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}

Table 2. Studies on the low mobilizability of pDm2. The final recipient was *E. coli* C spc^R, except in experiment 3, in which it was *E. coli* C str^R. Strain GM3 is *E. coli* C600 r_k^- m_k^- thr$^-$ leu$^-$ lac$^-$ Bl$^-$ (*9*). The construction and characterization of the recombinant plasmids has been described (*4*, *8*).

Donor	Intermediate	Frequency of mobilization (transconjugants per donor)
	Experiment 1	
GM4 (pSC101)	*S. typhimurium* (Col Ib)	0.8×10^{-4}
GM4 (pDm2)	*S. typhimurium* (Col Ib)	1.2×10^{-7}
GM3 (pSC101)	*S. typhimurium* (Col Ib)	3.2×10^{-4}
GM3 (pDm2)	*S. typhimurium* (Col Ib)	4.8×10^{-7}
	Experiment 2	
GM4 (pSC101)	*S. typhimurium* (Col Ib)	5.2×10^{-4}
GM4 (pDm2)	*S. typhimurium* (Col Ib)	7.4×10^{-7}
GM4 (pDm2/Eco RI → pSC101)	*S. typhimurium* (Col Ib)	6.8×10^{-4}
	Experiment 3	
C spc^R (pSC101, Col Ib)		8.1×10^{-3}
C spc^R (pDm2, Col Ib)		7.4×10^{-6}
	Experiment 4	
GM4 (pSC101)	*S. typhimurium* (Col Ib)	4.2×10^{-4}
	S. panama (I)	1.6×10^{-4}
GM4 (pML21)	*S. typhimurium* (Col Ib)	1.4×10^{-4}
	S. panama (I)	0.7×10^{-4}
GM4 (pML21/Eco RI–Dm2)	*S. typhimurium* (Col Ib)	2.1×10^{-4}
	S. panama (I)	0.6×10^{-4}
GM4 (pGM437 = pSC101/Sal I–pML21/Sal I)	*S. typhimurium* (Col Ib)	0.8×10^{-2}
	S. panama (I)	1.6×10^{-2}
GM4 (pGM439 = pSC101/Sal I–pML21/Sal I)	*S. typhimurium* (Col Ib)	1.2×10^{-2}
	S. panama (I)	0.9×10^{-2}
GM4 (pGM16 = pSC101/Eco RI–pML21/Eco RI)	*S. typhimurium* (Col Ib)	1.5×10^{-2}
	S. panama (I)	0.8×10^{-2}

Table 2, experiment 1, shows that this was not due to a host mutation, nor to a specific interaction with the host strain, since the same result was obtained after transformation into a new donor. Table 2, experiment 2, shows that the low mobilizability of pDm2 was not due to a mutation in the bacterial fragment, since a pSC101 plasmid generated by Eco RI cleavage of pDm2 was mobilized at the same frequency as the original pSC101. As shown in Table 2, experiment 3, the low mobilizability of pDm2 persisted in two parent crosses in which the donor harbored both the recombinant and Col Ib. Furthermore, strains harboring pDm2 were as good recipients for Col Ib as were strains harboring pSC101 (results not shown). This rules out the possibility that pDm2 renders its host resistant to infection by the sex factor. Thus, the low mobilizability of pDm2 must be attributed to some specific effect of the inserted *D. melanogaster* DNA.

Is this effect exerted only on pSC101, or is it general? To answer this, the Dm2 fragment was translocated to pML21 (*3*), a mini Col El–kanamycin resistance factor. Table 2, experiment 4, shows that this recombinant was mobilized at the same frequency as pML21 by both Col Ib and I. This suggests that pSC101 and pML21 are mobilized by different mechanisms. Further evidence for this comes from the surprising finding that hybrids between pSC101 and pML21 are mobilized up to 100 times more efficiently than either parental plasmid (Table 2, experiment 4).

Frequencies of Col Ib–mediated mobilization were also determined for 20 recombinants carrying Sal I fragments and for 22 recombinants carrying Bam I fragments of *D. melanogaster* DNA. The Sal I fragments were cloned on pML21 (*3*), whereas the Bam I fragments were cloned on pGM16 (*4*); both of these vehicles replicate under relaxed control. All of the recombinants were mobilized 20 to 100 percent as well as the parental plasmids (results not shown).

These limited data suggest that, in general, the insertion of foreign DNA into a plasmid has little effect on its mobilizability. In the one case where a significant change was noted, it was to decrease the mobilization frequency. No cases of increased mobilizability have been noted.

The transfer experiments described in this report were conducted under conditions of low risk as advised by the "Summary statement of the Asilomar conference on recombinant DNA molecules" (*5*). More stringent containment procedures are required by the National Institutes of Health guidelines (*1*).

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