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The kinetics of transfer of nonconjugative plasmids by mobilizing conjugative factors

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SUMMARY

A mathematical model for the kinetics of transfer of non-selftransmissible (nonconjugative) plasmids by mobilizing conjugative factors is presented and methods to estimate its parameters described. Using batch and chemostat cultures of *Escherichia coli* K-12 with the nonconjugative plasmid pCR1 and an F′ mobilizing factor, the parameters of this model were estimated. The observed changes in concentrations of the different parental and transconjugant cell types and the changes in these concentrations anticipated from the model are presented for two different 'mating' combinations in both exponentially growing and equilibrium chemostat populations of *E. coli*. The results of these experiments are interpreted to suggest that for bacterial populations dividing at a constant rate in liquid culture, the kinetics of mobilization transfer of nonconjugative plasmids can be reasonably well described by a simple set of mass action differential equations. These results also suggest that the carriage of the nonconjugative plasmid pCR1 has little, if any, effect on the capacity of a host bacterium to donate or receive conjugative *F′* plasmids.

1. INTRODUCTION

In the majority of higher organisms the exchange and reassortment of genes is a part of the reproductive process. Consequently, for the development of mathematical models of the genetics of populations of these 'true' sexually reproducing species, the principles of classical, transmission genetics can be directly applied at the population level (see for example, Crow & Kimura, 1970). In bacteria the transmission and exchange of genes between individuals of different clones is a contagious phenomenon which is not necessary for reproduction and population growth. For the development of mathematical models of the population genetics of bacteria that allow for interclone gene exchange and recombination, it is necessary to consider the population dynamics of the phage and plasmid vectors of gene transmission and/or the dynamics of the release and adsorption of free DNA for transformation.

Working in part towards this end of the development of a formal (mathematical) theory of the population genetics of infectious gene transmission in bacteria and in part to ascertain the conditions for the existence of extrachromosomal genetic

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elements in populations of bacteria, we have developed models of the population biology of conjugationally transmitted plasmids (Stewart & Levin, 1977; Levin & Stewart, 1977, 1980). To facilitate the construction and analysis of these models we assumed the transmission of plasmids to be a very simple process where 'mating' occurs by random union of donor and recipient cells and where the transmission of plasmids is instantaneous. These models of the population biology of plasmids neglected to consider the various known complexities and time delays associated with the processes of conjugation and the transfer of these extrachromosomal elements between cells (Curtiss *et al.* 1969; Meynell, 1973; Curtiss & Fenwick, 1975; Falkow, 1975; Achtman, 1975; Cullum, Collins & Broda, 1979). Nevertheless, for bacterial populations dividing at a constant rate in liquid culture the kinetics of the infectious transmission of conjugative plasmids can be reasonably well approximated by simple differential equation models analogous to those used in these population studies (Levin, Stewart & Rice, 1979).

In this report we consider the kinetics of infectious transmission of a second major class of bacterial plasmids, the non-selftransmissible (nonconjugative) (Novick *et al.* 1976) plasmids which can be transmitted through mobilization by conjugative factors (Smith, Ozeki & Stocker, 1963). We present a mathematical model for the kinetics of mobilization transfer and describe procedures to estimate its parameters. Using batch and chemostat populations of *E. coli* with a nonconjugative plasmid and a conjugative mobilizing factor we estimate the parameters of this model and compare the observed changes in 'parental' and transconjugant densities to those anticipated from the model. We also consider the effect carriage of the nonconjugative plasmid has on the ability of the host bacterium to transmit or receive the conjugative factor, and the effect carriage of the conjugative plasmid has on the recipient ability of its host.

2. A MODEL FOR THE MOBILIZATION TRANSFER OF NONCONJUGATIVE PLASMIDS

The model presented here is an extension of that in Levin *et al.* (1979).

We consider seven distinguishable bacterial populations:

D – donors: the original clone of bacteria carrying only the nonconjugative plasmid.

M – mobilizers: the original clone of bacteria carrying only the conjugative, mobilizing plasmid.

R – recipients: a plasmid-free clone capable of receiving both the conjugative and nonconjugative plasmids.

MD – mobilized donors: members of the donor clone that have received the conjugative plasmid and therefore carry both factors.

TD – transconjugant donors: members of the recipient clone that carry only the nonconjugative plasmid.

TM – transconjugant mobilizers: members of the recipient clone that carry only the mobilizing conjugative plasmid.

TMD – transconjugant mobilized donors: members of the recipient clone that carry both the nonconjugative and mobilizing conjugative plasmids.

In this model the symbols D, M, R, MD, TD, TM, and TMD represent the densities of these different cell types in units of bacteria per millilitre. We assume: (1) all bacterial cell types grow at the same rate, ψ h^{-1}, (2) during the period considered, plasmid loss by segregation occurs at a negligible rate, (3) conjugation occurs at random at a frequency which is jointly proportional to the concentrations of cells carrying mobilizing plasmids and their recipients, (4) the rate constant γ (ml/cell × hr) governing plasmid transfer is the same for all 'matings', (5) cells carrying mobilizing plasmids are unable to serve as recipients for either plasmid, (6) a constant proportion, α_D, of effective matings between mobilized donors or transconjugant mobilized donors (MD or TMD) and recipients (R) results in transfer of only the nonconjugative plasmid, in a proportion, α_M, of these matings, only the mobilizing plasmid is transmitted, and in a proportion, α_{MD}, both plasmids are transmitted ($\alpha_D+\alpha_M+\alpha_{MD}=1$), (7) in a constant proportion, $\alpha_M+\alpha_{MD}$, of matings between mobilized donors or transconjugant mobilized donors (MD or TMD) and donors or transconjugant donors (D or TD) the mobilizing plasmid is transmitted, and finally (8) the transfer of these plasmids and the acquisition of competence for retransfer are instantaneous processes. With these definitions and assumptions, the rates of change in the concentrations of the different cell types are given by

$$\dot{D} = \psi D-\gamma D[M+TM+(\alpha_M+\alpha_{MD})(MD+TMD)], \tag{2.1}$$

$$\dot{R} = \psi R-\gamma R(M+TM+MD+TMD), \tag{2.2}$$

$$\dot{M} = \psi M, \tag{2.3}$$

$$\dot{MD} = \psi MD+\gamma D[M+TM+(\alpha_M+\alpha_{MD})\,(MD+TMD)], \tag{2.4}$$

$$\dot{TD} = \psi TD+\gamma R\alpha_D(MD+TMD)-\gamma TD[M+TM+(\alpha_M+\alpha_{MD})\,(MD+TMD)], \tag{2.5}$$

$$\dot{TM} = \psi TM+\gamma R[M+TM+\alpha_M(MD+TMD)], \tag{2.6}$$

$$\dot{TMD} = \psi TMD+\gamma R\alpha_{MD}(MD+TMD)+\gamma TD[M+TM+(\alpha_M+\alpha_{MD})\,(MD+TMD)], \tag{2.7}$$

where a dot ($\cdot$) denotes differentiation with respect to time. To help clarify the derivation of this model, the various matings considered, the types of transconjugants produced and their relative frequencies are presented in Table 1. The validity and limitations of the assumptions upon which this model is based are considered in the discussion section.

3. MATERIALS AND METHODS

(i) *Bacterial strains and plasmids*

Strains of *E. coli* K-12 were used as donors, recipients and mobilizers in these experiments. For the nonconjugative plasmid we used a kanamycin resistance factor pCR1 (*Km*) (Covy, Richardson & Carbon, 1976) and for the mobilizing conjugative plasmid, *F-lac-pro*. The former plasmid was obtained from Dr R.

Table 1. *A model for the kinetics of mobilization transfer of nonconjugative plasmids, possible matings, transconjugants produced, and their relative frequencies*

	Transconjugants and frequencies			
Mating	*MD*	*TD*	*TM*	*TMD*
$D \times M$	1	.	.	.
$D \times TM$	1	.	.	.
$D \times MD$	$\alpha_M + \alpha_{MD}$	.	.	.
$D \times TMD$	$\alpha_M + \alpha_{MD}$	.	.	.
$R \times M$	.	.	1	.
$R \times TM$	.	.	1	.
$R \times MD$	.	α_D	α_M	α_{MD}
$R \times TMD$	.	α_D	α_M	α_D
$TD \times M$	.	.	.	1
$TD \times TM$	.	.	.	1
$TD \times MD$	.	.	.	$\alpha_M + \alpha_{MD}$
$TD \times TMD$	.	.	.	$\alpha_M + \alpha_{MD}$

$$\alpha_D + \alpha_M + \alpha_{MD} = 1.$$

Curtiss III and was originally carried on a X2114 host. The *F-lac-pro* plasmid used was that carried by CSH 23 (Miller, 1972). For the original donor, *D*, strain, the pCR1 plasmid was put in a CSH 7 F^- *lac*Y *rps*L *thi* clone to be designated CSH 7 (pCR1), the mobilizer, M, was CSH 23 F-*lac-pro*/Δ(*lac-pro*) *sup*E *rps*E *thi* and the recipient, *R*, a nalidixic acid resistant mutant of CSH 50 (ara Δ (*lac-pro*) *rps*L *thi*).

(ii) *Culture medium and sampling procedures*

All experimental cultures were maintained in a minimal salt solution containing (per litre) K_2HPO_4, 7 g; KH_2PO_4, 2 g; $(NH_4)_2SO_4$, 1 g; $Mg_2SO_4 \cdot 7H_2O$, 0·1 g and supplemented with 30 μg L-proline and 2 μg thiamine per ml. Glucose at 300 μg/ml was the limiting carbon source. The batch cultures were maintained in 50 ml Erlenmyer flasks stoppered with cotton plugs and incubated at 37 °C in constant temperature baths. These flasks were inoculated with fresh, overnight cultures from that medium for a total volume of 10 ml and shaken at approximately 200 rev/min to allow for proper aeration. The continuous culture populations were maintained in chemostats of a homemade variety, see the appendix to Chao, Levin & Stewart (1977). These chemostat cultures were initiated by adding cells from equilibrium chemostat populations to another chemostat already carrying one of the component populations.

At periodic intervals, samples were taken from these experimental cultures, diluted and plated on an array of media. Where possible, dilutions were chosen that would result in between 100 and 200 colonies on the sampling plates. The estimated numbers of each of the seven cell types were determined from colony count data using either direct colony counts or differences in colony counts on different plate types, see Table 2.

Table 2. *Plating media used for the estimates of cell densities from colony count data*

Cell type	Phenotype: kan	lac	str	nal	Estimate of density made from colony count or colony count differences
D	r	−	r	s	TLK (lac⁻) − TLKN(lac⁻)
M	s	+	s	s	Tl(lac⁺) − TLK(lac⁺) − MLPN + TLKN(lac⁺) or TL(lac⁺) − MLSP
R	s	−	r	r	TL(lac⁻) − TLK(lac⁻)
MD	r	+	r	s	TLK(lac⁺) − TLKN(lac⁺) or MLSP − MLPN
TD	r	−	r	r	TLKN(lac⁻)
TM	s	+	r	r	MLPN − TLKN(lac⁺)
TMD	r	+	r	r	TLKN(lac⁺)
Total cells					TL(lac⁺) + TL(lac⁻)

Plates used:

TL (all cell types grow) – tetrazolium lactose indicator medium.

TLK (D + MD + TD + TMD) – tetrazolium lactose indicator medium supplemented with 40 μg/ml kanamycin sulfate (Sigma)

TLKN (TD + TMD) – tetrazolium lactose indicator medium supplemented with 40 μg/ml kanamycin sulfate and 10 μg/ml nalidixic acid (Sigma)

MLPN (TM + TMD) – Lactose minimal medium supplemented with 30 μg/ml L-proline and 10 μg/ml nalidixic acid (Sigma)

MLSP (MD + TMD + TM) – Lactose minimal medium supplemented with 30 μg/ml L-proline and 100 μg/ml streptomycin (Sigma).

(iii) *Procedures for parameter estimation*

All of the parameters in equations (2.1) through (2.7) can be estimated from the results of matings between mobilized donors and recipients. The exponential growth rate, ψ, and transfer rate constant, γ, in the exponentially growing cultures are estimated in a manner similar to that considered for conjugative plasmids in Levin *et al.* (1979). The formulae derived are

$$\psi = \frac{1}{b-a} \ln \frac{N(b)}{N(a)} \tag{3.1}$$

and

$$\gamma = \frac{\psi}{N(b)-N(a)} \ln \frac{\beta+\rho(b)}{\beta+\rho(a)}, \tag{3.2}$$

where a and b are two time points during exponential growth (see Levin *et al.* 1979). The total cell densities, $N(a)$ and $N(b)$, are determined visually from best fit lines on graphs of the logarithm of the cell density plotted as a function of time. The parameter β, the ratio of mobilized donors to total cells, MD/N, was estimated from the mean donor/total cell ratio from the colony count data taken during the exponential growth period. The variables $\rho(a)$ and $\rho(b)$, the ratios of the total transconjugant densities, $T = TD + TM + TMD$, to recipient densities,

$\rho = T/R$, were estimated from best-fit lines. For the estimation of the proportions of the different kinds of transconjugants produced, α_D, α_M, and α_{MD}, we used the means of the ratios of the different types of transconjugants to total transconjugants calculated directly from colony count data during the exponential growth period,

$$\alpha_D = TD/T, \quad \alpha_M = TM/T, \quad \text{and} \quad \alpha_{MD} = TMD/T.$$

Although some of the increase in the concentration of TM transconjugants is due to transfer from TM to R, during the course of these experiments, TM cells remain very rare relative to the dominant plasmid transferring strain, MD, and consequently transfer from TM plays a negligible role in the buildup of transconjugants.

In a chemostat at equilibrium there is no net population growth, and $\psi = 0$. When the numbers of mobilized donors and recipients are large relative to those of the transconjugants, the transfer rate constant can be estimated directly from the rate of increase in the concentration of total transconjugants, i.e.

$$\dot{T} = \gamma MD \cdot R, \tag{3.3}$$

$$\gamma = \frac{\dot{T}}{MD \cdot R}. \tag{3.4}$$

For our estimates of MD and R in the above we used the mean value of these densities taken over the sampling period. For the estimate of the rate of change in transconjugants, we used the coefficient of regression of the concentration of total transconjugants as a linear function of time. The proportions of transconjugants of the different types, α_D, α_M, and α_{MD}, are calculated in the same way as for the $MD \times R$ mating in exponential culture.

The rate of cell growth, ψ, in the exponential three 'parent' $D \times M \times R$ mating is estimated in the same way as that for the two parent mating, i.e., from the change in the total cell density. However, for the $D \times M \times R$ mating we obtained two estimates of the transfer rate parameter, γ: (1) that for the mating between mobilizers and donors, $\gamma_{M \times D}$, and (2) that for the mating between mobilizers and recipients, $\gamma_{M \times R}$. The procedure used to estimate these rate parameters is similar to that described for the $MD \times R$ mating. However, in the case of $\gamma_{M \times R}$, M and R are the parental densities and TM the transconjugant density and in the case of $\gamma_{M \times D}$, M and D are the parental densities and MD the transconjugant density. During the course of these experiments, the TM population remains rare, and retransfer from the TM cells contributes very little to the buildup in the concentration of TM. Although it is possible to determine the values of α_D, α_M, and α_{MD} from three parent matings, it is difficult to obtain accurate, independent estimates of these proportions from matings of this type. For this reason, we rely on estimates of α_D, α_M, and α_{MD} from the $MD \times R$ matings. The artifacts associated with the use of colony count data to estimate these parameters are considered in Levin *et al.* (1979).

4. RESULTS

(i) *Exponentially growing cultures*

In Fig. 1 we present the results of an exponential growth and transfer rate experiment for cultures initiated with mobilized donors and recipients and the trajectories of the population densities anticipated from this model. The latter were obtained from numerical solutions to equations (2.1) through (2.7). For the parameter values for these numerical solutions we used those estimated from that experiment and for the initial value of the variables we used those for time a estimated from best fit lines in that experiment. In Fig. 2 we present the results of an exponential growth and plasmid transfer experiment from a $D \times M \times R$ mating and the theoretical trajectories anticipated from this model. For the estimate of the transfer rate constant in this experiment we used the mean of the estimated $\gamma_{M \times D}$ and $\gamma_{M \times R}$ obtained from the experimental trajectories. However, for the estimates of the proportions of the different transconjugants produced in this mating we used the mean of those values estimated in an array of $MD \times R$ exponential growth matings.

In the two parent, $MD \times R$ mating, the observed and expected trajectories of the concentrations of parental and transconjugant clones are reasonably close. In the three parent ($D \times M \times R$) mating a reasonably good fit of the expected and observed changes in cell concentrations obtains for the parental clones and the 'primary' transconjugants (MD and TM), see Fig. 2. However, although the general forms of the observed and expected changes in the concentrations of the secondary transconjugants (TD and TMD) in this mating are similar, the anticipated rate of increase in the densities of these cell types is significantly greater than that observed. We attribute this lower than anticipated rate of increase in the concentration of secondary transconjugants to a host effect on the rate at which donors transfer plasmids. The vast majority of primary transconjugants are the products of plasmid transfer between the CSH 23 (*F-lac-pro*) donor and the CSH 7 (pCR1) or the CSH 50 nal recipients, while the secondary transconjugants are primarily the products of plasmid transfer between the CSH 7 (*F-lac-pro*-pCR1) mobilized donor and the CSH 50 nal recipient. As we demonstrate in a later section of this paper, the rate of conjugative plasmid transmission from the CSH 23 host is about four times greater than that from the CSH 7 host (see Table 3). If we account for this host effect on transfer by altering the model so that the rate constant of plasmid transfer from the mobilized donors, MD, is about one quarter as great as that of the other cells carrying the mobilizing plasmid, M, TM and TMD, there is very little effect on the rate of buildup of primary transconjugants, but the trajectories of the concentration of secondary transconjugants becomes much more similar to that observed (see Fig. 2). Furthermore, some time transpires between the receipt of the mobilizing plasmid by donors and the point when they become competent to transmit the plasmid (see Achtman, Willetts & Clark, 1971 or Cullum *et al.* 1978). This *time delay* would also be reflected as a shift to the right in the observed trajectories of these second order transconjugants.

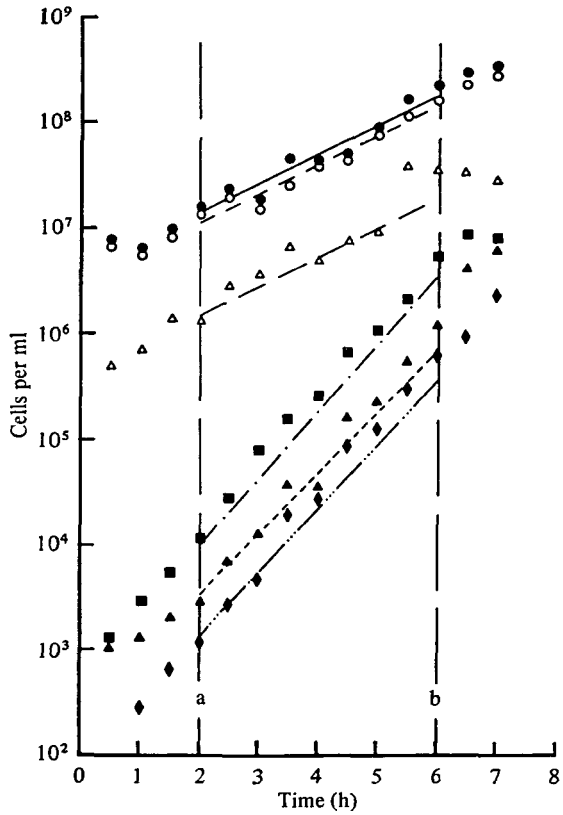

Fig. 1. Matings between CSH 7 (*F-lac-pro*-pCR1) mobilized donors and CSH 50 nal recipients in exponentially growing culture. Log of cell densities plotted as functions of time. ●—●: Total cells, N; ○– –○: mobilized donors, MD; △– – –△: recipients, R; ▲—·—▲, transconjugant donors, TD; ■—·—■, transconjugant mobilizers, TM; ◆—·–·—◆, transconjugant mobilized donors, TMD. The points in this and the other figures are experimental results and the lines are the trajectories anticipated from the model [equations (2.1)–(2.7)]. The parameters used for the latter numerical solutions to these equations are $\psi = 0{\cdot}78$, $\gamma = 4{\cdot}1 \times 10^{-10}$, $\alpha_D = 0{\cdot}21$, $\alpha_M = 0{\cdot}66$, and $\alpha_{MD} = 0{\cdot}13$. The cell densities used for the initial values of the variables (population densities) in these numerical solutions are those estimated from best fit lines taken at time 'a'.

(ii) *Cultures at the chemostat equilibrium*

For bacteria at lag phase the transfer of the conjugative plasmid commences before cell growth and during the period of no net population growth, the transfer rate parameter, γ, increases in value. This parameter remains constant during exponential phase, but as the cells approach stationary phase the magnitude of this rate parameter declines dramatically (Levin *et al.* 1979). Thus, although the assumption of a constant transfer rate parameter allows for a reasonably accurate analogue of the kinetics of conjugative plasmid transfer during exponential phase, this is not the case for lag or stationary. For this reason we did not anticipate that an extension of this conjugative plasmid model would serve as an accurate analogue

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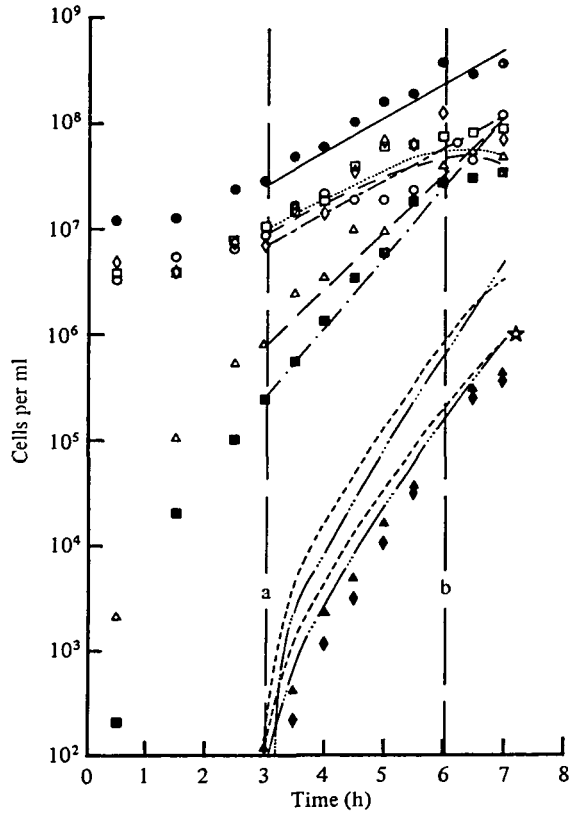

Fig. 2. Matings between CSH 23 (*F-lac-pro*) mobilizers, CSH 7 (pCR1) donors and CSH 50 nal recipients in exponentially growing culture. Logs of cell densities plotted as functions of time. The symbols and lines used for the different cell types are identical to those used in Fig. 1 with the addition of (□------□) donors, D and (◇—·—◇) mobilizers, M. The growth rate used for this numerical solution is that estimated from the changes in total cell density, $\psi = 0{\cdot}70$, and the transfer rate constant is the mean of those estimated for the mobilizer by donor, $\gamma_{M\times D}$, and for the mobilizer by recipient, $\gamma_{M\times R}$, matings in this experiment, $\gamma = 4{\cdot}0\times10^{-9}$. The proportions of the different types of transconjugants produced are the means of those independently estimated from an array of $MD\times R$ matings in exponential culture, $\alpha_D = 0{\cdot}17$, $\alpha_M = 0{\cdot}72$ and $\alpha_{MD} = 0{\cdot}11$. For the initial cell densities in these numerical solutions we use those estimated from best fit lines taken at time 'a'. The lower pair of TD and TMD theoretical trajectories, marked with a star, were calculated from a variant of equations (2.1)–(2.7) in which the transfer rate parameter of mobilized donors was $1{\cdot}0\times10^{-9}$, while that of other cells carrying the mobilizing plasmid was $4{\cdot}0\times10^{-9}$. The values of the other parameters and variables in these 'starred' solutions are identical to those presented above. Although, this change in the MD transfer rate constant has some effect on the trajectories of the other populations, the differences in the lines generated for these variables in the two solutions cannot be seen on figures of the present size and scale.

of mobilization transfer for lag phase or stationary phase cells. Consequently, for the present investigation, other than confirming this conjecture with some pilot experiments, we have not explored the kinetics of mobilization transfer in lag or stationary phase. However, the results of this earlier conjugative plasmid

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study suggested that mass action models offer a reasonable description of the kinetics of plasmid transfer in bacterial populations dividing at a constant rate in chemostats and consequently we have examined the mobilization transfer in chemostats.

In Fig. 3 we present the results of an $MD \times R$ mating in chemostat culture, and the trajectories of the concentrations of the different transconjugant types anticipated from the model. The changes in the concentrations of transconjugants produced by a three parent, $D \times M \times R$ mating in a chemostat and changes in transconjugant concentrations anticipated from the model are presented in Fig. 4. For the parameters used to generate the theoretical lines in Fig. 3, we used those estimated from the experiment depicted in that figure. For the theoretical trajectories in Fig. 4 we used the mean of $\gamma_{M \times R}$ and $\gamma_{M \times D}$ estimated from the three parent mating presented in that figure and for the values of α_D, α_M and α_{MD} we used the average of those estimated from a number of $MD \times R$ matings in exponential culture.

As anticipated from our studies of the kinetics of conjugative plasmid transfer (Levin *et al.*, 1979), the transfer rate constants in these chemostat cultures are considerably lower than those estimated in exponential phase. For the $MD \times R$ mating depicted in Fig. 3, there is reasonably close agreement between the theoretical and observed changes in transconjugant concentrations. However, as was the case with the $D \times M \times R$ mating in exponential culture, the fit of theory and experiment in the three parent mating in chemostat culture was not as good as that for the two parent mating. The changes in concentrations of the primary (TM and MD) transconjugants are pretty much consistent with those anticipated from the model, but the trajectories of the concentrations of second order transconjugants (TD and TMD) deviate significantly from those anticipated. Although, as anticipated, the densities of TD and TMD transconjugants increase exponentially, the rate of this increase and the timing of its onset differ considerably from theory. As was the case for this three parent mating in exponential culture, we believe that this deviation is, to a great extent, due to the fact that the rate constant of transfer from the CSH 7 host is less than that from the CSH 23 host (see Table 3). By altering the model to account for this host effect by making the transfer rate constant for mating with MD cells one fourth that of other cells carrying the conjugative plasmid, there is little change in the trajectories of the primary transconjugants relative to the case of equal rate constants, but the anticipated changes in the concentration of secondary transconjugants is somewhat closer to that observed (Fig. 4).

(iii) *The effects of the nonconjugative plasmid and host cell type on rates of plasmid transmission*

One of the assumptions made in the development of this model is that there is a unique transfer rate parameter. Implicit in this assumption is the assertion that neither the carriage of a nonconjugative plasmid nor the host cell type affects donor or recipient ability. The results of the transfer rate experiments we have

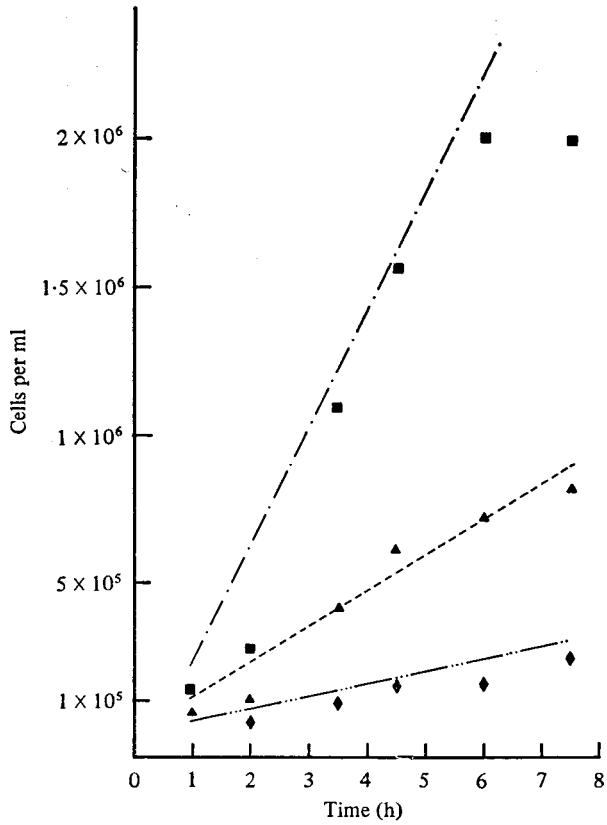

Fig. 3. Mating between CSH 7 (*F-lac-pro*-pCR1) mobilized donors and CSH 50 nal recipients in a chemostat with a 0·14 h^{-1} dilution rate. Densities of transconjugants plotted as linear functions of time. The points and lines in this figure are defined in the same way as those in the legend to Fig. 1. The mean concentrations of mobilized donors and recipients during the depicted period, $MD = 2{\cdot}53 \pm 0{\cdot}13$ ($\times 10^8$) and $R = 3{\cdot}02 \pm 0{\cdot}25$ ($\times 10^8$), were used as the parental densities for the calculations of the theoretical trajectories. The parameter values used were determined from the present experimental results, $\gamma = 6{\cdot}4 \times 10^{-12}$, $\alpha_D = 0{\cdot}26$, $\alpha_M = 0{\cdot}68$ and $\alpha_{MD} = 0{\cdot}06$. For the initial values of the variables we used those calculated from the coefficient of linear regression of points at one hour.

done in exponential culture are consistent with the hypothesis that the carriage of the nonconjugative plasmid does not affect the donor or recipient ability of a host bacterium. These results are summarized in Table 3.

Under the hypothesis that the nonconjugative plasmid has no effect on the recipient ability of a host bacterium, the transfer rate constant for a mating between mobilizers and plasmid-free recipients should be the same as that for a mating between mobilizers and recipients carrying the nonconjugative plasmid. The results of the experiments with the CSH 23 (*F-lac-pro*) host and the CSH 7 and CSH 7 (pCR1) recipients are consistent with this hypothesis. Also consistent are the results of the three parent matings with CSH 23 (*F-lac-pro*), CSH 7 (pCR1) and CSH 50 nal. Here $\gamma_{M\times D}$ is not significantly different from $\gamma_{M\times R}$.

Under the hypothesis that the nonconjugative plasmid has no effect on the

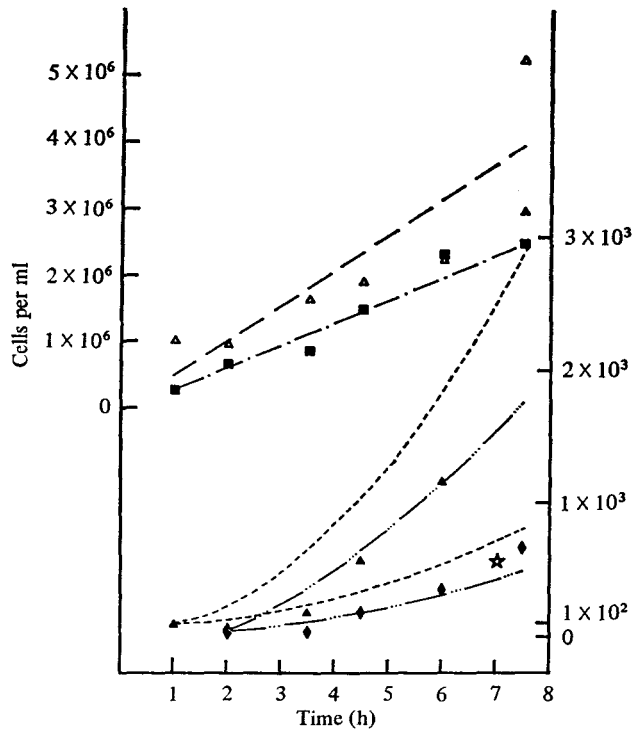

Fig. 4. Mating between CSH 23 (*F'-lac-pro*) mobilized donors, CSH 7 (pCR1) donors, and CSH 50 nal recipients in chemostats with 0·16 h^{-1} dilution rate. Densities of transconjugants are plotted as linear functions of time. The points and lines used in this figure are defined in the same way as those in the legends of Figs. 1 and 2. The concentrations of the primary (*MD* and *TM*) transconjugants are plotted on the right hand scale and the secondary transconjugants (*TD* and *TMD*) on the left-hand scale. The mean concentrations of the mobilizers, donors and recipients during the depicted period, $M = 2{\cdot}97 \pm 0{\cdot}23$ ($\times 10^8$), $D = 2{\cdot}72 \pm 0{\cdot}13$ ($\times 10^8$) and $R = 1{\cdot}73 \pm 0{\cdot}13$($\times 10^8$), were used as the parental densities to calculate the transconjugant trajectories. The transfer rate constant used for this numerical solution, $\gamma = 6{\cdot}7 \times 10^{-12}$, is the mean of that estimated for the $M \times D$ and $M \times R$ experiments depicted in this figure. For the proportions of the different kinds of transconjugants produced by mobilized donors we use the means of those independently estimated from an array of $MD \times R$ matings in exponential culture, $\alpha_D = 0{\cdot}17$, $\alpha_M = 0{\cdot}72$ and $\alpha_{MD} = 0{\cdot}11$. The initial *TM* and *MD* transconjugant densities are those calculated from the coefficients of linear regression at one hour, the initial *TD* concentration is the observed concentration at one hour and for *TMD* the initial value for one hour is zero. The lower pair of *TD* and *TMD* theoretical trajectories, those marked with a star, were calculated with a variant of equations (2.1)–(2.7) in which the transfer rate parameter for mobilized donors was $1{\cdot}7 \times 10^{-12}$ while that of other cells carrying the mobilizing plasmid was $6{\cdot}7 \times 10^{-12}$. The values of the other parameters and the variables in these 'starred' solutions are identical to those presented above. Although this change in the *MD* transfer rate constant has some effect on the trajectories of the other populations, the differences in the lines generated for these variables with these two solutions cannot be seen on figures of the present scale.

Table 3. *Exponential growth and transfer rate parameters for different matings*

Mating	Expts.	ψ (h^{-1})	γ (ml/cell × h)
(A) CSH 23 (F-*lac-pro*) × CSH 7	5	$0{\cdot}76 \pm 0{\cdot}02$	$5{\cdot}08 \pm 1{\cdot}47 \times 10^{-9}$
(B) CSH 23 (F-*lac-pro*) × CSH 7 (pCR1)	5	$0{\cdot}73 \pm 0{\cdot}03$	$4{\cdot}19 \pm 0{\cdot}69 \times 10^{-9}$
(C) CSH 7 (F-*lac-pro*) × CSH 50 nal	7	$0{\cdot}72 \pm 0{\cdot}01$	$1{\cdot}33 \pm 0{\cdot}42 \times 10^{-9}$
(D) CSH 7 (F-*lac-pro*-pCR1) × CSH 50 nal*	7	$0{\cdot}76 \pm 0{\cdot}03$	$6{\cdot}40 \pm 0{\cdot}47 \times 10^{-10}$
(E) CSH 23 (F-*lac-pro*) × CSH 7 (pCRl) × CSH 50 nal	4	$0{\cdot}79 \pm 0{\cdot}05$	$\gamma_{M\times D} = 4{\cdot}65 \pm 1{\cdot}52 \times 10^{-9}$ $\gamma_{M\times R} = 4{\cdot}03 \pm 1{\cdot}22 \times 10^{-9}$

*$\bar{\alpha}_D = 0{\cdot}174 \pm 0{\cdot}011$, $\bar{\alpha}_M = 0{\cdot}724 \pm 0{\cdot}019$, $\alpha_{MD} = 0{\cdot}102 \pm 0{\cdot}009$.

Results of the one factor analyses of variance:

(1) $H_0: \gamma_A = \gamma_B$, $F_{1,8} = 0{\cdot}28$, $P > 0{\cdot}25$

(2) $H_0: \gamma_C = \gamma_D$, $F_{1,12} = 2{\cdot}31$, $0{\cdot}10 < P < 0{\cdot}25$

(3) $H_0: \gamma_A = \gamma_C$, $F_{1,10} = 39{\cdot}80$, $P < 0{\cdot}005$

(4) $H_0: \gamma_{M\times D} = \gamma_{M\times R}$ in Set E, $F_{1,6} = 0{\cdot}052$, $P > 0{\cdot}25$

donor ability of a bacterium, the transfer rate constant estimated for an $M \times R$ mating should be equal to that for the $MD \times R$ mating. The results of the experiments with matings between CSH 7 (*F-lac-pro*) and CSH 50 nal and those between CSH 7 (*F-lac-pro*-pCR1) and CSH 50 nal are consistent with this hypothesis. Although the mean transfer rate constant for the $MD \times R$ mating is less than that for the $M \times R$ mating, this difference is not significant at the 0·05 level. However, in interpreting this, one should realize that in these experiments variance in the estimate of γ was quite large.

The results of the experiments summarized in Table 3 do suggest that there is a significant host effect on the rate at which conjugative plasmid transmission occurs. The transfer rate constant estimated in the CSH 23 (*F-lac-pro*) × CSH 7 mating is significantly greater than that estimated in the CSH 7 (*F-lac-pro*) × CSH 50 nal mating. The fact that in the three parent matings, where there is a unique donor, CSH 23 (*F-lac-pro*), and two different recipient clones, CSH 7 (pCR1) and CSH 50 nal, there is no significant difference in the primary transfer rate constants, $\gamma_{M\times D}$ and $\gamma_{M\times R}$ suggests that the preceding difference in transfer rate is a consequence of a donor rather than a recipient effect.

(iv) *The effect of the conjugative plasmid on the recipient ability of the host bacterium*

Bacteria carrying conjugative plasmids are known to be poor recipients in conjugation with cells carrying the same or similar sex factors, a phenomenon known as surface exclusion (Achtman & Helmuth, 1975; Achtman, Kennedy & Skurray, 1977). Nevertheless, we felt it necessary to evaluate the validity of our assumption of *no* transfer of the nonconjugative factor to cells carrying the mobilizing plasmid in a quantitative manner. For this we performed transfer experiments in exponentially growing cultures with CSH 7 (*F-lac-pro*-pCR1) as a mobilized donor and CSH 50 nal (*F-lac-pro*) as a recipient and also with CSH 7 nal (*F-lac-pro*) as a recipient. These exponentially growing cultures were continually sampled to determine the concentration of cells that were simultaneously resistant to both kanamycin and nalidixic acid.

The results of these experiments indicate that the assumption of no transfer of pCR1 to F-bearing cells may not be strictly correct. The density and rate of increase in the concentration of $kan^r nal^r$ cells was greater than that which can be explained by mutation and cell growth alone. Some of the cells of this transconjugant phenotype could have been the products of matings between mobilized donors and segregants of the F-*lac-pro*-bearing recipients. Since the sampling results indicate the existence of significant numbers of $lac^- nal^r$ cells in these cultures, it is reasonable to assume such segregant types are present. Nevertheless, we still cannot rule out the possibility of transfer of the nonconjugative plasmid to the *F*-bearing cell types. However, the results of these experiments suggest that even if *F*-bearing cells do serve as recipients, the rate at which they accept these factors is quite low when compared to recipients free of this conjugative plasmid. With a CSH 7 (F-*lac-pro*-pCR1) donor and a CSH 7 nal (F-*lac-pro*) recipient, assuming that about 30% of the transconjugants from mobilized donors get the nonconjugative plasmid, we estimate γ to be on the order of 2×10^{-12} ml/cell × h. In the experiment with this donor and the CSH 50 nal (*F-lac-pro*) recipient, the rate of increase in the concentration of cells of the transconjugant phenotype was too low and too erratic to obtain an accurate estimate of γ. Our 'ball park' estimates suggest this rate constant to be on the order of 10^{-14} ml/cell × h.

To ascertain the effect of transfer of the nonconjugative plasmid to cells carrying the mobilizing factor, we prepared a version of the model [equations (2.1)–(2.7)], which allows for transfer of the nonconjugative plasmid to the *M* and *TM* cell types. In this modified set of equations we assumed that the transfer of the nonconjugative plasmid to an *M* cell type would produce members of the *MD* cell population and that transfer to a *TM* cell type would produce members of the *TMD* cell population. We performed simulation experiments for both the $MD \times R$ and $M \times D \times R$ matings in exponentially growing situations. For the recipients free of the conjugative plasmid, we let $\gamma = 10^{-9}$ ml/cell × h and for the rate constant to recipients carrying the conjugative plasmid we let $\gamma = 5 \times 10^{-12}$ ml/cell × h. With cell densities and other parameters similar to those in the preceding exponential growth experiments and with figures of the present scale and size, it was not possible to distinguish the trajectories of the densities produced by the modified model and the original model. For this reason, we believe that the assumption of no transfer to cells carrying the conjugative plasmid is justified.

5. DISCUSSION

We interpret the results of these experiments as indicating that for bacterial populations dividing at a constant rate in liquid culture, the kinetics of mobilization transfer of nonconjugative plasmids can be reasonably well described by a set of simple, mass action differential equations. In rapidly dividing, exponential populations the rate constant of conjugative plasmid transfer is large relative to the corresponding value of this parameter in slowly dividing continuous cultures.

As a consequence, the absolute rate of transfer of the nonconjugative factor in exponentially growing bacterial populations is considerably greater than that which obtains in populations maintained in chemostats with low dilution rates. The relative proportions of the transconjugants produced in matings between mobilized donors and recipients which receive (i) just the nonconjugative plasmid, (ii) just the conjugative plasmid, and (iii) both plasmids appear to be constant in these 'steady-state' exponential phase or chemostat equilibrium cultures. In addition, the relative proportions of the three types of transconjugants are similar in exponentially growing and chemostat equilibrium cultures.

The results of these experiments suggest that the carriage of the nonconjugative pCR1 plasmid has little, if any, effect on the overall rate of transmission of plasmids by cells carrying the mobilizing conjugative *F′* factor. Analogously, the carriage of the nonconjugative plasmid appears to have little effect on the capacity of a bacterium to receive that conjugative plasmid. On the other hand, the host cell type carrying the mobilizing plasmid does have a significant effect on the rate of infectious transmission of plasmids by conjugation. Finally, as anticipated when an F′ factor is used as a mobilizing plasmid, the rate at which the nonconjugative plasmid is transmitted to cells carrying this mobilizer is sufficiently low to justify not considering these *F*-bearing cells as recipients.

It is unquestionably true that with a sufficient number of parameters, equations can be generated to fit pretty much any curve. However, we do not believe that a criticism of 'empirical curve fitting' applies here. The equations used were formulated *a priori* from specific, realistic, albeit simplifying, assumptions about population growth and the process of plasmid transfer and each of the parameters in these equations has biological meaning. Furthermore, concern was with changes in the values of four or seven variables using models with four parameters and not just accounting for the changes in single variables. On the other hand, it is important to point out that the apparent fit of this or any other model to a set of data is not sufficient evidence for the validity of the assumptions made in the construction of that model or that the conditions assumed will obtain in general. For example, the work of Achtman (1975) on mating aggregates and that of Cullum *et al.* (1978) on the kinetics of transconjugant formation as well as other studies indicate that plasmid transfer is more complex than the instantaneous random collision process assumed here. Although it is possible to obtain bacterial strains with nearly identical rates of growth, there is no justification for assuming that all strains of bacteria that exchange particular plasmids would have similar growth rates in general. Close fit of theory and experiment as obtained here in the mobilized donor by recipient matings could indicate that these and other deviations from the absolute validity of the assumptions made are minor, but this fit could also be a consequence of these deviations in some way compensating for each other.

For both the two parent (mobilized donor × recipient) and three parent (mobilizer × donor × recipient) matings the general forms of the observed and expected trajectories of transconjugant concentrations are similar. However, the precision of the fit of the observed and expected trajectories is better for the two parent

than it is for the three parent matings. We attribute the relatively poorer fit of theory and experiment in the latter matings primarily to the effect of the host clone on the rate of conjugative plasmid transfer. We also believe that some of this deviation of expected from observed behaviour in the mobilizer × donor × recipient matings is a consequence of the time delay associated with the achievement of competence for retransfer by donor cells receiving the mobilizing plasmid. Because of the close fit of theory and experiment in the two parent matings where the unfortunate complexities of host effect and time delays would not be very important, we do not interpret the poorer fit of the three parent experiments as evidence against the mass action assumption upon which these models are based. Nevertheless, this deviation from theory indicates that the assumptions of a unique value for the transfer rate parameter and instantaneous competence for retransfer cannot be generally supported. By modifying the model to allow for separate growth and plasmid transfer parameters for each of the cell lines and by incorporating time delays we believe that reasonably precise fit of theory and experiment could be obtained for these three parent matings, even if the strains involved differed considerably in their rates of growth and plasmid transfer. Needless to say, with a more complex model, the estimation of parameter values would be a more difficult task and one that may require trial and error procedures. However, it is our feeling that for the likely applications of this kind of theory, the generality of simple models is preferable to the precision of more complex ones (see Levins, 1966, for a discussion of this 'strategy' of model building).

In the present experiments we considered only the plasmid pCR1 mobilized by an F-*lac-pro* factor with strains of *E. coli* K-12 as hosts. However, we believe that to some extent the assumed generality of these results can be justified. In our earlier investigation (Levin *et al.* 1979), we presented evidence that the mass action equations from which the present mobilization transfer model was derived serve as a reasonably good analogue of the transfer kinetics of three conjugative plasmids in *E. coli* K-12 hosts. Two of these plasmids, *F-lac-pro* and R1 *drd* 19 (Km Cm Am), were permanently derepressed for conjugative pili formation while the third factor, R1 (Km Cm Am), was repressed for this function. Nevertheless, at this juncture, we consider the conclusion that the presented model serves as a reasonably good analogue of the mobilization transfer process for a wide variety of nonconjugative plasmid–mobilizer–donor–recipient combinations to be no more than a conjecture. We also believe that a considerable amount of additional work will be necessary to support the generality of the conclusions that the carriage of a nonconjugative plasmid would have little effect on the overall ability of a host bacterium to transmit or receive conjugative factors and that cells carrying the mobilizing plasmid need not be considered as recipients.

We would expect a fair amount of variation among different nonconjugative plasmid–mobilizer–donor–recipient combinations with respect to the specific values of the parameters of this model. It is, for example, reasonable to assume that plasmids with repressed conjugative pili formation would, as a class, transmit the nonconjugative plasmid at lower rates than mobilizing plasmids that are

derepressed for this character. In our earlier investigation of the kinetics of conjugative plasmid transmission (Levin *et al.* 1979) the estimated transfer rate constant for wild type, repressed R1 was between two and three orders of magnitude lower than that estimated for the permanently derepressed mutants of that plasmid, R1 *drd* 19, for both exponentially growing and chemostat equilibrium cultures. However, with repressed mobilizing plasmids it is possible that the rate at which second order transconjugants are produced in the three parent mating would be greater than that at which the first order transconjugants are produced. For plasmids with repressed conjugative pili formation the likelihood of a cell transmitting a plasmid declines with the time since that bacterium or its first plasmid-bearing ancestor acquired that factor (Meynell, 1973). Thus in three parent matings the average time since receipt of the mobilizing plasmid for a cell in the mobilized donor population would be expected to be less than that in the mobilizer population. Consequently, the conjugational transfer rate parameter would be higher in the mobilized donor population than it would be in the mobilizer population.

It seems reasonable to assume that different nonconjugative plasmid-mobilizer pairs would vary considerably in the proportions of the different types of transconjugants produced in matings between mobilized donors and recipients, i.e. α_D, α_M, and α_{MD}. The experiments we have done with the nonconjugative plasmid pSC101 (Tc) (Cohn & Chang, 1973) mobilized by R1 *drd* 19 (Km Cm Am) in *E. coli* K-12 hosts are very much consistent with this view. Although there was no evidence that the carriage of pSC101 had a significant effect on the capacity of hosts to receive or transmit R1 *drd* 19, the rate at which this nonconjugative factor is mobilized is obviously very low. Indeed, in matings between pSC101-R1 *drd* 19 mobilized donors and recipients in glucose limited minimal medium we failed to pick up any transconjugants carrying the pSC101 plasmid.

In our consideration of the kinetics of conjugative plasmid transfer (Levin *et al.* 1979) we stated that we did not believe that the fit of a simple mass action model offers very much information about the detailed mechanics of conjugative plasmid transfer. In the same way we do not believe that the apparent fit of a mass action model in the present investigation offers very much information about the mechanics of mobilization transfer of nonconjugative factors. Nevertheless, we do see this as a particularly useful relationship. From the perspective of population genetics, one value of this apparent fit of a simple mathematical model lies in the suggestion that analogous models can be employed for studies of the population biology and epidemiology of non-selftransmissible extrachromosomal elements. Models of this type could be used to evaluate the relative roles of selection and infectious transmission in the maintenance of nonconjugative plasmids in bacterial populations (Levin & Stewart, 1979) or to assess the contamination risk associated with the use of particular nonconjugative plasmids as cloning vectors in recombinant DNA procedures. Also of some interest from the perspective of population genetic theory is the suggestion that mass action assumptions of the type used here may also be applied to the mobilization of chromosomal genes in the con-

struction of models of infectious gene exchange and recombination in steady-state populations of bacteria.

This apparent fit to a simple mass action model is also useful for comparative studies of plasmid transmission rates and studies of factors affecting these rates. In this model, the rate of infectious transmission of the nonconjugative factor is defined with as few as three independent parameters, the transfer rate constant, γ, and any two proportions, α_D, α_M, and α_{MD}. As we pointed out in Levin *et al.* (1979), for a given set of physical culture conditions, the transfer rate constant, γ, is relatively insensitive to either absolute density or the relative frequency of donors and recipients. The present results suggest that the relative proportions of the different types of transconjugants produced in matings between mobilized donors and recipients would be similar in both rapidly and slowly dividing populations.

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