

A STOCHASTIC APPROACH TO THE KINETICS OF CONJUGATIVE TRANSFER OF BACTERIAL PLASMIDS

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A stochastic model is proposed for the kinetics of conjugative transmission of bacterial plasmids based on a Markov process with discrete states in continuous time. The kinetics of plasmid transfer in populations of *Escherichia coli* could be reasonably well approximated by the model. Using the stochastic model, rate constants of plasmid transfer could be estimated for three selftransmissible (R100, R100-1, RP4) plasmids and one non-selftransmissible, mobilizable (RSF2124) plasmid. The statistical significance of estimates of transfer rate constants is discussed, employing the normal distribution for the setting of significant level.

Introduction

Bacterial plasmids are supercoiled DNA molecules which are commonplace in numerous bacterial species. Many bacterial plasmids can move from one host to another via conjugation⁹⁾. Plasmid transmissibility allows exchange of genes among bacteria; it is an important means for *in vivo* gene manipulation, particularly in plant genetic engineering. The population dynamics of bacterial plasmids has occasionally been discussed^{6,7,10)}. Levin and co-workers¹⁰⁾ have developed a deterministic model for conjugative plasmid transfer based on mass action kinetics. Freter

and co-workers⁷⁾ have extended the model to estimate plasmid-transfer rate constants under the environmental conditions of mammalian guts. However, the conjugative transmission of plasmids is a complex process and is by nature stochastic rather than deterministic¹⁾. Collision between donors and recipients occurs at random. After the random collision between a donor and a recipient one can only predict the transfer of a plasmid with a certain probability in a given time interval. This is specially evident when the fertility of the plasmid, i.e. the intrinsic ability to transfer itself, is low. Therefore, it appears appropriate to investigate the kinetics of plasmid transfer from the stochastic viewpoint. Though many works have been reported on mathematical models for population

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dynamics of bacteria^{4,8,12}), only a few studies have employed stochastic approaches^{2,5}); no appropriate stochastic model is available for kinetic analyses of plasmid transmission. In this paper we explore some of the stochastic properties of the kinetics of conjugative plasmid transfer among bacterial strains.

1. Stochastic model

Consider a liquid culture containing plasmid-carrying donors and plasmid-free recipients. The transfer of plasmids from donors to recipients forms transconjugants. After accepting the plasmid, the transconjugant can also transfer it to plasmid-free recipients. The transmission of plasmids from transconjugants to recipients is called retransfer. The transconjugants can be distinguished phenotypically from both the donors and the recipients. When the concentration of transconjugants is $X(t)$ at time t , the possible transitions of $X(t)$ may be classified as follows:

- Chance of one birth = $g(t)X(t)\Delta t + o(\Delta t)$
- Chance of one death = $d(t)X(t)\Delta t + o(\Delta t)$
- Chance of retransfer = $\lambda(t)X(t)\Delta t + o(\Delta t)$
- Chance of more than one of these events = $o(\Delta t)$
- Chance of no change = $1 - \{g(t) + d(t) + \lambda(t)\}X(t)\Delta t + o(\Delta t)$

where $g(t)$ = the specific rate of growth at time t , $d(t)$ = the specific rate of death at time t and $\lambda(t)$ = the specific rate of plasmid retransfer at time t . Within the time interval $(t, t + \Delta t)$ the probability of transconjugant formation due to plasmid transfer from donors is

- Chance of transfer = $v(t)\Delta t + o(\Delta t)$
- Chance of no transfer = $1 - v(t)\Delta t + o(\Delta t)$

where $v(t)$ is the rate of plasmid transfer from donors at time t . Obviously $v(t)$ is a function of concentrations of donors and recipients as will be shown later. Let $p(x, t)$ be the probability that the concentration of transconjugants is x at any given time t . The stochastic model for $p(x, t)$ is a Markov process with discrete states in continuous time. The concentration of transconjugants is subject to a non-homogeneous birth-death-immigration process³. From the Kolmogorov forward equations (Appendix 1), we can readily deduce the equation for

$$F(s, t) = \sum_{x=0}^{\infty} p(x, t)s^x$$

$$\frac{\partial F(s, t)}{\partial t} = [\{\lambda(t) + g(t)\}s^2 - \{\lambda(t) + g(t) + d(t)\}s + d(t)] \frac{\partial F(s, t)}{\partial s} + v(t)(s-1)F(s, t) \quad (1)$$

If we start off with $X(0) = a$, the initial condition is

$$F(s, 0) = s^a \quad (2)$$

Eqs. (1) and (2) can be solved along the lines of Bailey³ (Appendix 2).

$$F(s, t) = \{Q(s, t; \theta)\}^a \exp \int_0^t v(\tau) \{Q(s, t; \tau) - 1\} d\tau \quad (3)$$

where

$$Q(s, t; \theta) = 1 + 1/R(s, t; \theta) \quad (4)$$

$$R(s, t; \theta) = \exp\{\rho(\theta, t)\}/(s-1) - \int_0^t g(\tau) \times \exp\{\rho(\theta, \tau)\} d\tau \quad (5)$$

$$\rho(\theta, t) = \int_0^t \{g(\tau) + \lambda(\tau) - d(\tau)\} d\tau \quad (6)$$

Under the conditions that the concentration change due to growth or death can be neglected and transfer and retransfer of plasmids are assumed to be independent of time, the formation of transconjugants is considered subject to a homogeneous process and the generating function of $p(x, t)$ with the initial condition $p(0, 0) = 1$ is reduced to

$$F(s, t) = [\exp(\lambda t) - \{\exp(\lambda t) - 1\}s]^{-y} \quad (7)$$

where $y = v/\lambda$; v is the time-independent rate of plasmid transfer. λ is the time-independent specific rate of plasmid retransfer. Eq. (7) is the generating function for the negative binominal distribution. We can expand Eq. (7) as a power series in s and the coefficients of s give

$$p(x, t) = [\Gamma(x+y)/\{\Gamma(x+1)\Gamma(y)\}](\varepsilon/y)^x \times \{1 + (\varepsilon/y)\}^{-(x+y)} \quad (8)$$

where $\Gamma(x) = (x-1)!$; ε = mean of $X(t)$. The mean and variance of $X(t)$ can be obtained by the differentiation of Eq. (7) by s and then

$$\varepsilon(x, t) = y\{\exp(\lambda t) - 1\} \quad (9)$$

$$\sigma^2(x, t) = \varepsilon(x, t) \exp(\lambda t) \quad (10)$$

If the retransfer of plasmids from transconjugants can be neglected, i.e. $\lambda t \ll 1$, Eqs. (9) and (10) are

$$\varepsilon(x, t) = vt \quad (11)$$

$$\sigma^2(x, t) = vt \quad (12)$$

Moreover, if $y \gg x$, the probability distribution of Eq. (8) becomes approximated by the Poisson distribution

$$p(x, t) = \{(vt)^x/(x!)\} \exp(-vt) \quad (13)$$

2. Experimental

Strains of *Escherichia coli* K12 are used as donors and recipients. Four plasmids were studied: R100,

R100-1, RP4 and RSF2124. RP4 (Ap-Tc), R100 (Cm-Sm-Tc) and its permanently derepressed transfer mutant R100-1 are selftransmissible R factors. RSF 2124 (Ap) is a non-selftransmissible, mobilizable plasmid. The conjugative transfer of RSF2124 was performed employing pRK2013 (Km) as a helper plasmid. In all experiments a nalidixic acid-resistant mutant of HB101 was used as the recipient strain. Donor and recipient cells were separately grown overnight at 37°C in L-broth. The plasmid-transfer experiments were performed in test tubes containing 10 ml of L-broth with gentle shaking at 37°C. The matings were initiated by mixing the fresh overnight cultures of donors and recipients. Viable cell densities of the different types of cells were estimated by diluting and plating on L agar containing a variety of antibiotics. When possible, dilutions were chosen so that there would be between 100 and 200 colonies on the sampling plates. The concentrations of antibiotics used for the selective agars were after Maniatis and co-workers¹¹.

3. Results and Discussion

3.1. Stochastic properties of plasmid transmission

Time courses of transconjugant formation due to conjugative transfer of R100-1 and R100 are shown in Fig. 1. Because of high bacterial cell densities, the concentrations of donors and recipients were nearly unchanged during the short-term transfer experiments. Only the concentrations of transconjugants increased due to transfer and retransfer of plasmids. The process of transconjugant formation was stochastic rather than deterministic. To illustrate this, we performed experiments where the mixture of donors and recipients were dispensed into 30 separate test tubes and the mating was allowed to proceed for 6 h (Fig. 2). The concentrations of transconjugants fluctuated from tube to tube and the dispersion became greater as the mating proceeded. For the sake of comparison 30 samples were taken from one of the 30 tubes at $t=6$ h (Fig. 2D). Clearly, the fluctuation about the mean was larger in Fig. 2C than Fig. 2D, though both the data were taken at the same time of $t=6$ h, showing that the stochastic fluctuation far surpassed the sampling errors. The stochastic fluctuation relative to the mean was more prominent for the slow transfer of R100 and RSF2124 than the derepressed R100-1 and RP4 (data not shown). These results indicated that the elements of chance and variation should be considered, particularly for analyzing the kinetics of slow plasmid transfer.

3.2 Determination of transfer rates

The rate of transconjugant formation was clearly dependent on the concentration of donors, provided that the density of recipient cells was constant (Fig. 3). Apparently the concentration of transconjugants

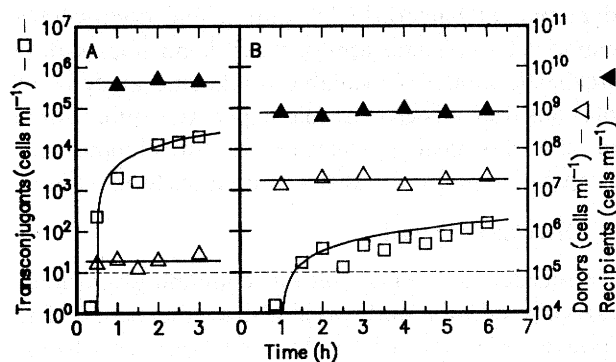


Fig. 1. Time courses of transconjugant formation due to transfer and retransfer of plasmids in matings between JE170 (R100-1) and HB101 nal^r (A) and JE51 (R100) and HB101 nal^r (B). The dotted line shows the limit of detection of transconjugant formation

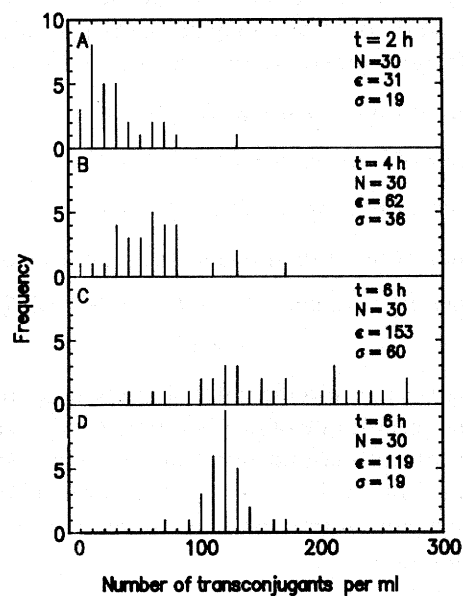


Fig. 2. Frequency distributions of the concentrations of transconjugants in matings between JE51 (R100) and HB101 nal^r. The samples were taken from a set of 30 separate test tubes at $t=2$ h (A), 4 h (B) and 6 h (C). For comparison 30 samples were taken from one of the 30 tubes at $t=6$ h (D). N: total number of samples; ϵ : mean; σ : standard deviation

increased in proportion to the concentration of donors up to 10^7 cells ml⁻¹. We interpret the results of this experiment as indicating that the kinetics of plasmid transmission can be approximated by a mass action formula such as previous authors employed^{7,10}. Therefore, the rates of plasmid transfer and retransfer were given by

$$v(t) = \gamma_{ab} C_{a0} C_{b0} \quad (14)$$

$$\lambda(t)X(t) = \gamma_{cb} C_{b0} X(t) \quad (15)$$

where γ_{ab} = transfer rate constant (ml cells⁻¹ h⁻¹), γ_{cb} = retransfer rate constant (ml cells⁻¹ h⁻¹), C_{a0} = concentration of donors (cells ml⁻¹) and C_{b0} = concentration of recipients (cells ml⁻¹). Transfer ex-

Table 1. Probability rate of conjugal plasmid transfer

	R100-1		R100		Plasmid		RP4		RSF2124 ^a	
C_{a0} (cells ml ⁻¹)	2×10^5		2×10^7				2×10^5		1×10^7	
C_{b0} (cells ml ⁻¹)	1×10^9		1×10^9				1×10^9		1×10^9	
Sampling time (h)	0.5	1	1.5	3	1	2	2	4		
$m \pm SD^b$ (cells ml ⁻¹)	170 ± 3.3	840 ± 40	32 ± 4.1	110 ± 7	130 ± 6	520 ± 29	23 ± 3.3	67 ± 4.4		
$\varepsilon \pm \sigma^c$ (cells ml ⁻¹)	160 ± 12	800 ± 28	40 ± 6.3	100 ± 10	150 ± 12	450 ± 21	20 ± 4.4	60 ± 7.7		
γ_{ab} (ml cell ⁻¹ h ⁻¹)	8×10^{-12}		2×10^{-15}		3×10^{-12}		2×10^{-15}			

^a mobilization by a helper plasmid pRK2013

^b sampled mean concentration of transconjugants \pm standard deviation

^c mean \pm standard deviation calculated from the stochastic model (Eqs. (11) and (12))

C_{a0} : concentration of donors; C_{b0} : concentration of recipients

γ_{ab} : probability rate constant of plasmid transfer

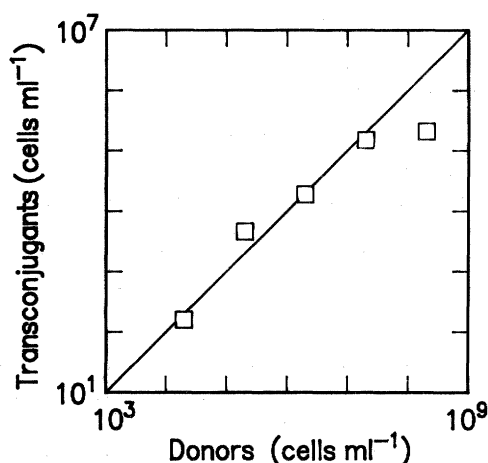


Fig. 3. Dependence of the rate of transconjugant formation on the concentration of donors. Concentration of recipients was 10^9 cells ml⁻¹. Donors: JE170 (R100-1); recipients: HB101 nal^r. Samples were taken one h after start of mating.

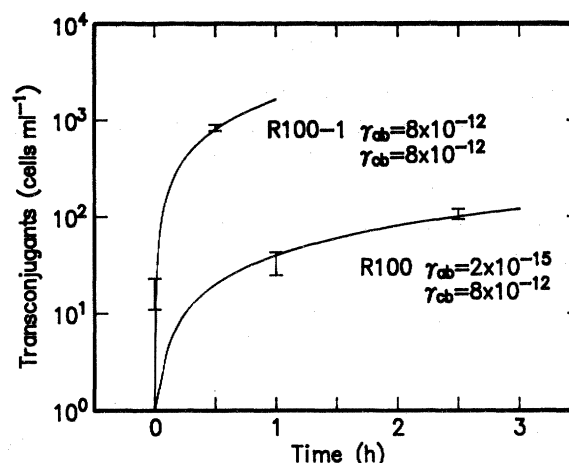


Fig. 4. Comparison between observed and calculated concentrations of transconjugants. Vertical bars are the 99% confidence intervals for sampled means. Donors: JE170 (R100-1) 2×10^5 cells ml⁻¹ and JE51 (R100) 2×10^7 cells ml⁻¹; recipients: HB101 nal^r 1×10^9 cells ml⁻¹.

periments were carried out with high cell densities and were finished within at most several hours, where the growth of bacterial cells was negligible. Therefore, C_{a0} and C_{b0} were assumed to be constant and the rate of plasmid transfer $v(t)$ and the specific rate of plasmid retransfer $\lambda(t)$ were considered to be independent of time.

Figure 4 shows a comparison between data and model fit for the experiments with R100 and R100-1. The vertical bars in Fig. 4 indicate 99% confidence intervals for the sampled means while the theoretical lines are generated by Eq. (9), assuming the values of γ_{ab} and γ_{cb} . For the sake of simplicity, the statistical fluctuation about the calculated mean is omitted in the figure. A comparison between measured and

calculated fluctuations is shown in **Table 1**. Since the transfer of R100-1 plasmid is permanently derepressed, the rate constants for transfer and retransfer are assumed to be equal. The retransfer of the R100 plasmid is transiently derepressed after entering the recipients. Thus the rate constant of retransfer of R100 plasmid is assumed to be equal to that of R100-1. As seen from this figure, the kinetics of plasmid transfer can be reasonably well approximated by the model (Eq. (9)). The concentration of transconjugants appeared to be independent of the retransfer of plasmids. The theoretical lines generated by Eq. (9) with and without consideration of retransfer of plasmids contributed little to the formation of transconjugants (data not shown). The transfer rate

constants γ_{ab} , and the mean and variance calculated by Eqs. (11) and (12) are summarized in Table 1.

3.3 Statistical test of transfer rate estimate

To test statistically the significance of estimates of transfer rate constants in Table 1, we define

$$P(L_1 \leq x \leq L_2, t) = \sum_{x=L_1}^{L_2} p(x, t) = 0.99 \quad (17)$$

This means that the probability P that the concentration of transconjugants $X(t)$ will be within the indicated boundary values L_1 and L_2 at time t is 0.99. Because the probability distribution of Eq. (13) approaches the normal distribution at t increases,

$$L_1 = \varepsilon - 2.58\sqrt{\varepsilon} \quad (18)$$

$$L_2 = \varepsilon + 2.58\sqrt{\varepsilon} \quad (19)$$

Consider three populations with different transfer rate constants $k\gamma_{ab}$, γ_{ab} and $(1/k)\gamma_{ab}$. Since ε can be calculated from Eq. (11), if

$$kvt - 2.58\sqrt{kvt} > vt + 2.58\sqrt{vt} \quad (20)$$

$$vt - 2.58\sqrt{vt} > (1/k)vt + 2.58\sqrt{(1/k)vt} \quad (21)$$

the regions of $P=0.99$ for the three populations do not overlap one another. If that is the case, one can say that the value of γ_{ab} estimated from a transfer experiment differs from $k\gamma_{ab}$ and $(1/k)\gamma_{ab}$ with the 99% confidence level. Solving the inequalities of (20) and (21) yields

$$vt > \frac{6.66k}{(k-1)^2} \quad (22)$$

Therefore

$$vt > 77.6 \quad (k=2) \quad (23)$$

$$vt > 14.2 \quad (k=10) \quad (24)$$

Thus the condition that the orders of magnitude of estimated γ_{ab} are considered to be statistically significant at 1% significance level ($k=10$) is given by the inequality (24). In Table 1 $\varepsilon = \gamma_{ab}C_{a0}C_{b0}t = vt > 14.2$ for all the mating experiments. Therefore, it is concluded that the orders of magnitude of estimated transfer rate constants are statistically significant at 1% significance level. The values of transfer rate constants for R100-1 and RP4 are apparently more accurate than those of R100 and RSF2124. Since $vt = 160 > 77.6$ for R100-1 at $t = 1$ h, we could expect that the transfer rate constant for R100-1 lay somewhere between $\gamma_{ab} \times 0.5$ and $\gamma_{ab} \times 2$ or between 4×10^{-12} and 1.6×10^{-11} ml cells $^{-1}$ h $^{-1}$.

Appendix 1

Taking into account the possible transitions of $X(t)$, the Kolmogorov forward equation is

$$\begin{aligned} \frac{dp(x, t)}{dt} = & g(t)(x-1)p(x-1, t) - g(t)xp(x, t) \quad \text{growth} \\ & + d(t)(x+1)p(x+1, t) - d(t)xp(x, t) \quad \text{death} \\ & + v(t)p(x-1, t) - v(t)p(x, t) \quad \text{transfer} \\ & + \lambda(t)(x-1)p(x-1, t) - \lambda(t)xp(x, t) \quad \text{retransfer} \end{aligned} \quad (\text{A-1})$$

When we introduce the generating function, $F(s, t) = \sum_{x=0}^{\infty} p(x, t)s^x$, Eq. (A-1) becomes Eq. (1) in the text.

Appendix 2

Consider the case where plasmid transfer from donors is absent, i.e. $v(t)=0$. In this case the generating function $G(s, t)$ becomes

$$\frac{\partial G(s, t)}{\partial t} = [\{\lambda(t) + g(t)\}s^2 - \{\lambda(t) + g(t) + d(t)\}s + d(t)] \times \frac{\partial G(s, t)}{\partial s} \quad (\text{A-2})$$

with the initial condition

$$G(s, 0) = s^a \quad (\text{A-3})$$

The partial difference equation (A-2) can be solved by the usual procedure, employing the subsidiary equation:

$$\begin{aligned} \frac{dt}{1 - \frac{-ds}{\{\lambda(t) + g(t)\}s^2 - \{\lambda(t) + g(t) + d(t)\}s + d(t)}} \\ = \frac{dG(s, t)}{0} \end{aligned}$$

Then the solution of Eq. (A-3) is

$$G(s, t) = 1 + \{1/R(s, t; 0)\}^a \quad (\text{A-4})$$

where $R(s, t; 0)$ was defined as Eq. (5) in the text.

Suppose that the probability-generating function at time t for the descendants of the transconjugants formed by a plasmid transfer between a donor and a recipient at time θ ($\theta \leq t$) is $Q(s, t; \theta)$. Then for a transconjugant existing at time $\theta=0$, we have

$$Q(s, t; 0) = G(s, t) \quad (\text{A-5})$$

Consider the interval $(\theta, \theta + \Delta\tau)$. The change of a plasmid transfer is $v(\theta)\Delta\tau$, and the generating function for the descendants of such a transconjugant at time t is $Q(s, t; \theta)$. The chance of no plasmid transfer is $1 - v(\theta)\Delta\tau$. Therefore the generating function at time t , related to the interval $(\theta, \theta + \Delta\tau)$ is

$$v(\theta)\Delta\tau Q(s, t; \theta) + \{1 - v(\theta)\Delta\tau\} = 1 + v(\theta)\Delta\tau\{Q(s, t; \theta) - 1\}$$

Then the generating function at time t over the whole range $(0, t)$ can be expressed as

$$\begin{aligned} F(s, t) = \{Q(s, t; 0)\}^a \lim_{\Delta\tau \rightarrow 0} \prod [1 + v(\theta)\Delta\tau\{Q(s, t; \theta) - 1\}] \\ = \{Q(s, t; 0)\}^a \exp \int_0^t v(\tau)\{Q(s, t; \tau) - 1\} d\tau \end{aligned}$$

Nomenclature

a	= initial concentration of transconjugant	[cells ml $^{-1}$]
C	= donor or recipient concentration	[cells ml $^{-1}$]
d	= specific death rate	[h $^{-1}$]
F	= probability-generating function	[—]
g	= specific growth rate	[h $^{-1}$]
p	= probability of transconjugant concentration	[—]
t	= time	[h]
X	= transconjugant concentration	[cells ml $^{-1}$]

γ	= plasmid transfer rate constant	[ml cells ⁻¹ h ⁻¹]
ε	= mean concentration	[cells ml ⁻¹]
λ	= specific plasmid retransfer rate	[h ⁻¹]
ν	= plasmid transfer rate	[h ⁻¹]
σ	= standard deviation of concentration	[cells ml ⁻¹]

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