6	1, instable	No	Yes, $[P_1]\succ[P_2]=[P_3]$
	No 1.3	No	Yes 1.3.1	1, stable	No	No
			No 1.3.2	1, stable	No	Yes, $[P_1]\succ[P_2]=[P_3]$
			No 1.3.3	1, stable	No	Yes, 2: $[P_1]\succ[P_2]=[P_3]$ and $[P_1]=[P_2]\succ[P_3]$
			No 1.3.4	1, instable	No	No
No 2	Yes 2.1	Yes $[P_1]=[P_2]$	Yes 2.1.1	1, stable	No	No
			Yes 2.1.2	1, instable	Yes, $[P_1]=[P_2]\succ[P_3]$	Yes, $[P_1]\succ[P_2]=[P_3]$
			No 2.1.3	1, instable	No	Yes, $[P_1]\succ[P_2]=[P_3]$
	No 2.2	Yes $[P_1]=[P_2]$	Yes 2.2.1	1, stable	No	No
			Yes 2.2.2	2, stable & inst.	No	No
			Yes 2.2.3	2, both inst.	Yes, $[P_1]=[P_2]\succ[P_3]$	Yes, $[P_1]\succ[P_2]=[P_3]$
			No 2.2.4	2, both inst.	No	Yes, $[P_1]\succ[P_2]=[P_3]$
	No 2.3	No	Yes 2.3.1	1, stable	No	No
			No 2.3.2	2, stable & inst.	No	No
			No 2.3.3	0	No	No

TABLE II

TYPES OF QUALITATIVELY DIFFERENT DYNAMICAL BEHAVIOUR OF THE MODEL OF COMPETITION BETWEEN THREE, IN PARAMETERS IDENTICAL, COMPATIBLE PLASMIDS

$[P_i]$ is the concentration of bacteria carrying plasmid P_i. Since the plasmids have identical parameter values, the indices in the result can be interchanged. For example, if an equilibrium with $[P_1] = [P_2] > [P_3]$ is described, the corresponding equilibria $[P_1] = [P_3] > [P_2]$ and $[P_2] = [P_3] > [P_1]$ also exist. Each different dynamical behaviour has been given a number of the form $i.j.k.$, where k, or both j and k may be absent; i refers to the situation with only one plasmid, j refers to the situation when a second plasmid is added, while k refers to the situation when a third plasmid is also present. An increase in one of the indices i, j and k, while the other two indices remain constant, indicates a decrease in the growth rate of bacteria carrying one, two or three plasmids.

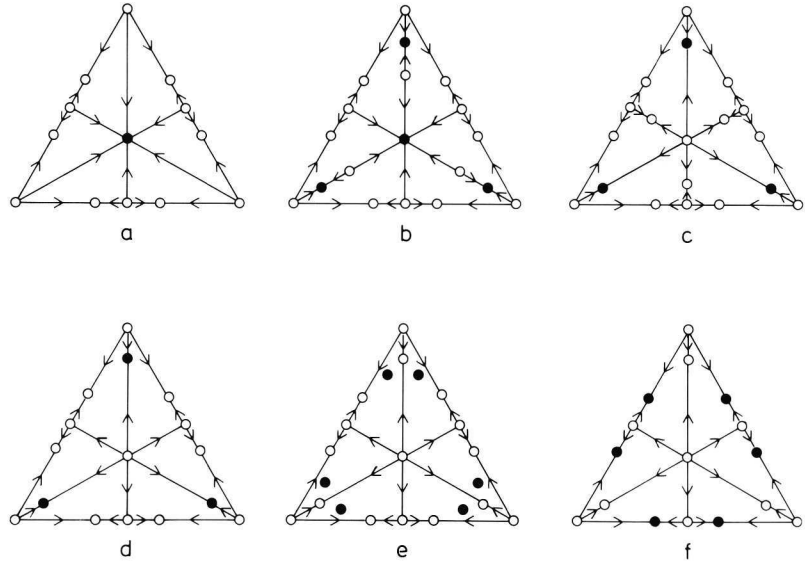


Fig. 7. Diagram of the different possible equilibrium situations of the three plasmid model in case that two plasmids can coexist while they have different equilibrium concentrations. All three plasmids are identical in parameter values. At the angular points only one plasmid is present, on the sides two, and on the perpendiculars two plasmids are present in equal concentration while the third may occur in another concentration. o: unstable equilibrium; •: stable equilibrium. The corresponding situations described in table II are a: 1.2.1; b: 1.2.2; c: 1.2.3; d: 1.2.4; e: 1.2.5; f: 1.2.6.

combination of two of them cannot coexist, or when a single plasmid cannot be maintained.

- Plasmids, identical in parameters will not necessarily reach equal concentrations. Sometimes two plasmids will have the same concentration at equilibrium, whereas the third has a higher, or lower concentration, and sometimes each plasmid will have a different concentration at equilibrium (fig. 7e). Which plasmid will predominate in the population depends on the initial concentrations.
- Contrary to the two-plasmid case, stable coexistence of

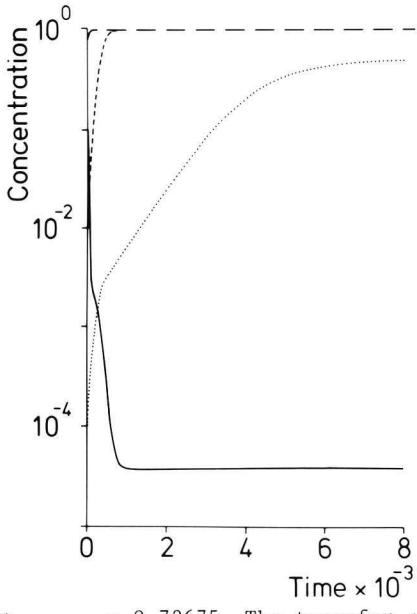


Fig. 8. Dynamics of the competition of three compatible plasmids. The growth rate of bacteria bearing the third plasmid P_3 is lower than that of bacteria bearing P_2 which is in its turn lower than the growth rate of bacteria bearing P_1. The effects on the bacterial growth rate of the three plasmids are multiplicative. $w_{(1,0,0)} = 0.95$; $w_{(0,1,0)} = 0.9$; $w_{(0,0,1)} = 0.85$; $w_{(1,1,0)} = 0.855$; $w_{(1,0,1)} = 0.8075$; $w_{(0,1,1)} = 0.765$; $w_{(1,1,1)} = 0.72675$. The transfer and rates of loss of all three plasmids are identical, independent of the presence of other plasmids (γ=0.05; $\tau = 10^{-4}$). The plasmids do not exclude each other (α_i^{ξ}=1 for all ξ and i). ———: b_0; —— ——: concentration of bacteria bearing P_1; - - - - -: concentration of bacteria bearing P_2; .....: concentration of bacteria bearing P_3.

three plasmids may be possible in cases, where neither of them can enter a bacterium carrying the other two plasmids. This can occur irrespective of the growth rate of bacteria bearing all three plasmids.

DISCUSSION

The model analysed in this paper shows that two or three compatible plasmids can easily coexist in one bacterial population. There are no indications that this result is limited to three plasmids. Not only is it possible for two or more plasmids to coexist, but the presence of one plasmid species can even facilitate the entrance of a second plasmid in a bacterial population. Exclusion of compatible plasmids does not seem very profitable, especially not when it brings

along a decrease in the bacterial growth rate.

Some special cases of the two plasmid model have already been analysed previously. The case of complete surface exclusion ($\alpha^1=\alpha^2=0$) was investigated (van der Hoeven, 1984: Chapter 2) with special reference to incompatible plasmids. In that case bacteria carrying both plasmids P_1 and P_2 will not occur. It was shown that P_1 and P_2 can only coexist in a bacterial population if the plasmid with the highest transfer rate reduces the fitness of its bearer more than the plasmid with the lower transfer rate. It appeared to be impossible for three plasmids to coexist under these circumstances.

Levin & Stewart (1980) have studied a model for the maintenance of a mobilizable non-conjugative plasmid. In their model one of the plasmids, say P_2, is mobilizable by the other plasmid (P_1), but cannot induce transfer itself ($\gamma_2=0$, whereas $\gamma_2'>0$). Their model is a special case of the model presented in this paper with one exception. In their model the mobilizing (P_1) and mobilizable (P_2) plasmid can be transferred together, converting a plasmid-free bacterium into a bacterium carrying both plasmids in one step.

Levin & Stewart showed that a mobilizable non-conjugative plasmid will not only be maintained if it is advantageous for its bacterial bearer, but can even be maintained if it is disadvantageous, although only for a very narrow parameter range.

Surprisingly, if two plasmids differ only slightly in parameter values, the predominant plasmid in the population does not have to be the fittest. When one of the plasmids was favoured in the past, for instance by resistance to antibiotics, that plasmid may remain predominant in the population although at present it is almost neutral compared with the other plasmids.

Plasmids are found in many natural bacterial populations, and the coexistence of several different plasmids is no exception (Christiansen et al., 1973; Datta et al., 1979; Richards & Datta, 1982; Hedges, Smith & Brazil, 1985; among many others). However, it is often not clear whether all of these plasmids are conjugative. Some authors claim that the

conjugation rate in vivo is negligible (for instance Anderson, 1975), although Anderson leaves open the possibility that conjugational transfer of resistance plasmids may occur if antibiotics are used. In vitro, however, the transfer rate can be reasonably high. (Bennet & Richmond, 1978, between 6.10^{-6} and 2.10^{-2} transfers per donor per hour; Cullum, Collins & Broda, 1978b, about 0.2 transfers per efficient donor per hour). Besides, the fact that a mechanism to promote transfer has been developed, suggests that it will at least sometimes be used in nature. One of the reasons why plasmid transfer cannot easily be detected in vivo is that the natural bacterial population density is often much lower than in vitro. Freter, Freter & Brickner (1983) state that transfer rate of a plasmid in a mouse gut is about the same as in a chemostat, but that the resulting population of bacteria, which have acquired a plasmid by means of conjugation is too small to be detected with the normal culture methods.

It is well established that the presence of a plasmid in a bacterium affects its growth rate (e.g. Zünd & Lebek, 1980). What the effect will be of a second or even a third plasmid in a bacterium is not known. It could be that the growth rate reduction, caused by both plasmids together, is the product of the reductions caused by each plasmid separately ($w_{1,2}=w_1w_2$). This assumption is made in some of the examples in this chapter, for instance in the example of coexistence of three compatible plasmids (fig. 8). Given this assumption, two plasmids are not able to coexist if neither of them can become established on its own. Another reasonable assumption for the effect of two plasmids together on the bacterial growth is that each plasmid increases the generation time of the bacterial host with a certain length. In that case, $w_{1,2} > w_1w_2$. The real effect of plasmid interaction in a bacterium will, however, probably be more complicated. It might be either that the effect for the bacterium of two plasmids together is worse than multiplicative ($w_{1,2}<w_1w_2$), as is assumed in the examples in this chapter of the coexistence of two compatible plasmids (fig. 2 and 4), or that this effect is less than multiplicative ($w_{1,2}>w_1w_2$) (fig. 5).

The conclusion that several plasmid species can coexist is not in accordance with the one-niche-one-species principle (Gause, 1934; Gilbert, Reynoldsen & Hobart, 1952). Of course, one could argue that, since the usage by one plasmid species of a bacterium does not prevent that bacterium to serve as a host for another plasmid, a niche includes more for a plasmid than a well defined bacterial species in a well defined bacterial environment. It should also include the direct plasmid environment, i.e. the content of the bacterial cell, and the way a plasmid uses that environment to regulate its replication and segregation mechanism. If the replication and segregation regulation of a plasmid are considered as part of that plasmid's niche, two compatible plasmids employ, by definition, never the same niche. However, this argument has two major drawbacks. In the first place refining the definition of a niche with properties of the inhabiting species holds the danger of making the differences between niches coincide with the differences between the inhabiting species. In terms of Rescigno & Richardson (1965) this would imply that each additional species adds a new niche (niche function) to the total number of niches (niche functions), since the reaction of each individual of that species to conspecifics differs from that to individuals of any of the other species. In this way the one-niche-one-species principle becomes a tautology. In the second place, the fact that different compatible plasmids use different mechanisms to regulate their replication and segregation is not used in the model. And, according to the model, two plasmid species, which are in every respect identical, can stably coexist (of course, a model is the only place where two species can be exactly identical). Therefore, it seems more sensible to consider the model developed in this paper as an illustration of the idea that the one-species-one-niche principle does not hold if the density of one species is restricted by the number of territories (bacteria) instead of the food supply, in which case competition might be (almost) exclusively intraspecific (Hutchinson, 1957).

A great deal of plasmid ecology both in vivo and in vitro

is still unknown. The model in this paper is a first theoretical exploration of the behaviour of coexisting compatible plasmids. It indicates that many different compatible conjugative plasmids can, at least theoretically, coexist without periodic selection for the different species, cooperation between different species or different bacterial hosts as refuges for the different plasmids.

APPENDIX A

CONDITIONS FOR THE INVASION OF A SECOND COMPATIBLE PLASMID P_2 IN AN EQUILIBRIUM WITH PLASMID P_1

P_2 can invade an equilibrium of P_1 if the jacobian matrix, in which $\dot{b} = db/dt$,

$$\begin{pmatrix} \dot{\partial b}_0/\partial b_0 & \dot{\partial b}_0/\partial b_1 & \dot{\partial b}_0/\partial b_2 & \dot{\partial b}_0/\partial b_{1,2} \\ \dot{\partial b}_1/\partial b_0 & \dot{\partial b}_1/\partial b_1 & \dot{\partial b}_1/\partial b_2 & \dot{\partial b}_1/\partial b_{1,2} \\ \dot{\partial b}_2/\partial b_0 & \dot{\partial b}_2/\partial b_1 & \dot{\partial b}_2/\partial b_2 & \dot{\partial b}_2/\partial b_{1,2} \\ \dot{\partial b}_{1,2}/\partial b_0 & \dot{\partial b}_{1,2}/\partial b_1 & \dot{\partial b}_{1,2}/\partial b_2 & \dot{\partial b}_{1,2}/\partial b_{1,2} \end{pmatrix} \qquad \text{(A-1)}$$

at equilibrium with P_1 has at least one eigenvalue with positive real part (and corresponding eigenvector $(a_1,a_2,a_3,a_4)^T$ with either $a_3 \neq 0$ or $a_4 \neq 0$).

At the equilibrium with P_1

$\dot{\partial b}_2/\partial b_0 = \dot{\partial b}_2/\partial b_1 = \dot{\partial b}_{1,2}/\partial b_0 = \dot{\partial b}_{1,2}/\partial b_1 = 0$,

therefore, the eigenvalues of the matrix A-1 are the combined eigenvalues of the matrices

$$\begin{pmatrix} \dot{\partial b}_0/\partial b_0 & \dot{\partial b}_0/\partial b_1 \\ \dot{\partial b}_1/\partial b_0 & \dot{\partial b}_1/\partial b_1 \end{pmatrix} \qquad \text{(A-2)}$$

and

$$\begin{pmatrix} \dot{\partial b_2}/\partial b_2 & \dot{\partial b_2}/\partial b_{1,2} \\ \dot{\partial b_{1,2}}/\partial b_2 & \dot{\partial b_{1,2}}/\partial b_{1,2} \end{pmatrix} \tag{A-3}$$

The sign of the real parts of the eigenvalues of matrix A-2 determines the stability of an equilibrium with plasmid P_1 in absence of plasmid P_2, and are irrelevant for the ability of P_2 to invade the equilibrium with P_1.

At that equilibrium

$$\frac{\dot{\partial b_2}}{\partial b_2} = w_2\hat{h} - \rho - \tau_2 + \gamma_2\hat{b}_0 - \alpha^2\gamma_1\hat{b}_1 =$$

$$= (w_2-w_1)\hat{h} + (\gamma_2-\gamma_1)\hat{b}_0 + (\tau_1-\tau_2) - \alpha^2\gamma_1\hat{b}_1 \tag{A-4a}$$

since at the equilibrium

$$w_1\hat{h} - \rho - \tau_1 + \gamma_1\hat{b}_0 = 0,$$

$$\frac{\dot{\partial b_2}}{\partial b_{1,2}} = \tau_1' + \gamma_2'\hat{b}_0 \quad , \tag{A-4b}$$

$$\frac{\dot{\partial b_{1,2}}}{\partial b_2} = (\alpha^2\gamma_1+\alpha^1\gamma_2)\hat{b}_1 \tag{A-4c}$$

and

$$\frac{\dot{\partial b_{1,2}}}{\partial b_{1,2}} = w_{1,2}\hat{h} - \rho - (\tau_1'+\tau_2') + \alpha^1\gamma_2'\hat{b}_1 =$$

$$= (w_{1,2}-w_1)\hat{h} + \tau_1 - (\tau_1'+\tau_2') - \gamma_1\hat{b}_0 + \alpha^1\gamma_2'\hat{b}_1 \tag{A-4d}$$

At least one of the eigenvalues of matrix A-3 has a positive real part if either the trace of the matrix is positive or its determinant negative. Since both $\dot{\partial b_2}/\partial b_{1,2} \geq 0$ and $\dot{\partial b_{1,2}}/\partial b_2 \geq 0$ this condition implies that either

$$(w_2-w_1)\hat{h} + (\gamma_2-\gamma_1)\hat{b}_0 + (\tau_1-\tau_2) - \alpha^2\gamma_1\hat{b}_1 > 0 \tag{A-5a}$$

or

$$(w_{1,2}-w_1)\hat{h} + \tau_1 - (\tau_1'+\tau_2') - \gamma_1\hat{b}_0 + \alpha^1\gamma_2'\hat{b}_1 > 0 \tag{A-5b}$$

or

$$\{(w_2-w_1)\hat{h}+(\gamma_2-\gamma_1)\hat{b}_0+(\tau_1-\tau_2)\}\ \{(w_{1,2}-w_1)\hat{h}+\tau_1-(\tau_1'+\tau_2')-\gamma_1\hat{b}_0+\alpha^1\gamma_2'\hat{b}_1\}$$

$$-\alpha^2\gamma_1\hat{b}_1.((w_{1,2}-w_1)\hat{h}+(\tau_1-\tau_2')+(\gamma_2'-\gamma_1)\hat{b}_0+\alpha^1\gamma_2'\hat{b}_1)\ -\alpha^1\gamma_2\hat{b}_1(\tau_1'+\gamma_2'\hat{b}_0)\ <\ 0 \qquad \text{(A-5c)}$$

Provided that at least one of the two plasmids does not induce complete surface exclusion (either $\alpha^1>0$ or $\alpha^2>0$), inequality A-5c is true if only one of the other two inequalities A-5a and A-5b holds, therefore one of these inequalities is redundant.

If both plasmids are identical in parameters ($w_1=w_2\equiv w$; $\gamma_1=\gamma_2\equiv\gamma$; $\gamma_1'=\gamma_2'\equiv\gamma'$; $\tau_1=\tau_2\equiv\tau$; $\tau_1'=\tau_2'\equiv\tau'$; $\alpha^1=\alpha^2\equiv\alpha$) the conditions of inequalities A-5 for invasion of the second plasmid reduce to

$$w_{1,2} > w - \{(2\gamma'-\gamma)\hat{b}_0+\alpha\gamma'\hat{b}_1+\tau\}\ /\ \hat{h} \qquad \text{(A-6)}$$

APPENDIX B

EQUILIBRIUM CONCENTRATIONS OF TWO COEXISTING PLASMIDS WHICH ARE IDENTICAL IN PARAMETERS

If the two plasmids P_1 and P_2 are identical in parameters ($w_1=w_2\equiv w$; $\gamma_1=\gamma_2\equiv\gamma$; $\gamma_1'=\gamma_2'\equiv\gamma'$; $\tau_1=\tau_2\equiv\tau$; $\tau_1'=\tau_2'\equiv\tau'$ and $\alpha^1=\alpha^2\equiv\alpha$) the equilibrium values of system (1) with both plasmids are given by the solutions of

$$(\hat{h}-\rho-\gamma(\hat{b}_1+\hat{b}_2)-2\gamma'\hat{b}_{1,2})\hat{b}_0 + \tau(\hat{b}_1+\hat{b}_2) = 0 \qquad \text{(B-1a)}$$

$$(w\hat{h}-\rho-\tau+\gamma\hat{b}_0-\alpha\gamma\hat{b}_2)\hat{b}_1 + (\gamma'\hat{b}_0+\tau'-\alpha\gamma'\hat{b}_1)\hat{b}_{1,2} = 0 \qquad \text{(B-1b)}$$

$$(w\hat{h}-\rho-\tau+\gamma\hat{b}_0-\alpha\gamma\hat{b}_1)\hat{b}_2 + (\gamma'\hat{b}_0+\tau'-\alpha\gamma'\hat{b}_2)\hat{b}_{1,2} = 0 \qquad \text{(B-1c)}$$

and

$$(w_{1,2}\hat{h}-\rho-2\tau'+\alpha\gamma'(\hat{b}_1+\hat{b}_2))\hat{b}_{1,2} + 2\alpha\gamma\hat{b}_1\hat{b}_2 = 0 \qquad \text{(B-1d)}$$

Equation (B-1d) leads to

$$\hat{b}_{1,2} = 2\alpha\gamma\hat{b}_1\hat{b}_2/\{\rho+2\tau'-w_{1,2}\hat{h}-\alpha\gamma'(\hat{b}_1+\hat{b}_2)\} \qquad \text{(B-2)}$$

and this equation, combined with equation (B-1c) leads to

$$\hat{b}_1^2(\alpha)^2\gamma\gamma' - \hat{b}_1\hat{b}_2(\alpha)^2\gamma\gamma' + \hat{b}_1\{\alpha\gamma'(\rho+\tau-\omega\hat{h}-\gamma\hat{b}_o)+\alpha\gamma(w_{1,2}\hat{h}-\rho+2\gamma'\hat{b}_o)\}$$

$$+ \hat{b}_2\alpha\gamma'(\rho+\tau-\omega\hat{h}-\gamma\hat{b}_o) + (\omega\hat{h}-\rho-\tau+\gamma\hat{b}_o)(\rho+2\tau'-w_{1,2}\hat{h}) = 0 \qquad \text{(B-3)}$$

The combination of equation (B-2) with equation (B-1b) gives of course the same result, with $\hat{b}_1$ and $\hat{b}_2$ interchanged. Therefore, the combination of B-1b, B-1c and B-1d gives

$$\{(2\alpha\gamma'(\omega\hat{h}-\rho-\tau)+\alpha\gamma(\rho-w_{1,2}\hat{h}))y-(\omega\hat{h}-\rho-\tau+\gamma\hat{b}_o)(\rho+2\tau'-w_{1,2}\hat{h})\}.$$

$$\{(\alpha)^2\gamma\gamma'y^2+\alpha\gamma(w_{1,2}\hat{h}-\rho+2\gamma'\hat{b}_o)y+(\omega\hat{h}-\rho-\tau+\gamma\hat{b}_o)(\tau+\gamma'\hat{b}_o)\} = 0 \qquad \text{(B-4)}$$

with either $y = \hat{b}_1$ or $y = \hat{b}_2$. This implies that at equilibrium either

$$\hat{b}_1 = \hat{b}_2 = (\omega\hat{h}-\rho-\tau+\gamma\hat{b}_o)(\rho+2\tau'-w_{1,2}\hat{h}) \;/\; \{2\alpha\gamma'(\omega\hat{h}-\rho-\tau)+\alpha\gamma(\rho-w_{1,2}\hat{h})\}, \qquad \text{(B-5)}$$

in which case

$$\hat{b}_{1,2} = \frac{2(\omega\hat{h}-\rho-\tau+\gamma\hat{b}_o)^2(\rho+2\tau'-w_{1,2}\hat{h})}{(2\alpha\gamma'(\omega\hat{h}-\rho-\tau)+\alpha\gamma(\rho-w_{1,2}\hat{h}))(\rho-w_{1,2}\hat{h}-2\gamma'\hat{b}_o)} \qquad \text{(B-6)}$$

and (equation B-1a), $\hat{b}_o$ is the solution of

$$\hat{b}_o^2\Big[2\gamma'(\hat{h}-\rho)(2\alpha\gamma'(\omega h-\rho-\tau)+\alpha\gamma(\rho-w_{1,2}\hat{h}))$$

$$+2\gamma(\rho+2\tau'-w_{1,2}\hat{h})(2\gamma'(\omega\hat{h}-\rho)+\gamma(\rho-w_{1,2}\hat{h}))\Big]$$

$$-\hat{b}_o\Big[(\rho-w_{1,2}\hat{h})(\hat{h}-\rho)(2\alpha\gamma'(\omega\hat{h}-\rho-\tau)+\alpha\gamma(\rho-w_{1,2}\hat{h}))$$

$$-2(2\gamma'(\omega\hat{h}-\rho)+\gamma(\rho-w_{1,2}\hat{h}))(\omega\hat{h}-\rho-\tau)(\rho+2\tau'-w_{1,2}\hat{h})$$

$$+2\tau\gamma(\rho+2\tau'-w_{1,2}\hat{h})(\rho-w_{1,2}\hat{h})\Big]$$

$$+ 2\tau(\omega\hat{h}-\rho-\tau)(\rho+2\tau'-w_{1,2}\hat{h})(w_{1,2}\hat{h}-\rho) = 0 \qquad \text{(B-7)}$$

or $\hat{b}_1$ and $\hat{b}_2$ differ, in which case one of $\hat{b}_1$ and $\hat{b}_2$ is equal to

$$\frac{\{\gamma(\rho-w_{1,2}\hat{h}-2\gamma'\hat{b}_o)+\sqrt{\gamma^2(\rho-w_{1,2}\hat{h}-2\gamma'\hat{b}_o)^2-4\gamma\gamma'(\gamma'\hat{b}_o+\tau')(\omega\hat{h}-\rho-\tau+\gamma\hat{b}_o)}\}}{2\alpha\gamma\gamma'} \qquad \text{(B-8a)}$$

and the other equals

$$\frac{\{\gamma(\rho-w_{1,2}\hat{h}-2\gamma'\hat{b}_0)-\sqrt{\gamma^2(\rho-w_{1,2}\hat{h}-2\gamma'\hat{b}_0)^2-4\gamma\gamma'(\gamma'\hat{b}_0+\tau')(w\hat{h}-\rho-\tau+\gamma\hat{b}_0)}\}}{2\alpha\gamma\gamma'} \qquad \text{(B-8b)}$$

In this case,

$$\hat{b}_{1,2} = (w\hat{h}-\rho-\tau+\gamma\hat{b}_0) \;/\; \alpha\gamma' \qquad \text{(B-9)}$$

and (equation B-1a)

$$\hat{b}_0 = \tau(\rho-w_{1,2}\hat{h}) \;/\; \{\alpha\gamma'(\rho-\hat{h})+\gamma(\rho-w_{1,2}\hat{h})+2\gamma'(w\hat{h}-\rho)\} \qquad \text{(B-10)}$$

The asymmetrical equilibria (B-8) exist only if both

$$w_{1,2}\hat{h} < \rho - 2\gamma' b_0$$

and $w\hat{h} > \rho + \tau - \gamma\hat{b}_0$

As long as the presence of the second plasmid does not diminish the capacity of the bacterium to donate any plasmid ($\gamma' > \frac{1}{2}\gamma$), this implies that these asymmetric equilibria can only occur if the growth rate of bacteria bearing both plasmids is (considerably) less than the growth rate of bacteria with only one plasmid ($w_{1,2} \ll w$).

APPENDIX C

THE FATE OF A MUTANT P_{2m} OF P_2

Can a mutant P_{2m} of P_2 invade if the plasmid P_2 and its mutant are mutually exclusive, and the mutant only differs from the original in the recipient competence of its host (α^{2m} instead of α^2) and in the relative growth rate of its host (w_{2m} instead of w_2; $w_{1,2m}$ instead of $w_{1,2}$)? The dynamics of bacteria carrying only P_{2m} (concentration b_{2m}) and of bacteria carrying both P_1 and P_{2m} (concentration $b_{1,2m}$) in the neighbourhood of the equilibrium with P_1 and P_2 but without P_{2m} are

$$\frac{db_{2m}}{dt} = (w_{2m}h(\bar{b})-\rho-\tau_2-\alpha^{2m}(\gamma_1 b_1+\gamma_1' b_{1,2}+\gamma_1' b_{1,2m}))b_{2m}$$

$$+ b_0(\gamma_2 b_{2m}+\gamma_2' b_{1,2m}) + \tau_1' b_{1,2m}$$

$$\approx \{w_{2m}\hat{h}-\rho-\tau_2+\gamma_2\hat{b}_0-\alpha^{2m}(\gamma_1\hat{b}_1+\gamma_1'\hat{b}_{1,2})\}b_{2m} + \{\gamma_2'\hat{b}_0+\tau_1'\}b_{1,2m}$$

$$\equiv A_{11}b_{2m} + A_{12}b_{1,2m} \qquad \text{(C-1a)}$$

$$\frac{db_{1,2m}}{dt} = (w_{1,2m}h(\bar{b})-\rho-\tau_1'-\tau_2'+\alpha^{2m}\gamma_1' b_{2m}+\alpha^1\gamma_2' b_1)b_{1,2m}$$

$$+ b_{2m}(\alpha^{2m}(\gamma_1 b_1+\gamma_1' b_{1,2})+\alpha^1\gamma_2 b_1) \approx \{\alpha^{2m}(\gamma_1\hat{b}_1+\gamma_1'\hat{b}_{1,2})+\alpha^1\gamma_2\hat{b}_1\}b_{2m}$$

$$+ \{w_{1,2m}\hat{h}-\rho-(\tau_1'+\tau_2')+\alpha^1\gamma_2'\hat{b}_1\}b_{1,2m} \equiv A_{21}b_{2m} + A_{22}b_{1,2m}$$

(C-1b)

The mutant P_{2m} can invade if the matrix

$$\begin{pmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{pmatrix} \qquad \text{(C-2)}$$

has at least one eigenvalue with positive real part. At the equilibrium with P_1 and P_2

$$A_{11} = (w_{2m}-w_2)\hat{h} + (\alpha^2-\alpha^{2m})(\gamma_1\hat{b}_1+\gamma_1'\hat{b}_{1,2}) - (\gamma_2'\hat{b}_0+\tau_1')\hat{b}_{1,2}/\hat{b}_1 \qquad \text{(C-3a)}$$

and

$$A_{22} = (w_{1,2m}-w_{1,2})\hat{h} - \{\alpha^2\gamma_1'\hat{b}_{1,2}+(\alpha^2\gamma_1+\alpha^1\gamma_2)\hat{b}_1\}\hat{b}_2 / \hat{b}_{1,2} \qquad \text{(C-3b)}$$

Both A_{12} and A_{21} are positive, therefore a necessary and sufficient condition for P_{2m} to be able to invade is that either $A_{11} > 0$ and $A_{22} > 0$ or $A_{11}A_{22} - A_{12}A_{21} < 0$. A_{22} can only be positive if $w_{1,2m}$ is considerably larger than $w_{1,2}$, therefore, the interesting condition for the effect of surface exclusion on the ability to invade is $A_{11}A_{22} - A_{12}A_{21} < < 0$

$$A_{11}A_{22} - A_{12}A_{21} = (w_{2m}-w_2)(w_{1,2m}-w_{1,2})\hat{h}^2 -$$

$$- (w_{2m}-w_2)\hat{h}.\{\alpha^2\gamma_1'\hat{b}_{1,2}+(\alpha^2\gamma_1+\alpha^1\gamma_2)\hat{b}_1\}\hat{b}_2 / \hat{b}_{1,2} -$$

$$- (w_{1,2m}-w_{1,2})\hat{h}\{\gamma_2'\hat{b}_0+\tau_1'\}\hat{b}_{1,2} / \hat{b}_2 +$$

$$+ (\alpha^2-\alpha^{2m}).(\gamma_1\hat{b}_1+\gamma_1'\hat{b}_{1,2}).\ (w_{1,2m}-w_{1,2})\hat{h}+\gamma_2'\hat{b}_0+\tau_1'-$$
$$[\alpha^2\gamma_1'\hat{b}_{1,2}+(\alpha^2\gamma_1+\alpha^1\gamma_2)\hat{b}_1]\hat{b}_2/\hat{b}_{1,2} \qquad \text{(C-4)}$$

If the mutant P_{2m} has the same effect on the growth rate of the bacterial host as P_2 ($w_2=w_{2m}$; $w_{1,2}=w_{1,2m}$), it depends on the sign of

$$\gamma_2'\hat{b}_0 + \tau_1' - [\alpha^2\gamma_1'\hat{b}_{1,2}+(\alpha^2\gamma_1+\alpha^1\gamma_2)\hat{b}_1]\hat{b}_2 \,/\, \hat{b}_{1,2} \equiv D \qquad \text{(C-5)}$$

whether the mutant can invade when it has stronger surface exclusion ($\alpha^{2m}<\alpha^2$) or weaker surface exclusion ($\alpha^{2m}>\alpha^2$). If $D > 0$ the mutant can invade if $\alpha^{2m} > \alpha^2$, whereas if $D < 0$ the mutant can invade if $\alpha^{2m} < \alpha^2$. Some elaborate calculations show that

$$D < 0 \iff w_{1,2} < w_2\gamma_2'/\gamma_2 + \rho(1-\gamma_2'/\gamma_2) \,/\, \hat{h} + (\tau_2'-\tau_2)\gamma_2' \,/\, (\gamma_2\hat{h}) \qquad \text{(C-6)}$$

If neither the transfer rate nor the rate of loss of the second plasmid P_2 is influenced by the presence of the first plasmid P_1 in its host ($\gamma_2=\gamma_2'$; $\tau_2=\tau_2'$), a mutant of P_2 with a higher degree of surface exclusion than P_2, but with the same effect on the bacterial growth rate as P_2, can invade if $w_{1,2} < w_2$, in other words if P_1 has a negative effect on the growth rate of bacteria already carrying P_2.

CHAPTER 7:

SOME GENERAL CONSIDERATIONS

The results of the analyses reported in this thesis are discussed in the previous chapters. In this chapter some of the links between the different chapters will be discussed while also some still unanswered questions concerning selection on plasmids will be formulated.

COEXISTENCE OF INCOMPATIBLE PLASMIDS WITH INCOMPLETE SURFACE EXCLUSION

In Chapters 2 and 3 it is shown that two plasmid types exhibiting complete surface exclusion can only coexist if one plasmid type has a higher transfer rate and the other is more beneficial or less harmful to its bacterial bearer. However, surface exclusion is never absolute. What will happen if two incompatible plasmids only partly exclude each other?

In Chapter 6 it was shown that two or three compatible plasmids can coexist, if exclusion is ineffective. Two compatible plasmids can even coexist if one of them has a lower transfer rate and its host has a lower growth rate, or, in other words, if one of the plasmids is less fit than the other. Can two incompatible plasmids, which exclude each other incompletely, coexist if one of them is less fit?

As long as two incompatible plasmids exclude each other equally well and have equal transfer rates their ratio will not change by infecting once in a while bacteria already bearing a plasmid. This holds only if, as supposed in Chapter 4, the transfer rate of a plasmid from a donor is proportional to the number of copies of that plasmid in the donor. It should therefore be expected that for two incompatible plasmids with equal exclusion and transfer rates the one whose host possesses the highest growth rate will be able to expel the other. On the other hand, if the growth rates are equal, the plasmid with the highest transfer rate will sur-

vive. Some preliminary calculations show that this is indeed the case. Two incompatible plasmids with an equal degree of surface exclusion appear only to be able to coexist if one has a higher transfer rate and if the bacterial host of the other has a higher growth rate. Of course, this conclusion will no longer hold if bacteria bearing both plasmids have a higher growth rate than bacteria carrying only one. Three incompatible plasmids probably can not coexist if they exhibit an equal degree of surface exclusion.

In Chapter 4 it was demonstrated that a high degree of exclusion against incompatible plasmids may compensate for a lower growth rate of the plasmid's host. Sometimes two incompatible plasmids with equal transfer rates can coexist if one of them has a higher degree of exclusion whereas the growth rate of the bacterial host of the other is higher. Therefore, there are two cases in which two incompatible plasmids can coexist. The first case is when both plasmids only differ in their transfer rate and in the growth rate of their bacterial host (Chapters 2 and 3). In the second case both plasmids only differ in their degree of surface exclusion and also in the growth rate of their bacterial host (Chapter 4). What will happen if plasmids differ in all these three properties (transfer rate, surface exclusion, bacterial growth rate)? Can three incompatible plasmids coexist in that case?

WHY ARE THE EXCLUSION GENES LOCATED ON THE TRANSFER GENE COMPLEX?

In Chapter 4 it is shown that surface exclusion against incompatible plasmids will be advantageous for low copy number plasmids with a high transfer rate. It appears that the genes, responsible for exclusion are often situated in the gene complex encoding for conjugative transfer (Alfaro & Willetts, 1972; Achtman, Kennedy & Skurray, 1977; Barth, 1979). The expression of the transfer (*tra*) gene complex of many conjugative plasmids can be repressed (Willetts & Skurray, 1980). In that case the exclusion genes will also be repressed, and other, incompatible plasmids may enter. If

an incompatible plasmid enters such a bacterium, its transfer genes will immediately be repressed by the repression products of the plasmid already present. Both the resident plasmid and the invading plasmid will therefore have a low (re)transfer rate. In Chapter 5 it is demonstrated that under some growth conditions, regulation of transfer rate can be advantageous. In that case, the transfer from most of the plasmid bearing bacteria will be repressed, and the overall transfer rate will be low. Since exclusion is not very advantageous if the transfer rate is low, it might be advantageous to repress exclusion together with transfer. Close linkage between both genes would then facilitate regulation of both characters. Whether selection will really favour such a close linkage between transfer and exclusion genes is, however, still an open question.

THE MAP LOCATION OF THE TRANSFER GENES

When plasmid transfer is interrupted, only part of the plasmid DNA reaches the recipient cell. Only those genes, which have been transferred already, can become expressed in the recipient cell. The order of transfer is therefore of great importance for a plasmid. It appears that most conjugative plasmids transfer their replication genes first (Guyer & Clark, 1977; Al-Doori, Watson & Scaife, 1982) and their transfer genes only at last (Walker & Pittard, 1972; Guyer & Clark, 1977; Al-Doori, Watson & Scaife, 1982; Guiney & Yakobson, 1983). Between the replication region and the transfer region several genes of less importance for the maintenance of the plasmid may be situated (Barth, 1979; Willetts & Skurray, 1980). Sometimes these genes are situated inside the transfer gene complex, dividing it into two separate regions (Barth, Richards & Datta, 1978). Can this order be determined by selection or is it just an arbitrary one? It seems reasonable that the replication genes, being the most essential genes for the maintenance of a plasmid, are transferred first. But then it can be asked why the transfer genes are not transferred immediately afterwards?

GENES SITUATED ON PLASMIDS

On plasmids several genes can be located. Some of these genes are necessary for the maintenance of the plasmid, such as replication and partitioning genes. Other genes encode for special plasmid properties, such as transfer and surface exclusion. Many plasmids also possess genes which may influence the fitness of the bacterial host, but do not affect the plasmid dynamics in any other way. Examples of such genes are genes encoding for resistance to antibiotics, and for resistance against heavy metals (Foster, 1983). These genes could probably function equally well when they had been situated on the bacterial chromosome. Many of these genes are situated on transposable elements, enabling them to switch from the plasmid genome to the bacterial chromosome and vice versa (Cohen, 1976; Campbell, 1981). Which specific properties of these genes determine their frequent location on plasmids? It is remarkable that many of these plasmid borne genes encoding, for instance, for resistance to antibiotics, are only once in a while favourable for the bacterial host. Several investigators have argued that this is not accidental since plasmids enable bacteria to acquire their genes quickly when needed, and lose them easily afterwards (Clowes, 1972; Koch, 1981). However, this argument is questionable, since it implies that a few bacteria should sacrifice themselves and keep plasmids under unfavourable circumstances, in order to let other bacteria profit from their altruism in periods when the plasmid genes are needed. It can also be imagined that all kinds of bacterial genes have a nearly equal probability of being transposed to a plasmid and vice versa. Plasmids, adding a favourable property to their bacterial carriers, will tend to predominate in the plasmid population. Genes which had been already necessary for a long time, may have entered the bacterium on a plasmid. However, after some time, the gene will have been transposed to the bacterial chromosome, whereafter the plasmid can be dispensed with. Therefore, the genes discovered on plasmids will be mainly genes which have become useful for the bacterium only

recently or genes which are only once in a while favourable. On the other hand, the discovery of genes, occurring on many plasmids, that are only occasionally useful could be an artifact resulting from a bias of investigators to search for antibiotic resistance genes, which are easily detected and have medical importance. The question, therefore, whether individual selection on plasmids and bacteria can explain the presence of genes on plasmids that are only once in a while useful for the bacteria cannot yet be answered conclusively.

THE SPREAD OF GROWTH RATE REDUCING PLASMIDS IN NATURE

Stewart & Levin (1977) have calculated under which circumstances a growth rate reducing plasmid can invade a bacterial population. They have analysed the most simple cases of only one plasmid with a constant transfer rate. They concluded that there exists a broad range of parameter values for which conjugative plasmids can become established and for which plasmid-bearing bacteria will maintain high frequencies, even when these factors considerably reduce the fitness of their host cells. This implies that antibiotic resistance factors may be maintained on plasmids in the bacterial population, even when no antibiotics are used. This means that even a severe reduction in the use of antibiotics will not necessarily lead to a lower frequency of antibiotic resistance. Attempts to estimate the transfer rate in natural bacterial populations indicate, however, that this rate may be even too low to satisfy the broad conditions for plasmid maintenance (Anderson, 1975; Caugant, Levin & Selander, 1981). This may be due to the fact that most plasmid-bearing bacteria in nature did acquire that plasmid many generations ago, and are therefore transfer-repressed. However, in Chapter 5 it is shown that transfer regulating plasmids can, under some growth conditions, become established and be maintained at an even broader range of parameter values than non-regulating plasmids. In Chapter 6 it is demonstrated that in a bacterial population, already containing several plasmids, a new compatible plasmid can invade more easily. Therefore,

the maintenance of plasmids, which are undesirable from an human point of view because they confer antibiotic resistance may be even more easy than the models of Stewart & Levin (1977) indicate.

SUMMARY AND GENERAL CONCLUSIONS

In this thesis several mathematical models are formulated to analyse the population dynamics of plasmids. Furthermore it is investigated how selection affects the characteristics of the population dynamics of plasmids. In the Introduction (*Chapter 1*) a survey is given of the principal properties of plasmids. In the Chapters 2, 3, 4 and 5 the fate of a mutant plasmid in a plasmid population is investigated. A mutant will in most cases be incompatible to the corresponding wild-type plasmid, because a plasmid and its mutant mostly use the same mechanism to regulate their replication and partitioning.

First the question is answered whether one, two or three incompatible plasmids, which exclude each other completely, can coexist. In *Chapter 2* this is done for plasmids in a bacterial population kept in a chemostat and in *Chapter 3* for the case that the bacterial population is periodically transferred to fresh medium. It appears that under both growth conditions two plasmids may be able to coexist if one of them has a higher transfer rate, whereas bacteria bearing the other plasmid possess a higher growth rate. In a chemostat the concentrations of both plasmids will converge to stable equilibria concentrations. In a periodically transferred bacterial population the frequency of plasmids may oscillate, both when one plasmid type is present and when two plasmid types are competing. Three plasmids are able to coexist under neither growth conditions.

Occasionally plasmid mutants with a different transfer rate will arise. It is assumed that as the transfer rate of the plasmid mutant is the higher, the more negative the effect of the plasmid on the growth rate of its bearer will be. In that case selection will ultimately lead to the establishment of a plasmid with an optimal transfer rate, or to a situation in which two plasmids, one with a high transfer rate and the other non-conjugative, will coexist. The first situation will occur if the relation between the transfer rate and the bacterial growth rate is convex, and the second

if this relation is concave (see fig. 2, p. 30).

Plasmids often exclude other incompatible plasmids from their hosts by changing some of the bacterial membrane properties. This change has probably a negative effect on the bacterium. Since survival and growth of the bacterial host are of great importance for the survival and spread of the plasmid, a property disadvantageous for the bacterial host tends also to be detrimental to the plasmid. So it can be asked why plasmids exclude other incompatible plasmids. This question is dealt with in *Chapter 4*. It appears that exclusion is advantageous for a plasmid if its transfer rate is high and its copy number low. For plasmids with a high copy number exclusion does not seem to be profitable since the entering plasmid will probably disappear out of the majority of the descendants of the invaded bacterium by incompatibility segregation.

Many plasmids regulate their ability to induce transfer. Plasmids have only an efficient transfer in newly infected hosts. After several generations in a bacterial cell line, the ability to transfer becomes repressed. In *Chapter 5* the dynamics of transfer regulation is modelled, both in a chemostat and in a bacterial population with serial transfer. It appears that competition between a transfer regulating plasmid and its mutants in a chemostat will lead to a situation of neutrality for a non-regulating plasmid, provided that the ability to regulate transfer has no costs. In the case where bacteria, bearing non-regulating plasmids, have a slightly higher growth rate than bacteria bearing derepressed regulating plasmids (i.e. if regulation has some costs), the non-regulating plasmid will eventually win. In a serially transferred bacterial population optimal regulation dynamics exist. A regulating plasmid with optimal regulation is able to compete successfully with a non-regulating plasmid, even if regulation has some costs. How great the costs of regulation may become without regulation becoming disadvantageous, depends on several factors. One of these is the extent of the environmental differences the plasmid has to cope with during its stay at each growth site. More particularly, how

much the bacterial concentration differs between the start and the end of each growth period. A greater difference leads to a higher advantage for transfer regulation.

The dynamics of competition between compatible plasmids differs from those of incompatible plasmids. Compatible plasmids often do not exclude each other and from the moment they are combined in a bacterium they stay together in that bacterial cell line. In *Chapter 6* the competition between compatible plasmids is analysed. It appears that it is possible for at least three compatible plasmids to coexist; this probably also holds for higher numbers. Two (or three) plasmids can coexist, although one of them is superior to the other, i.e. when it has a higher transfer rate and its bacterial host has a higher growth rate. Sometimes it depends on the initial plasmid concentrations which equilibrium concentration will be reached. When a bacterial population carrying a plasmid is invaded by a second compatible plasmid, which is slightly superior to the resident plasmid, it may occur that the less fit plasmid, which was present first, remains predominant. The competition between two (or three) compatible plasmids can be considered as an example of the possibility of stable coexistence of two (or three) species in the same niche.

In *Chapter 7* some unanswered questions about plasmid dynamics are discussed:

- How are the population dynamics of competing incompatible plasmids affected by incomplete surface exclusion?
- Is the structure of the plasmid genome arbitrary or influenced by selection?
- Why do plasmids carry so often genes coding for properties which are only once in a while favourable for bacteria?
- How are the (theoretical) conditions for plasmid spread in nature affected by the ability of a plasmid to regulate its transfer rate?

SAMENVATTING EN ALGEMENE CONCLUSIES

In dit proefschrift zijn verscheidene mathematische modellen opgesteld om de populatiedynamica van plasmiden te analyseren. Tevens is onderzocht wat het effect van selectie is op de populatiedynamische eigenschappen van plasmiden. In de Inleiding (*Hoofdstuk 1*) wordt een overzicht gegeven van de voornaamste kenmerken van plasmiden. In de Hoofdstukken 2, 3, 4 en 5 is onderzocht wat het lot is van een gemuteerd plasmide in een plasmidepopulatie. Zo'n mutant zal doorgaans incompatibel zijn met het oorspronkelijke plasmide, aangezien een plasmide en zijn mutant meestal over dezelfde mechanismen beschikken voor het reguleren van hun replicatie en segregatie.

In de eerste plaats wordt de vraag beantwoord hoeveel incompatibele plasmiden samen voor kunnen komen in een bacteriepopulatie, indien ieder van deze plasmiden verhindert dat een van de andere in hun gastheer binnendringt. In *Hoofdstuk 2* is dit onderzocht voor plasmiden in een bacteriepopulatie die zich in een chemostaat bevindt en in *Hoofdstuk 3* voor het geval dat eens in de zoveel tijd een (random) fractie van de gastheren wordt overgeënt naar een nieuw voedingsmedium. In beide gevallen blijken twee plasmiden samen voor te kunnen komen. Dit is alleen mogelijk indien één van de twee een hogere (infectieuze) transfer snelheid heeft, terwijl de groeisnelheid van de drager van het andere plasmide hoger is. De concentraties van beide plasmiden zullen in een chemostaat naar stabiele evenwichtconcentraties convergeren. Bij periodieke overenting kunnen de frequenties van de plasmiden ook gaan oscilleren. Dit kan optreden zowel indien er maar één plasmide aanwezig is, als in het geval waarin twee plasmiden met elkaar concurreren. Noch in een chemostaat, noch bij periodieke overenting kunnen drie plasmiden samen voorkomen. Zo nu en dan zullen er mutanten van plasmiden ontstaan met een andere transfersnelheid. Indien wordt verondersteld dat een toename in de transfersnelheid gepaard gaat met een verlaging van de groeisnelheid van de bacteriële gastheer, kan selectie tot twee verschillende eindtoestanden leiden. Er kan een toestand ontstaan waarin maar één plasmide met opti-

male transfersnelheid overblijft. Anderzijds kan selectie ook leiden tot het samen voorkomen van twee plasmiden, één met een maximale transfersnelheid, en de andere met een minimale. De eerste situatie zal ontstaan als de relatie tussen de transfersnelheid en de bacteriële groeisnelheid convex is, en de tweede als die relatie concaaf is (zie fig. 2, blz. 30).

Plasmiden voorkomen vaak dat andere, incompatibele plasmiden in hun gastheer binnendringen. Hiertoe veranderen zij enige membraaneigenschappen van hun gastheer. Zo'n verandering zal vermoedelijk ongunstig zijn voor de bacterie. Immers, indien deze verandering gunstig zou zijn voor de bacterie, dan zou een bacteriemutant, die zelf voor deze verandering codeert, spoedig de oorspronkelijke bacteriepopulatie verdringen. Aangezien het voor de verspreiding van plasmiden van groot belang is dat hun bacteriële gastheren overleven en groeien, zal een eigenschap die nadelig is voor de gastheer ook ongunstig zijn voor het plasmide. In *Hoofdstuk 4* wordt onderzocht waarom plasmiden desalniettemin verhinderen dat andere, incompatibele, plasmiden hun gastheer binnen gaan. Het blijkt dat deze buitensluiting voordelig is voor plasmiden met een hoge transfersnelheid en een laag aantal kopieën per gastheercel. Buitensluiting is niet erg voordelig voor plasmiden met een groot aantal kopieën per cel. In dat geval immers zal een plasmide dat net is binnengedrongen in een bacterie, daar sterk in de minderheid zijn. Daarom zal het binnengedrongen plasmide, als gevolg van incompatibiliteits-segregatie, in het merendeel van de nakomelingen van die bacterie afwezig zijn.

Veel plasmiden reguleren het vermogen om hun eigen transfer te bewerkstelligen. De transfersnelheid uit pas geïnfecteerde bacteriën is hoog. Na enkele generaties in een bacteriële cellijn wordt het vermogen om transfer te induceren onderdrukt. In *Hoofdstuk 5* is een model opgesteld dat de populatiedynamica beschrijft van een plasmide dat zijn transfersnelheid reguleert. Dit is zowel gedaan voor het geval dat de bacteriële gastheerpopulatie in een chemostaat groeit, als voor het geval waarin deze periodiek wordt overgeënt. Wanneer een plasmide, dat zijn transfersnelheid reguleert,

moet concurreren met mutanten, die een andere regulatiedynamica hebben, dan zal in een chemostaat een toestand ontstaan, waarin een niet-regulerend plasmide selectief neutraal is. Dit alles indien het vermogen om de transfersnelheid te reguleren geen extra kosten in de vorm van een verlaging van de fitness van de gastheer met zich meebrengt. Als bacteriën met een niet-regulerend plasmide een iets hogere groeisnelheid hebben dan bacteriën met een regulerend plasmide met dezelfde transfersnelheid (dus als regulatie kosten met zich meebrengt), dan zal het niet-regulerende plasmide uiteindelijk "winnen". In een periodiek overgeënte bacteriepopulatie blijkt er een optimale regulatiedynamica voor het plasmide te bestaan. Een regulerend plasmide met optimale regulatie kan succesvol concurreren met een niet-regulerend plasmide. Dit is zelfs mogelijk als het vermogen de transfersnelheid te reguleren enige kosten met zich meebrengt. Hoe groot die kosten moeten worden, wil niet-reguleren voordeliger zijn, hangt af van verscheidene factoren. Een daarvan is hoe variabel het milieu van de plasmiden is gedurende iedere groeiperiode. Als de totale bacterieconcentratie aan het begin en het einde van de groeiperiode zeer verschillend is, hebben de plasmiden te maken met een sterk variërend milieu. Naarmate het verschil groter wordt, wordt regulatie van de transfersnelheid voordeliger.

De dynamica van de competitie tussen compatibele typen plasmiden verschilt van die tussen incompatibele plasmiden. Het binnendringen van een plasmide in een bacterie wordt vaak niet gehinderd door de aanwezigheid van een ander compatibel plasmide in die bacterie. Als twee compatibele plasmiden eenmaal samen voorkomen in een bacterie, blijven zij ook samen aanwezig in de nakomelingen van die bacterie. In *Hoofdstuk 6* wordt de competitie tussen compatibele plasmiden onderzocht. Het blijkt mogelijk te zijn dat tenminste drie compatibele plasmiden samen voorkomen in één bacteriepopulatie. Waarschijnlijk kunnen ook meer dan drie compatibele plasmiden samen voorkomen. Twee (of drie) compatibele plasmiden kunnen ook samen voorkomen als één superieur is aan de andere, dus de hoogste transfersnelheid heeft, terwijl de groeisnelheid

van zijn drager eveneens hoger is. Soms bestaan er meerdere verschillende stabiele evenwichtconcentraties van de bacterien. Welke van die evenwichten wordt bereikt, hangt af van de beginconcentraties. Als er reeds een plasmide aanwezig is in een bacteriepopulatie, kan het voorkomen dat een tweede plasmide, dat in de bacteriepopulatie binnendringt, in de minderheid blijft, zelfs als het tweede plasmide superieur is aan het eerste. Competitie tussen twee (of drie)compatibele plasmiden kan worden opgevat als een voorbeeld van stabiele coexistentie van twee (of drie) soorten in dezelfde niche.

In *Hoofdstuk 7* worden enkele nog te beantwoorden vragen betreffende de dynamica van plasmiden uitgewerkt:

- Wat is het effect van het verschijnsel, dat incompatibele plasmiden elkaar niet volledig uit hun gastheer buiten kunnen sluiten, op hun populatiedynamica?
- Is de structuur van het plasmidegenoom willekeurig, of is die structuur ontstaan onder invloed van selectie?
- Waarom bevatten plasmiden zo vaak genen, die coderen voor eigenschappen die slechts zo nu en dan nuttig zijn voor een bacterie?
- Welke zijn de (theoretische) voorwaarden voor de verspreiding van plasmiden in natuurlijke bacteriepopulaties, gezien het vermogen van het plasmide om de transfersnelheid te reguleren?

REFERENCES

Achtman, M., N. Kennedy & R. Skurray, 1977. Cell-Cell interactions in conjugating Escherichia coli: Role of *traT* protein in surface exclusion. Proc.Natl.Acad.Sci.USA 74: 5104-5108

Achtman, M., N.S. Willetts & A.J. Clark, 1971. Beginning a Genetic analysis of Conjugational Transfer Determined by the F-factor in E. coli by Isolation and Characterization of Transfer Deficient Mutants. J.Bacteriol. 106: 529-538

Al-Doori, Z., M. Watson & J. Scaife, 1982. The Orientation of Transfer of the Plasmid RP4. Genet.Res. 39: 99-103

Alfaro, G. & N. Willetts, 1972. The Relationship between the Transfer Systems of some Bacterial Plasmids. Genet.Res. 20: 279-289

Anderson, E.S., 1975. Viability of, and Transfer of a Plasmid from, E. coli K12 in the Human Intestine. Nature 255: 502-504

Austin, S. & A. Abeles, 1983a. Partition of Unit-Copy Miniplasmids to Daughter cells. I. P1 and F miniplasmids contain discrete, interchangeable sequences sufficient to promote equipartitioning. J.Mol. Biol. 169: 353-372

Austin, S. & A. Abeles, 1983b. Partition of Unit-Copy Miniplasmids to Daughter cells. II. The Partition Region of Miniplasmid P1 encodes an essential Protein and a Centromere-like Site at which it acts. J.Mol.Biol. 169: 373-387

Barth, P., 1979. RP4 and R300B as wide Host-range Plasmid cloning Vehicles. In: Plasmids of Medical, Environmental and Commercial Importance, eds. K.N. Timmis & A. Pühler; North-Holland. p 399-410

Barth, P. & N. Datta, 1977. Two Naturally Occurring Transposons Indistinguishable from Tn7. J.Gen.Microbiol. 102: 129-134

Barth, P., N.J. Grinter & D.E. Bradley, 1978. Conjugal Transfer System of Plasmid RP4: Analysis by transposon 7 insertion. J.Bacteriol. 133: 43-52

Barth, P.T., H. Richards & N. Datta, 1978. Copy numbers of Coexisting Plasmids in Escherichia coli K-12. J.Bacteriol. 135: 760-765

Bennett, P.M. & M.H. Richmond, 1978. Plasmids and their possible influence on Bacterial Evolution. In: The bacteria, a treatise on structure and function, Vol. VI, eds. L.N. Ornston & J.R. Sokatch; Acad.Press, New York, p 1-69

Benveniste, R. & J. Davies, 1973a. Mechanisms of Antibiotic Resistance in Bacteria. Ann.Rev.Biochem. 43: 471-506

Benveniste, R. & J. Davies, 1973b. Aminoglycoside Antibiotic-Inactivating Enzymes in Actinomycetes Similar to Those Present in Clinical Isolates of Antibiotic-Resistant Bacteria. Proc.Natl.Acad.Sci.USA 70: 2276-2280

Boorman, S.A. & Levitt, P.R., 1973. Group Selection on the Boundary of a Stable Population. Theor.Popul.Biol. 4: 85-128

Bradley, D.E., 1976. Adsorption of the R-specific Bacteriophage PR4 to Pili determined by a Drug Resistance Plasmid of the W Compatibility group. J.Gen.Micribiol. 95: 181-185

Bradley, D.E., 1980a. Morphological and Serological Relationships of Conjugative Pili. Plasmid 4: 155-169

Bradley, D.E., 1980b. Determination of Pili by Conjugative Bacterial Drug Resistance Plasmids of Incompatibility Groups B, C, H, J, K, M, V and X. J.Bacteriol. 141: 828-837

Bradley, D.E., 1981. Conjugative Pili of Plasmids in Escherichia coli K-12 and Pseudomonas species. In: Molecular Biology, Pathogenicity, and Ecology of Bacterial Plasmids, eds. S.B. Levy, R.C. Clowes & E.L. Koenig; Plenum Press. p 217-226

Brinton, C.C., P. Gemski & J. Carnahan, 1964. A new type of Bacterial Pilus Genetically controlled by the Fertility Factor of E. coli K12 and its role in Chromosome Transfer. Proc.Natl.Acad.Sci.USA 52: 776-783

Broda, P., 1979a. Molecular Relationships between Plasmids. In: Plasmids of Medical, Environmental and Commercial Importance, eds. K.N. Timmis & A. Pühler; North-Holland. p 23-27

Broda, P., 1979b. Plasmids. Freeman; Oxford.

Broda, P., 1979c. Conjugation in Bacteria. In: Plasmids of Medical, Environmental and Commercial Importance, eds. K.N. Timmis & A. Pühler; North-Holland. p 47-54

Broda, P., P. Meacock & M. Achtman, 1972. Early Transfer of Genes determining Transfer functions of some H*fr* Strains in E. coli K12. Mol.Gen. Genet. 116: 336-347

Bukhari, A.I., J.A. Shapiro & S.L. Adhya, 1977. DNA Insertion Elements, Plasmids, and Episomes. Cold Spring Harbor Laboratory.

Campbell, A., 1981. Evolutionary significance of Accessory DNA Elements in Bacteria. Ann.Rev.Microbiol. 35: 55-83

Caro, L.G. & M. Schnös, 1966. The attachment of the Male-Specific Bacteriophage F1 to sensitive strains of Escherichia coli. Proc.Natl. Acad.Sci.USA 56: 126-132

Caugent, D.A., B.R. Levin & R.K. Selander, 1981. Genetic Diversity and Temporal Variation in the E. coli population of a Human Host. Genetics 89: 467-490

Chandler, P.M. & V. Krishnapillai, 1974. Phenotypic Properties of R factors of Pseudomonas aeruginosa: R factors readily transferable between Pseudomonas and the Enterobacteriaceae. Genet.Res. 23: 239-250

Christiansen, C., G. Christiansen, A. Lethbak & A. Stenderup, 1973. Extrachromosomal Deoxiribonucleic Acid in different Enterobacteria. J.Bacteriol. 114: 367-377

Clark, A.J. & G.J. Warren, 1979. Conjugal Transmission of Plasmids. Ann.Rev.Genet. 13: 99-125

Clowes, R.C., 1972. Molecular Structure of Bacterial Plasmids. Bacteriol. Rev. 36: 361-405

Cohen, S.N., 1976. Transposable Genetic Elements and Plasmid Evolution. Nature 263: 731-738

Collins, J.F. & P. Broda, 1975. Mobility, Diffusion and Cell Concentration affect Pair formation in Escherichia coli. Nature 258: 722-723

Cress, D.E. & B.C. Kline, 1976. Isolation and Characterization of Escherichia coli chromosomal mutants affecting Plasmid copy number. J. Bacteriol. 125: 635-642

Cullum, J. & P. Broda, 1979. Rate of Segregation due to plasmid incompatibility. Genet.Res. 33: 61-79

Cullum, J., J.F. Collins & P. Broda, 1978a. Factors Affecting the Kinetics of Progeny Formation with F'*lac* in Escherichia coli K12. Plasmid 1: 536-544

Cullum, J., J.F. Collins & P. Broda, 1978b. The spread of plasmids in model populations of Escherichia coli K12. Plasmid 1: 545-556

Datta, N., 1979. Plasmid Classification: Incompatibility Grouping. In: Plasmids of Medical, Environmental and Commercial Importance, eds. K.N. Timmis & A. Pühler; North-Holland. p 3-12

Datta, N. & R.W. Hedges, 1972. Host Range of R factors. J.Gen.Microbiol. 70: 453-460
Datta, N., V.M. Hughes, M.E. Nugent & H. Richards, 1979. Plasmids and Transposons and their Stability and Mutability in Bacteria isolated during an outbreak of Hospital Infection. Plasmid 2: 182-196
Eshel, I., 1972. On the Neighbor Effect and the Evolution of Altruistic Traits. Theor.Popul.Biol. 3: 258-277
Farrar, W.E.jr., 1981. Evolution among Antibiotic Resistance Plasmids in the Hospital Environment. In: Molecular Biology, Pathogenicity, and Ecology of Bacterial Plasmids, eds. S.B. Levy, R.C. Clowes & E.L. Koenig; Plenum Press. p 1-10
Feller, W., 1968. An Introduction to Probability Theory and Its Applications, Vol 1. Wiley & Sons.
Finger, J. & V. Krishnapillai, 1980. Host Range, Entry Exclusion, and Incompatibility of Pseudomonas aeruginosa FP plasmids. Plasmid 3: 332-342
Finnegan, D.J. & N.S. Willetts, 1971. Two Classes of F-lac Mutants Insensitive to Transfer Inhibition by an F-like R factor. Mol.Gen.Genet. 111: 256-264
Finnegan, D. & N.S. Willetts, 1972. The Nature of the Transfer Inhibition of Several F-like Plasmids. Mol.Gen.Genet. 119: 57-66
Finnegan, D. & N.S. Willetts, 1973. The Site of Action of the F transfer Inhibition. Mol.Gen.Genet. 127: 307-316
Foster, T.J., 1983. Plasmid-Determined Resistance to Antimicrobial Drugs and Toxic Metal Ions in Bacteria. Microbiol.Rev. 47: 361-409
Freter, R., R.R. Freter & H. Brickner, 1983. Experimental and Mathematical Models of Escherichia coli Plasmid Transfer in Vitro and in Vivo. Infect.Immun. 39: 60-84
Gause, G.F., 1934. The Struggle for Existence. Hafner, New York.
Gilbert, O., T.B. Reynoldsen & J. Hobart, 1952. Gause's Hypothesis; an Examination. J.Anim.Ecol. 21: 310-312
Godwin, D. & J.H. Slater, 1979. The Influence of the Growth Environment on the Stability of a Drug Resistance Plasmid in Escherichia coli K12. J.Gen.Microbiol. 111: 201-210
Goursot, R., A. Goze, B. Niadet & S.D. Ehrlich, 1982. Plasmids from Staphylococcus aureus replicate in Yeast Saccharomyces cerevisiae. Nature 298: 488-490
Grindley, N.D.F., J.N. Grindley, H.R. Smith & E.S. Anderson, 1973a. Characterization of Derepressed Mutants of an F-like R factor. Mol.Gen.Genet. 120: 27-34
Grindley, N.D.F., G.O. Humphreys & E.S. Anderson, 1973b. Molecular Studies of R Factor Compatibility Groups. J.Bacteriol. 115: 387-398
Guiney, D.G. & E. Yakobson, 1983. Location and Nucleotide Sequence of the Transfer Origin of the Broad Host Range Plasmid RK2. Proc.Natl. Acad.Sci.USA 80: 3595-3598
Gustafsson, P., H. Dreisig, S. Molin, K. Nordström & B.E. Uhlin, 1978. DNA Replication Control: Studies of Plasmid R1. Cold Spring Harbor Symp.Quant.Biol. 43: 419-425
Gustafsson, P. & K. Nordström, 1975. Random Replication of the Stringent Plasmid R1 in Escherichia coli K12. J.Bacteriol. 123: 443-448
Gustafsson, P. & K. Nordström, 1980. Control of Plasmid R1 Replication: Kinetics of Replication in Shifts Between Different Copy Number Levels. J.Bacteriol. 141: 106-110
Gustafsson, P., K. Nordström & J.W. Perran, 1978. Selection and Timing of Replication of Plasmids R1*drd*-19 and F'*lac* in Escherichia coli. Plasmid 1: 187-203

Guyer, M.S. & A.J. Clark, 1977. Early and late Transfer of F genes by Hfr donors of E. coli K12. Mol.Gen.Genet. 157: 215-222
Hartskeerl, R., J. Tommassen & W. Hoekstra, 1985. Relationship between the proteins encoded by the exclusion determining locus of the IncI plasmid R144 and the cellular localization of these proteins in Escherichia coli K12. Mol.Gen.Genet. 200: 138-144
Hedges, R.W. & N. Datta, 1973. Plasmids Determining I pili constitute a Compatibility Complex. J.Gen.Microbiol. 77: 19-25
Hedges, R.W., P. Smith & G. Brazil, 1985. Resistance Plasmids of Aeromonads. J.Gen.Microbiol. 131: 2091-2095
Heffron, F., R. Sublett, R.W. Hedges, A. Jacob & S. Falkow, 1975. Origin of the Tem Beta-Lactamase Gene found on Plasmids. J.Bacteriol. 122: 250-256
Helling, R.B., T. Kinney & J. Adams, 1981. The maintenance of Plasmid-Containing Organisms in Populations of Escherichia coli. J.Gen.Microbiol. 123: 129-141
Holloway, B.W., 1979. Plasmids that Mobilize Bacterial Chromosome. Plasmid 2: 1-19
Hooykaas, P.J.J., H. Den Dulk-Ras & R.A. Schilperoort, 1980. Molecular Mechanism of Ti Plasmid Mobilization by R Plasmids: Isolation of Ti Plasmids with Transposon-Insertions in Agrobacterium tumefaciens. Plasmid 4: 64-75
Hughes, V.M. & N. Datta, 1983. Conjugative Plasmids in Bacteria of the "pre-antibiotic" era. Nature 302: 725-726
Hutchinson, G.E., 1957. Concluding Remarks. Cold Spring Harbor Symp.Quant. Biol. 22: 415-427
Ishii, K., T. Hashimoto-Gotoh & K. Matsubara, 1978. Random Replication and Random Assortment Model for Plasmid Incompatibility in Bacteria. Plasmid 1: 435-445
Jones, D. & P.H.A. Sneath, 1970. Genetic Transfer and Bacterial Taxonomy. Bacteriol.Rev. 34: 40-81
Jørgensen, S.T. & V.W. Sørensen, 1979. Spread of an R plasmid among Antigen Types of Escherichia coli pathogenic in Piglets. Plasmid 2: 290-292
Kennedy, N., L. Beutin, M. Achtman, R. Skurray, U. Rahmsdorf & P. Herrlich, 1977. Conjugation Proteins encoded by the F Sex Factor. Nature 270: 580-585
Koch, A.L., 1981. Evolution of Antibiotic Resistance Gene Function. Microbiol.Rev. 45: 355-378
Kopecko, D.J., 1980. Involvement of Specialized Recombination in the Evolution and Expression of Bacterial Genomes. In: Plasmids and Transposons: Environmental Effects and Maintenance Mechanisms, eds. C. Stuttard & K.R. Rozee; Acad.Press, New York. p 165-205
Lederberg, J., L.L. Cavalli & E.M. Lederberg, 1952. Sex compatibility in Escherichia coli. Genetics 37: 720-730
Lee, S.C., D.N. Gerding & P.P. Cleary, 1984. Plasmid Macroevolution in a Nosocomial Environment: Demonstration of a Persistent Molecular Polymorphism and Construction of a Cladistic Phylogeny on the basis of Restriction Data. Mol.Gen.Genet. 194: 173-178
Levin, B.R. & F.M. Stewart, 1980. The population biology of Bacterial Plasmids: A priori conditions for the Existence of Mobilizable Nonconjugative factors. Genetics 80: 425-443
Levin, B.R., F.M. Stewart & V.A. Rice, 1979. The Kinetics of Conjugative Plasmid Transmission: Fit of a simple Mass Action Model. Plasmid 2: 247-260

Macrina, F.L., G.G. Weatherly & R. Curtiss III, 1974. R6K Plasmid Replication: Influence of Chromosomal Genotype in Minicell-Producing Strains of Escherichia coli K12. J.Bacteriol. 120: 1387-1400
Maynard Smith, J., 1982. Evolution and the Theory of Games. Cambridge University Press.
Miki, T., A.M. Easton & R.H. Rownd, 1980. Cloning of Replication, Incompatibility, and Stability functions of R-plasmid NR1. J.Bacteriol. 141: 87-99
Monod, J., 1949. The Growth of Bacterial Cultures. Ann.Rev.Microbiol. 3: 371-394
Nordström, K. & H. Aagaard-Hansen, 1984. Maintenance of Bacterial Plasmids: Comparison of Theoretical Calculations and Experiments with Plasmid R1 in Escherichia coli. Mol.Gen.Genet. 197: 1-7
Nordström, K., L.C. Ingram & A. Lundbäck, 1972. Mutations in R factors of Escherichia coli causing an increased number of R-factor copies per chromosome. J.Bacteriol. 110: 562-569
Nordström, K., S. Molin & H. Aagaard-Hansen, 1980. Partitioning of plasmid R1 in Escherichia coli. 1. Kinetics of loss of Plasmid derivates deleted of the *par* region. Plasmid 4: 215-227
Nordström, K., S. Molin & J. Light, 1984. Control of Replication of Bacterial Plasmids: Genetics, Molecular Biology, and Physiology of the Plasmid R1 System. Plasmid 12: 71-90
Novick, R.P., R.C. Clowes, S.N. Cohen, R. Curtiss III, N. Datta & S. Falkow, 1976. Uniform Nomenclature for Bacterial Plasmids: A Proposal. Bacteriol.Rev. 40: 168-189
Novick, R.P. & F.C. Hoppensteadt, 1978. On Plasmid Incompatibility. Plasmid 1: 421-434
Ogura, T. & S. Hiraga, 1983. Mini-F Plasmid Genes that Couple Host Cell Division to Plasmid Proliferation. Proc.Natl.Acad.Sci.USA 80: 4784-4788
Ohki, M. & J. Tomizawa, 1968. Asymmetric Transfer of DNA Strands in Bacterial Conjugation. Cold Spring Harbor Symp.Quant.Biol. 33: 651-658
Ou, J.T. & T.F. Anderson, 1970. Role of Pili in Bacterial Conjugation. J.Bacteriol. 102: 648-654
Ozeki, H., B.A.D. Stocker & S.M. Smith, 1962. Transmission of Colincinogeny between Strains of Salmonella typhimurium grown together. J.Gen.Microbiol. 28: 671-687
Polak, J. & R.P. Novick, 1982. Closely Related Plasmids from Staphylococcus aureus and Soil Bacilli. Plasmid 7: 152-162
Pritchard, R.H. & N.B. Grover, 1981. Control of Plasmid Replication and its Relationship to Incompatibility. In: Molecular Biology, Pathogenicity, and Ecology of Bacterial Plasmids, eds. S.B. Levy, R.C. Clowes & E.L. Koenig; Plenum Press. p 271-278
Projan, S.J., S. Carleton & R.P. Novick, 1983. Determination of Plasmid Copy Number by fluorescense Densiometry. Plasmid 9: 182-190
Reanney, D., 1976. Extrachromosomal Agents as possible Agents of Adaptation and Development. Bacteriol.Rev. 40: 552-590
Rescigno, A. & J.W. Richardson, 1965. On the competitive exclusion principle. Bull.Math.Biophys. 27: 85-89
Richards, H. & N. Datta, 1982. Plasmids and Transposons Acquired by Salmonella typhi in Man. Plasmid 8: 9-14
Roussel, A.F. & Y.A. Chabbert, 1978. Taxonomy and Epidemiology of Gram-negative Bacterial Plasmids Studied by DNA-DNA Filter Hybridization in Formamide. J.Gen.Microbiol. 104: 269-276

Rowbury, R.J., 1977. Bacterial Plasmids with Particular Reference to their Replication and Transfer Properties. Prog.Biophys.Mol.Biol. 31: 271-317
Scott, J.R., 1984. Regulation of Plasmid Replication. Microbiol.Rev. 48: 1-23
Shapiro, J.A., 1980. A model for the Genetic Activity of Transposable Elements involving DNA Replication. In: Plasmids and Transposons: Environmental Effects and Maintenance Mechanisms, eds. C. Stuttard & K.R. Rozee, Acad.Press, New York. p 229-244
Steinberg, D.A. & C.E. Helmstetter, 1981. F Plasmid Replication and the Division Cycle of Escherichia coli B/r. Plasmid 6: 342-353
Stewart, F.M. & B.R. Levin, 1977. The Population Biology of Bacterial Plasmids: A priori conditions for the Existence of Conjugationally Transmitted Factors. Genetics 87: 209-228
Stocker, B.A.D., S.M. Smith & H. Ozeki, 1963. High Infectivity of Salmonella typhimurium newly infected by the ColI factor. J.Gen.Microbiol. 30: 201-221.
Tietze, E., R. Prager & H. Tschäpe, 1982. Characterization of the Transposons Tn1822 (Tc) and Tn1824 (TpSm) and the light they throw on the Natural Spread of Resistance Genes. Plasmid 8: 253-260
Timmis, K.N., 1979. Mechanism of Plasmid Incompatibility. In: Plasmids of Medical, Environmental and Commercial Importance, eds. K.N. Timmis & A. Pühler, North-Holland. p 13-22
Timmis, K.N. & U. Winkler, 1973. Gene Dosage Studies with Pleiotropic Mutants of Serratio macrescens Superactive in the synthesis of Macrescin A and certain other exocellular proteins. Mol.Gen.Genet. 124: 207-217
Tomoeda, M., M. Inuzuka & T. Date, 1975. Bacterial Sex Pili. Prog.Biophys.Mol.Biol. 30: 23-56
Van der Hoeven, N., 1984. A mathematical model for the Co-existence of Incompatible, Conjugative Plasmids in Individual Bacteria of a Bacterial Population. J.Theor.Biol. 110: 411-423
Van der Hoeven, N., in press. Evolution of Bacterial Surface Exclusion against Incompatible Plasmids. J.Theor.Biol. (in press).
Van der Pol, H., E. Veltkamp & H.J.J. Nijkamp, 1978. Genetic Analysis of the Mobilization of the non-conjugative Plasmid ClоDF13. Mol.Gen. Genet. 160: 139-149
Vapnek, D., M.B. Lipman & W.D. Rupp, 1971. Physical Properties and Mechanism of Transfer of R factors in Escherichia coli. J.Bacteriol. 108: 508-514
Walker, E.M. & J. Pittard, 1972. Conjugation in E. coli: Failure to confirm the transfer of part of the Sex Factor at the leading end of the donor chromosome. J.Bacteriol. 110: 516-522
Warren, G.J., A.J. Twigg & D.J. Sherratt, 1978. ColE1 Plasmid Mobility and Relaxation Complex. Nature 274: 259-261
Warren, G.J., M.W. Saul & D.J. Sherratt, 1979. ColE1 Plasmid Mobility: Essential and Conditional Functions. Mol.Gen.Genet. 170: 103-107
Willetts, N. & J. Maule, 1974. Interaction between the Surface Exclusion Systems of some F-like Plasmids. Genet.Res. 24: 81-89
Willetts, N. & J. Maule, 1979. Investigation of the F conjugation gene *traI*: *traI* mutants and λ*traI* transducing phages. Mol.Gen.Genet. 169: 325-336
Willetts, N. & R. Skurray, 1980. The Conjugation System of F-like Plasmids. Ann.Rev.Genet. 14: 41-76
Willetts, N. & B. Wilkins, 1984. Processing of Plasmid DNA During Bacterial Conjugation. Microbiol.Rev. 48: 24-41

Winans, S.C. & G.C. Walker, 1985. Entry Exclusion Determinant(s) of IncN plasmid pKM101. J.Bacteriol. 161: 411-416

Zünd, P. & G. Lebek, 1980. Generation Time-Prolonging R Plasmids: Correlation between Increases in the Generation Time of Escherichia coli caused by R Plasmids and their Molecular Size. Plasmid 3: 65-69