since feeding in normal humans stimulates both pancreatic and bile secretions into the duodenum (*7*). This last suggestion, although somewhat contrary to the advice given in the National Institutes of Health recombinant DNA research guidelines, may prove to be a safer practice when conducting recombinant DNA research with disabled hosts such as χ1776.

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10. The rats were provided by J. McGhee. Supported by NSF grant GB-37546 and NIH grants AI-11456 and DE-02670.

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Probability of Establishing Chimeric Plasmids in Natural Populations of Bacteria

Abstract. *Formulas for estimating the probability that chimeric plasmids carried by disarmed hosts will become established in natural populations of bacteria are presented and their use illustrated with a series of realistic numerical examples. The implications of these a priori probability estimates for the problem of containment for recombinant DNA research is discussed.*

At the very best, physical containment will retard the rate at which the bacteria, phage, and plasmids used in recombinant DNA research enter the environment. Some, and potentially great numbers, of these cloning vectors will escape. However, escape from containment does not necessarily imply that these renegade organisms and plasmids or their chimeric DNA will become established in natural populations of bacteria, or that carriers of this synthetic genetic material will achieve and maintain sufficient densities to represent a threat to existing communities. This contamination of natural populations of bacteria with chimeric DNA ultimately depends on the population biology of the cloning vectors. In recognition of the need for biological containment, "disarmed" strains of bacteria with low fitness have been developed, and phage and plasmids with limited infectious capabilities have been identified for use in recombinant DNA research (*1*). Although there is no question that employing the recommended cloning vectors offers a much lower likelihood of contaminating natural populations with chimeric DNA than that associated with the use of normal laboratory strains, just what this contamination probability is remains uncertain. We believe that there are two difficulties with the estimates of it that have been offered (*1, 2*): (i) failure of the estimation procedure to consider the population dynamics of the contamination process and (ii) inability to obtain accurate estimates of some of the parameters necessary to compute the contamination probability.

In this report we present a stochastic model of the establishment of a plasmid carried by a disarmed host into a natural population of bacteria. We do consider the population dynamics of the contamination process and, we believe, reasonably accurate estimates of all the parameters of this model could be obtained. Although we restrict this formal consideration to conjugative factors, the theory is appropriate to certain situations where nonconjugative plasmids can be mobilized (*3*). We present formulas for the probability of establishment of a chimeric plasmid and illustrate their use with a series of realistic numerical examples.

In this treatment we define establishment as the indefinite persistence of the chimeric plasmid in a natural population of bacteria. That is, bacteria carrying that plasmid will continue to exist and increase in numbers after all of the original, disarmed, hosts are eliminated. What densities "wild" bacteria carrying that plasmid will maintain and whether these factors will persist if the plasmid reduces the fitness of its host cell have been considered elsewhere (*4*).

Consider the release of m_0 disarmed, plasmid-bearing bacteria into a natural population containing r (bacteria per milliliter) potential hosts of that plasmid. The original hosts are incapable of growth in the natural habitat and, in the course of time, die or effectively die by losing the plasmid (segregation) or the capacity to transmit it. Let the probability of any particular disarmed plasmid-carrying bacterium dying or becoming infertile during the short period δt be $d\delta t$. We assume that all the wild bacteria have the same rates of cell division and cell mortality and that these rates are not influenced by carrying the chimeric plasmid. Let the probabilities of a particular wild bacterium dividing or dying during the period δt be $b\delta t$ and $e\delta t$, respectively. We further assume that transmission of the plasmid by conjugation occurs at random at a frequency that is proportional to the concentration of potential recipients. We let the probability that a single disarmed host will transmit the plasmid during time δt be $g\delta t = \gamma_d r\delta t$, where γ_d (milliliters per cell per hour) is the conjugational transfer rate constant (*4*). The analogous transmission probability for a single wild bacterium carrying that factor is $h\delta t = \gamma_h r\delta t$.

With the above definitions and assumptions, the establishment of the plasmid can be regarded as a population process (*5*) to be analyzed by means of generating functions. Using this approach, and after some relatively straightforward, albeit cumbersome, calculations, we concluded that the probability that the plasmid will be lost after the escape of a *single*, disarmed plasmid-bearing cell is

$$P_{loss} = \frac{d(b + h)}{d(b + h) + g(b + h - e)} \quad (1)$$

and hence the probability that the plasmid will become established is

$$P_{est} = 1 - P_{loss} = \frac{g(b + h - e)}{d(b + h) + g(b + h - e)} \quad (2)$$

The calculations that formally prove these results can be obtained from (*6*). In this report we restrict the justification of this probability estimator to an intuitive argument.

With biologically realistic values of the

parameters, the establishment probability is small and Eq. 2 can be very closely approximated by

$$P_{est} \approx \frac{g}{d} \cdot \frac{b + h - e}{b + h} \quad (3)$$

In this form, the formula more closely approximates one's biological intuition. The first factor, g/d, is the probability that a copy of the plasmid will be transmitted from the original, disarmed host to a wild cell before the original host dies or becomes infertile. The second factor, $(b + h - e)/(b + h)$, is the conditional probability that the wild bearer of this single copy will leave copies of the plasmid in all subsequent generations, given that the copy was transmitted to some wild bacterium. The quotient $1/d$ is the average persistence time of the disarmed host, so g/d is the expected number of copies of that plasmid that will be transmitted by the disarmed host before it dies or becomes infertile. However, it is most unlikely that it will transmit more than one copy during this period. Consequently, this average number of transmitted copies is very nearly the probability that it will transmit one copy. The second factor, $(b + h - e)/(b + h)$, is analogous to the probability that a family name will survive indefinitely in Galton's classical problem (*7*). In this situation the plasmid is the analog of the family name. When a change in the number of plasmid copies occurs, it occurs either because one copy has doubled (by cell division or conjugational transmission) or because the plasmid is lost (due to its host's death or infertility), the probability of doubling being $B = (b + h)/(b + h + e)$ and the probability of loss being $D = e/(b + h + e)$. The probability that the plasmid will be established, given that a wild bacterium has acquired it, is then

$$\frac{B - D}{B} = \frac{b + h - e}{b + h}$$

If, instead of a single disarmed, plasmid-bearing bacterium, a number m_0 escaped, establishment of the plasmid would be a consequence of m_0 stochastically independent trials. The probability of establishment is then

$$P_{est|m_0} = 1 - (P_{loss})^{m_0}$$
$$= 1 - (1 - P_{est})^{m_0}$$

which, for the anticipated small values of P_{est}, is

$$P_{est|m_0} \approx 1 - \exp(-m_0 P_{est}) \quad (4)$$

For very low values of P_{est}

$$P_{est|m_0} \approx m_0 P_{est}$$

Table 1. Probability that chimeric plasmids will be established in natural populations of bacteria following the release of different numbers of disarmed carriers. The parameters are g, the product of the concentration of potential recipients, r, and the transfer rate constant of the plasmid in a disarmed host, γ_d; h, the product of r and the transfer rate constant of the plasmid in a wild host, γ_n; d, the death rate of disarmed cells; b, the probability of a wild bacterium dividing; and e, the probability of a wild bacterium dying. The choice of these parameter values is justified in the text. The probability of establishment is computed for single cells ($m_0 = 1$) from Eq. 2 and extended for the release of multiple cells ($m_0 > 1$) with Eq. 4.

Parameter values			Probability of establishment			
g,h	d	b,e	$m_0 = 1$	$m_0 = 10^2$	$m_0 = 10^5$	$m_0 = 10^8$
5×10^{-2}	0.58	0.20	1.7×10^{-2}	8.2×10^{-1}	1.0*	1.0*
		0.02	5.8×10^{-2}	1.0*	1.0*	1.0*
	2.30	0.20	4.3×10^{-3}	3.5×10^{-1}	1.0*	1.0*
		0.02	1.5×10^{-3}	8.6×10^{-1}	1.0*	1.0*
5×10^{-4}	0.58	0.20	2.1×10^{-6}	2.1×10^{-4}	7.0×10^{-1}	1.0*
		0.02	2.1×10^{-5}	2.1×10^{-3}	8.7×10^{-1}	1.0*
	2.30	0.20	5.4×10^{-7}	5.4×10^{-5}	5.3×10^{-2}	1.0*
		0.02	5.3×10^{-6}	5.3×10^{-4}	4.1×10^{-1}	1.0*
5×10^{-5}	0.58	0.20	2.2×10^{-8}	2.2×10^{-6}	2.1×10^{-3}	8.8×10^{-1}
		0.02	2.2×10^{-7}	2.2×10^{-5}	2.1×10^{-2}	1.0*
	2.30	0.20	5.4×10^{-9}	5.4×10^{-7}	9.4×10^{-4}	4.2×10^{-1}
		0.02	5.4×10^{-8}	5.4×10^{-6}	5.4×10^{-3}	9.9×10^{-1}
5×10^{-7}	0.58	0.20	2.2×10^{-12}	2.2×10^{-10}	2.2×10^{-7}	2.2×10^{-4}
		0.02	2.2×10^{-11}	2.2×10^{-9}	2.2×10^{-6}	2.2×10^{-3}
	2.30	0.20	5.4×10^{-13}	5.4×10^{-11}	5.4×10^{-8}	5.4×10^{-5}
		0.02	5.4×10^{-12}	5.4×10^{-10}	5.4×10^{-7}	5.4×10^{-4}

*The probability of this event exceeds .995.

The numerical values used to generate the examples presented in Table 1 were chosen to illustrate the relative contributions of the different parameters to the establishment probability. However, these sample parameter values are within a realistic range for natural populations of enteric bacteria and some of their conjugative plasmids. In these examples, the conjugational transfer rate constants of the plasmid in the disarmed host and in the wild host are equal, $\gamma_d = \gamma_n = \gamma$. The two values of γ used, 5×10^{-10} and 5×10^{-13} ml per cell per hour, are, respectively, the value we estimated for an F-lac$^+$pro$^+$ plasmid in an *Escherichia coli* K12 host growing exponentially in glucose-limited minimal medium at 37°C, and the value for these bacteria at equilibrium in chemostats at a dilution rate of 0.2 hour^{-1} with that medium and at that temperature. We used two values for the concentration of potential recipients $r = 10^6$ and $r = 10^8$ cell/ml. The first is the density considered by Curtiss and co-workers (*1*, *2*) in their estimates of the contamination probability. However, we believe that the higher value, $r = 10^8$, is realistic for some natural populations of enteric bacteria. The two mortality probabilities for disarmed bacteria that we used, $d = 0.58$ and $d = 2.30$ hour^{-1}, are those estimated by Curtiss and his colleagues (*8*) for EK2 (strain χ1776) in minimal medium and in broth without the specific EK2 supplements, respectively. In these examples we consider the natural population to be at a steady state so that the probability of a wild bacterium dividing is equal to the probability of a wild cell dying, $b = e$. The two values of these parameters used, 0.20 and 0.02 hour^{-1}, specify steady-state generation times of 5 and 50 hours, respectively.

In interpreting the estimates presented in Table 1, it is necessary to consider that the probabilities of establishment given are those for single escape events. The inadvertent release of bacteria carrying chimeric plasmids is, however, an event that can be anticipated to occur many times and from many different laboratories doing recombinant DNA research. Nevertheless, even for single escape events, the establishment probabilities for the conjugative plasmids considered in the numerical examples above are extremely high. These results unquestionably support the existing ban on using conjugative plasmids as cloning vectors. These theoretical considerations also illustrate the utility of efforts at biological containment. In the range of parameter values we anticipate for most recombinant DNA research, the probability of establishment of the chimeric plasmid is directly proportional to the fertility of the host-plasmid combination, as measured by the transfer rate constant, γ_d, and inversely proportional to the death rate of the host cell, d. An order of magnitude decrease in this fertility or an order of magnitude increase in the death rate of the disarmed host is reflected by an order of magnitude decline in

the establishment probability. These results also illustrate the value of combining biological containment with strict physical containment. In the anticipated parameter range, the establishment probability is directly proportional to the number of plasmid-carrying bacteria released into the environment.

Since the rate of transfer of nonconjugative plasmids is proportional to the density of bacteria carrying mobilizing conjugative plasmids (*1*, *2*), the model used here is not appropriate as a general analog of this situation. However, this model and the probability estimators derived from its analysis do apply to two special cases of nonconjugative plasmids. (i) In steady-state natural populations it is reasonable to assume that the frequency of bacteria carrying potentially mobilizing plasmids would be relatively constant and, as a result, the average transfer rate parameters, γ_d and γ_n, would also be relatively constant. Although it is reasonable to assume that, in general, these "mobilization-transfer" rate parameters would be lower than the analogous parameters for conjugative plasmids, there is evidence that for some nonconjugative plasmids transfer by mobilization can occur at substantial rates (*2*). (ii) It is conceivable that chimeric DNA carried by a nonconjugative plasmid will become incorporated into a conjugative factor. After such an event, the probabilities that chimeric DNA will persist could well be as high as or even higher than those presented in the numerical examples. At this juncture it is not at all clear just how great the probability is that DNA from a nonconjugative plasmid will be permanently incorporated into a conjugative factor. It is, however, our contention that estimates of this probability are essential to a full evaluation of the dangers of recombinant DNA research.

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Interbacterial Transfer of *Escherichia coli*–*Drosophila melanogaster* Recombinant Plasmids

Abstract. *Recombinants were constructed between various* Escherichia coli *plasmids and fragments of* Drosophila melanogaster *DNA. These recombinant plasmids are nonconjugative, but can be mobilized from one cell to another by conjugative sex factors. Of 47 recombinants studied, 46 were mobilized at approximately the same or slightly lower frequencies than the parental plasmids, whereas one was mobilized 1000 times less efficiently.*

Most of the plasmids used as molecular cloning vehicles are nonconjugative; that is, they are not transferred from one cell to another under normal mating conditions. However, when a cell harboring a nonconjugative plasmid also carries a conjugative sex factor, the nonconjugative plasmid can be passively transferred to an appropriate recipient. This process, referred to as mobilization, is one possible route by which recombinant plasmids might be disseminated in animal and human populations (*1*).

Does foreign DNA inserted into a plasmid affect its mobilization? To approach this question, I determined mobilization frequencies for a series of recombinants between various *Escherichia coli* plasmids and fragments of *Drosophila melanogaster* DNA. Of 47 recombinants studied, 46 were mobilized with approximately the same or slightly lower efficiencies than the parental plasmids, whereas one was mobilized very poorly. The low mobilizability of the latter recombinant was shown to be due to some specific effect of the inserted *D. melanogaster* DNA.

The first determinations were made on recombinants between pSC101 (*2*), a tetracycline resistance factor that replicates under stringent control, and Eco RI fragments of *D. melanogaster* DNA. Preliminary experiments showed that pSC101 could be mobilized readily by the *Salmonella* sex factors Col Ib and I. Table 1 presents the results of triparental matings in which the donor harbored pSC101 or a pSC101/Eco RI–*D. melanogaster*/Eco RI recombinant, and the intermediate carried Col Ib or I. Of the five recombinants studied, four were mobilized at about the same frequency as the parental plasmid. However, one of the recombinants, pDm2, was mobilized 500 to 1000 times less efficiently by both sex factors.

Why is pDm2 mobilized so poorly?

Table 1. Mobilization of pSC101 and pSC101/Eco RI–*Drosophila melanogaster*/Eco RI recombinants by Col Ib and I. Triparental matings were performed by the method of Anderson and Lewis (*6*), using *E. coli* C spc^R as the final recipient. No transconjugants were observed in control matings in which one of the parents was omitted, or in which the donor carried no plasmid. Strain GM4 is *E. coli* HB101 r_B^- m_B^- pro$^-$ str^R gal$^-$ lac$^-$ ara$^-$ arg$^-$ *rec*A (*7*). The construction and characterization of the recombinant plasmids has been described (*8*).

Donor	Length of *D. melanogaster* DNA fragment (kilobase pairs)	Intermediate	Frequency of mobilization (transconjugants per donor)
GM4 (pSC101)	0	*S. typhimurium* (Col Ib)	3.0×10^{-4}
		S. panama (I)	1.4×10^{-4}
GM4 (pDm1)	4.4, 3.3	*S. typhimurium* (Col Ib)	1.2×10^{-4}
		S. panama (I)	0.8×10^{-4}
GM4 (pDm2)	3.1	*S. typhimurium* (Col Ib)	2.8×10^{-7}
		S. panama (I)	3.1×10^{-7}
GM4 (pDM3)	2.9	*S. typhimurium* (Col Ib)	1.8×10^{-4}
		S. panama (I)	1.2×10^{-4}
GM4 (pDm4)	0.3	*S. typhimurium* (Col Ib)	2.7×10^{-4}
		S. panama (I)	1.2×10^{-4}
GM4 (pDm5)	1.5	*S. typhimurium* (Col Ib)	2.8×10^{-4}
		S. panama (I)	0.9×10^{-4}