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THE EVOLUTION OF TRANSPOSABLE ELEMENTS: CONDITIONS FOR ESTABLISHMENT IN BACTERIAL POPULATIONS

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Abstract.—Previous theoretical studies have shown that bacterial transposons can become established in populations by infectious transfer, even if they reduce the fitness of their host cells. Conditions for the persistence of "parasitic" transposons are, however, restrictive: i) transposition must be replicative, rather than conservative; ii) the rate of transposition must be greater than the loss in host fitness caused by the transposon; and iii) cells must exchange plasmids at rates greater than the fitness cost of the transposon.

I sought to test the validity of the model underlying this theory by performing experiments with laboratory populations of the bacterium *Escherichia coli*, the conjugative plasmid R100, and the transposons Tn3 and Tn5. A plasmid-borne transposon was introduced at low frequency into a population of bacteria carrying the same plasmid without the transposon in a habitat where the transposon offered no benefit to its host. The fate of the invading transposon was followed by tracking the various bacterial populations appearing in the cultures. Using independent estimates of the parameters of the model, predicted population changes were generated with numerical solutions of the model, and these were compared to experimental results.

Plasmids transferred into new hosts as predicted by the model, and the resulting transconjugant populations either maintained a steady low density or rose slowly in abundance. Transposition appeared to play no role in population changes. Abundance of all cell types fit theoretical predictions of a system with no transposition, despite evidence that transposition was taking place. This is exactly what the model predicted. It thus appears unlikely that deleterious or neutral transposons have much impact on the genetics of bacterial populations. This is consistent with the hypothesis that most bacterial transposons are not parasitic DNA, but rather invade and persist in populations by providing a fitness advantage to cells carrying them.

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Transposable elements are segments of DNA that are capable of moving from one site to another in the genome of a single cell (Kleckner, 1981; Iida et al., 1983; Shapiro, 1983). From the perspective of evolutionary biology, the issue of primary interest is how transposons invade and persist in populations. Are they maintained because they enhance the fitness of their hosts, or are they "selfish" DNAs that reduce host fitness but are maintained by infectious transfer (Orgel and Crick, 1980; Doolittle and Sapienza, 1980; Campbell, 1981; Charlesworth, 1987)? In bacteria, there is evidence supporting the former view, since transposons can benefit their host cells by carrying antibiotic-resistance genes or other beneficial traits or by causing mutations at other loci (Cohen, 1976; Kleckner, 1981; Biel and Hartl, 1983; Hartl et al., 1983; Chao et al., 1983; Chao and McBroom, 1985). On the other hand, Sawyer et al. (1987) concluded that the distribution of transposable elements among strains of *Escherichia coli* is consistent with their maintenance as selfish elements, and there are many transposons for which no beneficial effect has been demonstrated.

To evaluate the plausibility of the hypothesis that bacterial transposons are parasitic DNA, Condit et al. (1988) examined conditions for their maintenance by infectious transfer in a population of bacteria and conjugative plasmids. Plasmids are important as vehicles of horizontal transfer, since by themselves transposons are capable only of intracellular movement (there are a few exceptions; see Gawron-Burke and Clewell, 1982). The results of the analysis by Condit et al. indicated that conservative transposons, which do not leave a copy behind when moving between sites, cannot become established in a population if they are neutral or deleterious. On the other hand, replicative transposons, which are copied during transfer, can invade and persist as "parasites." However, conditions allowing invasion are very restrictive: 1) a transposon cannot diminish fitness by more than its transposition rate, which is generally very

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low for bacterial transposons; 2) plasmids which are capable of transmitting themselves between bacterial cells (conjugative plasmids) must be present as vectors; and 3) the rate of plasmid exchange must exceed the fitness cost of the transposon. (Other vectors for horizontal transfer, such as bacteriophage, might replace plasmids but were not considered by Condit et al. [1988].) We interpreted such restrictive invasion conditions as evidence against the generality of the "selfish DNA" hypothesis.

While some may be willing to accept a conclusion based on purely theoretical analyses, others might question arguments lacking empirical verification. Is the model realistic? Was the range of parameter values used in its analysis appropriate for real bacteria, transposons, and plasmids? In an effort to test the accuracy of the model and the validity of its conclusions and to uncover ruses real transposons may employ to ensure their maintenance, I examined conditions for the invasion of transposons in experimental populations of *E. coli*.

The experiments were designed to mimic conditions described by the model of Condit et al. (1988). A small inoculum of bacteria with a transposon-bearing, conjugative plasmid was introduced into a culture dominated by cells carrying the same plasmid without the transposon. Culture conditions did not provide any known selective advantage for the transposon. The bacteria, plasmids, and transposon were genetically marked so that it was possible to follow changes in the frequencies of different cell lines. Parameters of the model were independently estimated, and observed population dynamics were compared to numerical solutions of the equations in Condit et al. (1988). In analyzing these data, two questions were asked: 1) did simulations based on independent parameter estimates match the observed population dynamics? 2) how large was the contribution of transposition to the fate of the invading transposon, relative to the contributions of plasmid transfer and selection?

Materials and Methods

Media, Strains, and Plasmids.—Either minimal liquid medium (7 mg ml^{-1} K_2HPO_4, 2 mg ml^{-1} KH_2PO_4, 1 mg ml^{-1} $(NH_4)_2SO_4$, 0.5 mg ml^{-1} Na citrate, 0.1 mg ml^{-1} $MgSO_4$, 2 μg ml^{-1} vitamin B1, and 0.6 mg ml^{-1} glucose) or Luria Broth (Miller, 1972) was used in all experiments. Plating was done on agar medium (16 mg ml^{-1} agar and 0.05 mg ml^{-1} antifoam B) mixed with minimal lactose medium (4 mg ml^{-1} lactose substituted for glucose) or broth (10 mg ml^{-1} tryptone, 1 mg ml^{-1} yeast extract, and 5 mg ml^{-1} NaCl). Antibiotic concentrations used in plates were: 20 μg ml^{-1} nalidixic acid, 25 μg ml^{-1} kanamycin, 25 μg ml^{-1} chloramphenicol, and 32 μg ml^{-1} ampicillin. Triphenyltetrazolium chloride (50 μg ml^{-1}) and lactose (10 mg ml^{-1}) were added to broth plates so that lactose-utilizing strains (lac^+ strains) could be distinguished from lac^- strains (Levin et al., 1977).

E. coli K12 strain CSH7 (Miller, 1972), which is lac^+, was used in population experiments. A spontaneous mutant unable to utilize lactose (lac^-) was collected, and a strain resistant to nalidixic acid was isolated from it by spreading about 10^{10} cells on a plate with medium containing nalidixic acid. The CSH7 lac^+, nalidixic-acid-sensitive strain (7^+) and its lac^-, nalidixic-acid-resistant derivative ($7n^-$) were used in all invasion experiments. At the start of each experiment, one strain carried the transposon, and the other did not.

The conjugative plasmid R100.1*drd* (Nakaya et al., 1960; Iida, 1980) was used in the population experiments. This plasmid is a derepressed mutant of the wild-type R100.1 and carries resistance to chloramphenicol; I will always refer to it simply as "R." The transposons used were Tn3 and Tn5 (Heffron et al., 1979; Beck et al., 1982). Tn3 carries resistance to ampicillin, and Tn5 carries resistance to kanamycin.

Construction of Transposon-Bearing Plasmids.—To isolate derivatives of the plasmid R that carried Tn3 and Tn5, a mating-out procedure was used (Morisato et al., 1983). First, R was transferred into strain UB1731, which carries Tn3 on the chromosome (Bennett and Richmond, 1976), and the resulting strain was used as the donor in a mating with $7n^-$. From this mating, $7n^-$ cells that acquired R were tallied on plates carrying the antibiotics chloramphenicol and nalidixic acid; $7n^-$ cells that acquired both a plasmid and Tn3 were se-

lected on plates carrying ampicillin and nalidixic acid. Colonies from the latter plates were tested for sensitivity to chloramphenicol, and a sensitive colony was used as donor in a subsequent mating to confirm that the transposon's resistance gene was transferring with the plasmid. A plasmid that transmitted ampicillin-resistance (and presumably carried Tn3) but did not carry chloramphenicol-resistance was collected and named "R3." A similar mating-out was done using Tn5, starting with strain DD1420 (Hartl et al., 1983), which carries Tn5 on the chromosome. A plasmid carrying Tn5 but which had lost chloramphenicol-resistance was isolated and named "R5."

It was necessary to use chloramphenicol-sensitive, transposon-bearing plasmids for invasion experiments in which R was the "resident" plasmid, because only then was it possible to distinguish cells with two different plasmids (heteroplasmid cells). For example, in an experimental population carrying R and R5, R-bearing cells can be selected on plates with chloramphenicol, R5 cells on kanamycin plates, and heteroplasmid cells (R and R5) on chloramphenicol-kanamycin plates. This would not be possible if the transposon-bearing plasmids still carried chloramphenicol resistance.

Maintaining Populations.—All experiments were carried out in one of two liquid-culture regimes: serial transfer or chemostats. Under a serial-transfer regime (Atwood et al., 1951), bacteria are kept in "batch" culture; they are provided with liquid medium and grow until a carbon source is exhausted. Each day, a small aliquot is transferred to a fresh batch of the resource. Batch cultures were always kept in 10 ml of liquid medium in 50-ml flasks, shaken at about 150 rpm at 37°C. For serial transfer, 0.1 ml of maximum-density cultures was transferred into 10 ml of fresh medium every day; 24 hours was more than enough time for cultures to reach saturation density.

In chemostats, a culture is maintained by constant inflow of growth medium and constant outflow of spent medium and bacteria; cell density can be maintained at equilibrium (Novick, 1955; Dykhuizen and Hartl, 1983). I used chemostats of the homemade variety described by Chao et al. (1977), with minimal glucose as the medium. Effluent was collected in flasks, and its volume was measured every 2–3 days to calculate the exact flow rate of each chemostat culture.

All experiments described below were initiated with cultures that were grown overnight from a single bacterial colony, which in turn had been grown from a single cell. This minimizes variation within populations of the same plasmid and host.

Parameter Estimates.—Growth rates in batch cultures were estimated from plate counts made at roughly hourly intervals during one growth period (4–5 counts per experiment). The estimates for growth rates presented here are taken from conjugation and segregation experiments described below. In chemostats, the growth rate was assumed to equal the flow rate.

The relative fitnesses of two coexisting populations were estimated from the rate of change of the ratio of the rare population to the total population (Dykhuizen and Hartl, 1983) in either chemostats or serial transfer. Estimates for fitness differences were taken from the invasion experiments themselves (described below).

Conjugation parameters were estimated in both batch and chemostat cultures in matings that were independent of the invasion experiments. For batch cultures, a plasmid-bearing culture of the 7^+ host and a plasmid-free culture of $7n^-$, both at maximum density, were diluted 100-fold, mixed into fresh medium, and sampled every 30–60 minutes for 2–3 hours. In a chemostat, the entire contents of tubes holding equilibrium populations of donors and recipients were mixed and sampled hourly for up to eight hours. Conjugation parameters were estimated from changes in the densities of donors, recipients, and transconjugants (recipients that acquired a plasmid); Levin et al. (1979) give formulae for the calculations.

Segregation rates were also measured independently of invasion experiments. There are two different segregation rates in the model described by Condit et al. (1988): The first, τ_0, is the rate at which plasmid-bearing cells become plasmid-free. To estimate its rate, colonies of an R-bearing culture were grown on nonselecting medium, and single colonies were patched to plates with chloramphenicol, where R-free colonies would fail to grow.

The second segregation parameter is τ_p, or the rate at which heteroplasmid cells (cells with two different plasmids) are converted to homoplasmid cells by loss of one plasmid type; this rate is high when two plasmids are incompatible. I estimated τ_p as follows (Nordstrom et al., 1980; Novick and Hoppensteadt, 1978; Novick, 1987). Broth was inoculated with 5×10^4 heteroplasmid cell ml^{-1}, and the density of total cells and heteroplasmid cells was estimated hourly. As long as total density was less than 10^7 cell ml^{-1}, plasmid transfer would be insignificant, so the difference between the rate of increase of total cells and heteroplasmid cells equaled the segregation rate. Regression was done on 4–7 sampling points per experiment to estimate the two growth rates.

Transposition rate from chromosome to plasmid was estimated during mating-out experiments, (see above). The ratio of transconjugants that acquired a transposon to total transconjugants was calculated at four sampling times during exponential growth of a mating culture. The slope of a regression of this ratio against time is an approximation of the transposition rate.

The ability of Tn3 and Tn5 (on the plasmids) to transpose was tested by screening for strains in which the transposon had moved to the chromosome. This was accomplished by growing a heteroplasmid culture (R/R5 or R/R3) in antibiotics selecting for resistance genes on both plasmids. After several transfers under selection, colonies derived from single cells in the culture were used as donors in matings to a $7n^-$ recipient. Colonies that still manifested drug resistance but were unable to transfer it would provide evidence that the transposons had moved to the chromosome. Such colonies were further examined by comparing *Bam*H I restriction digests of their plasmid DNA to digests from the original plasmid-bearing strains (see Condit and Levin [1990] for details). It was not possible to estimate transposition rate using this procedure, so I assumed that the plasmid-to-chromosome transposition rate was the same as the chromosome-to-plasmid rate.

Statistics of parameter estimates were based on replicate experiments; multiple sampling points within an experiment (to generate regression lines) were not used to calculate standard deviations. For conjugation and transposition parameters, log-transformed data were used for statistics because the estimates appeared to be distributed log-normally.

Population Dynamics.—Six invasion experiments were carried out: two in chemostats and four in serial transfer, with two of the latter in broth and two in minimal medium. Invasion in serial transfer was initiated with overnight cultures of plasmid R on host 7^+ ($R/7^+$) and $R3/7n^-$, or conversely, $R/7n^-$ and $R3/7^+$. The transposon-bearing culture was diluted by 10^4, and 0.1 ml of the dilution was mixed with 0.1 ml of the undiluted R culture in 10 ml of fresh medium. The mixed culture was grown overnight, and 0.1 ml was retransferred into fresh medium the following day, and so on for 11 days. Invasion experiments in chemostats were started with equilibrium chemostat cultures. A 10^4 dilution of the transposon-bearing culture was added to the culture of the plasmid R.

Experimental cultures were sampled daily, and appropriate dilutions were plated on various combinations of antibiotics on broth plates and minimal lactose plates. This allows density estimates to be made for the two cell types in the original inoculum, as well as for new types formed by exchange of the plasmids and transposition (see below).

Modeling.—The original model used in Condit et al. (1988) was based on chemostat dynamics; for the present study, slight modifications were incorporated to make a serial-transfer version as well. In addition, the original model was altered to accommodate the use of genetically distinctive hosts. This required doubling the eight-cell-type model of Condit et al. (1988) to a 16-cell model, but this change is minor. The models and their associated differential equations are presented in the Appendix. Simulations of the differential equations were run using the Euler method (Ritger and Rose, 1968, pp. 374–377) with step size set at 0.02 hours.

Output of simulations included 16 cell types, eight on each of the two host strains (these are diagrammed in Condit et al. [1988]). Only eight of these were detectable in experiments, because it is not possible to separate a strain with a resistance gene on

TABLE 1. Growth rates (Ψ), maximum cell densities (N), and relative fitnesses (W; mean ± SD). W is defined as the fitness of the 7^+ strain relative to $7n^-$ and is dimensionless. In serial transfer, 7^+ had a lower fitness (W is significantly different from 1.0), but in chemostats, it did not.

Parameter	Serial transfer		Chemostat
	Minimal medium	Luria broth	
Ψ (hr^{-1})	0.72	1.59	0.13
N (cell ml^{-1})	5.1×10^8	3.0×10^9	7.8×10^8
W	0.94 ± 0.01	0.85 ± 0.01	0.99 ± 0.13

a plasmid from one with the same gene on both plasmid and chromosome. Populations of cell types that could not be distinguished on plates were combined in the output of simulations (see Appendix). In all simulations, the densities of cell types with the transposon on the chromosome were much lower than the densities of cell types with which they were combined.

Serial-transfer experiments were simulated using the independently derived estimates of conjugation, segregation, and transposition parameters (see above) and estimates of fitness taken from the experiments themselves. Simulations of chemostat experiments were run with the independently derived conjugation and transposition parameters, the approximations for segregation described below, and with no fitness difference assigned. Additional simulations were run with the transposition rate set to zero or as high as 0.1 hr^{-1}, with all other parameters kept the same.

RESULTS

Parameter Estimates.—Growth and fitness parameters are presented in Table 1, and estimates for conjugation parameters are given in Table 2. In populations of cells carrying a single plasmid type, segregants were not observed, so I assumed a value of 10^{-4} hr^{-1} for τ_0, as reported in Falkow (1975) for resistance plasmids in general and in Gerdes et al. (1985) for R1, a relative of R100. Segregation rate from a heteroplasmid cell line to form cells with only one plasmid type was 0.44 hr^{-1} (SD = 0.08, N = 6 experiments, combining estimates for R/R3 and R/R5 in minimal and LB). Segregation-rate constants were not measured in chemostats, so I used the serial-transfer value of 10^{-4} for τ_0. For τ_p, it was necessary to assume that segregation is directly proportional to growth rate (see Novick and Hoppensteadt, 1978). Based on growth rates given in Table 1, this leads to an estimate of 0.05 for τ_p in chemostats.

TABLE 2. Conjugation-rate constants for plasmid R (R100.1*drd*) and its derivatives. In all cases, the strain 7^+ acted as donor and $7n^-$ acted as recipient, or vice versa. Transfer to a plasmid-bearing recipient was measured using a recipient carrying one of the other two plasmids; for example, transfer of R was measured into recipients with R5 or R3, with the data for both combined. CI's are confidence intervals, and N gives the number of independent experiments.

Culture regime	Recipient	Plasmid	Mean	CL	N
Batch, minimal medium	plasmid-free	R	1.2×10^{-9}	$0.40–3.6 \times 10^{-9}$	3
		R3	2.9×10^{-9}	$1.0–8.3 \times 10^{-9}$	3
	plasmid-bearing	R	9.4×10^{-13}	$2.0–43.1 \times 10^{-13}$	5
		R3	2.4×10^{-11}	$1.1–5.2 \times 10^{-11}$	8
Batch, Luria broth	plasmid-free	R	6.5×10^{-9}	—	1
		R5	2.7×10^{-10}	$0.80–9.5 \times 10^{-10}$	3
	plasmid-bearing	R	4.2×10^{-10}	$1.6–11.2 \times 10^{-10}$	4
		R5	1.9×10^{-12}	$0.28–12.6 \times 10^{-12}$	4
Chemostat	plasmid-free	R	4.6×10^{-13}	—	1
		R3	4.3×10^{-13}	$2.2–10.1 \times 10^{-13}$	2
		R5	6.0×10^{-14}	$4.3–7.8 \times 10^{-14}$	3
	plasmid-bearing	R	1.3×10^{-14}	$0.30–5.1 \times 10^{-14}$	2
		R3	2.4×10^{-13}	$0.59–7.9 \times 10^{-13}$	3
		R5	7.0×10^{-17}	—	1

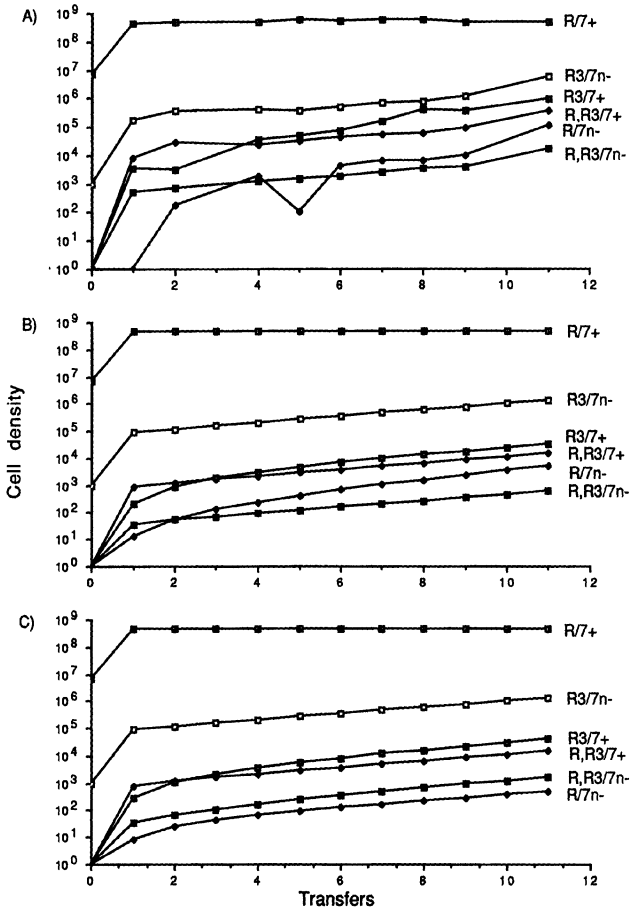

FIG. 1. Invasion of R3/7n⁻ into R/7⁺ in minimal medium. There are 6.6 cell generations per transfer. A) Experimenal results; B) simulation with transposition rate set at the estimate given in the text (a simulation with transposition rate set to zero looked indistinguishable and is not shown); C) simulation with transposition rate set very high (0.05) but all other parameters identical to those in B.

The mean transposition rate for Tn5 was 7.1×10^{-7} hr^{-1} (95% confidence interval = 4.1–12.1 $\times$ 10^{-7}, $N = 5$ experiments), and that for Tn3 was 6.3×10^{-6} (1.5–12.0 $\times$ 10^{-6}, $N = 4$). These estimates exclude any transpositions into the plasmid's transfer region that prevented subsequent plasmid transfer, but such events would be unimportant from the perspective of population dynamics. I must also assume that there is no fitness effect of the transposon-bearing plasmid relative to the transposon-free plasmid, but over a short experiment even a substantial effect would not alter estimates much.

After growing heteroplasmid populations in medium selecting for both plasmids, it was common to find strains that still carried kanamycin-resistance but could not transfer it to recipients, and I also located two strains for which the same was true of ampicillin-resistance. Restriction digests of the plasmid DNA from these strains confirmed that the plasmid with Tn5 or Tn3 was no longer present. Apparently, these resistance genes had moved from the plasmid onto the chromosome, and the plasmid had been lost (the second plasmid carrying a different resistance gene was still present; see Condit and Levin [1990] for further explanation).

Population Dynamics.—Since the two plasmid types started on different hosts, four new strains could be formed by plasmid transfer. For example, when the original

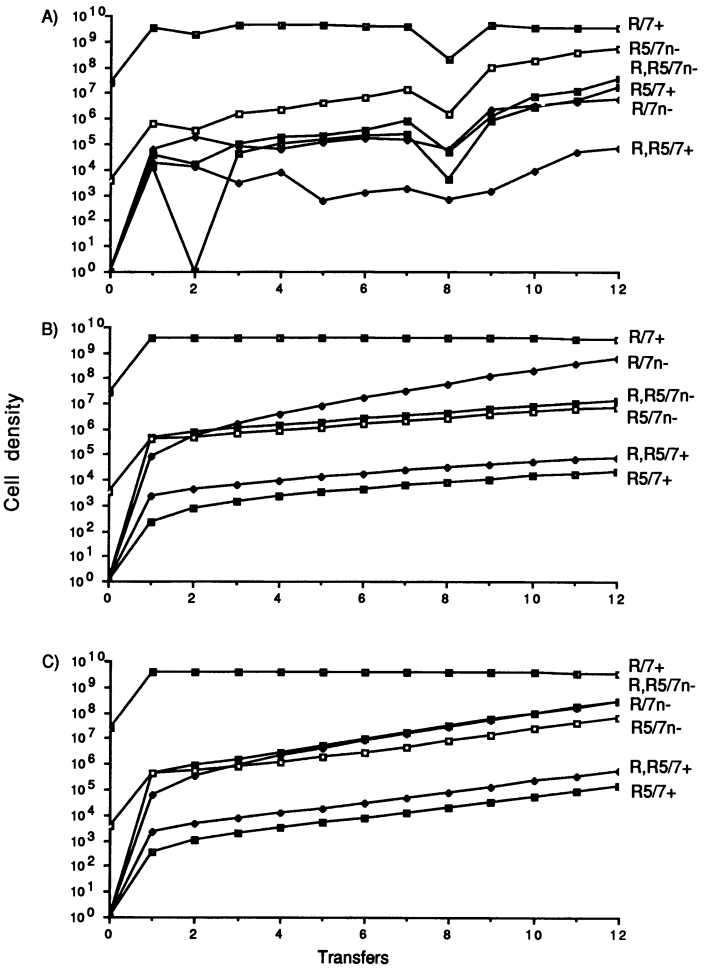

FIG. 2. R5/7n$^-$ invading R/7$^+$ in broth. There are 6.6 cell generations per transfer. A) Experimental results; B) simulation with transposition rate set at the estimate given in the text (a simulation with transposition rate set to zero looked indistinguishable and is not shown); C) simulation with transposition rate set very high (0.05) but all other parameters identical to those in B.

cultures were R/7$^+$ and R3/7n$^-$, the four new strains were R,R3/7$^+$, R,R3/7n$^-$, R/7n$^-$, and R3/7$^+$. Plasmid-free strains were always very rare (they were never detected) and will be ignored in subsequent discussion. As discussed above, eight other strains may have been formed by a transposon moving onto the chromosome, but these were not detectable in plate counts.

In serial-transfer experiments, the 7n$^-$ host increased in frequency relative to the 7$^+$ host (Figs. 1a–3a). The dynamics were consistent with growth differences of 6% in minimal medium and 15% in broth (Table 1). Simulations were run using these selection coefficients. The fitness difference between hosts did not depend on which plasmid was initially on which host, demonstrating that the transposons and plasmids did not affect host fitness.

In two experiments in which the invading plasmid started on the 7n$^-$ host, the four transconjugants rose in frequency (Figs. 1a, 2a); conversely, when the invading plasmid started on the 7$^+$ host, all transconjugants waned after they first appeared (Fig. 3a). Results from a fourth serial-transfer experiment in broth medium, with R5/7$^+$ invading R/7n$^-$, are not shown but were quite similar to those in Figure 3a in that transconjugants appeared on the first day but waned in frequency thereafter. In general,

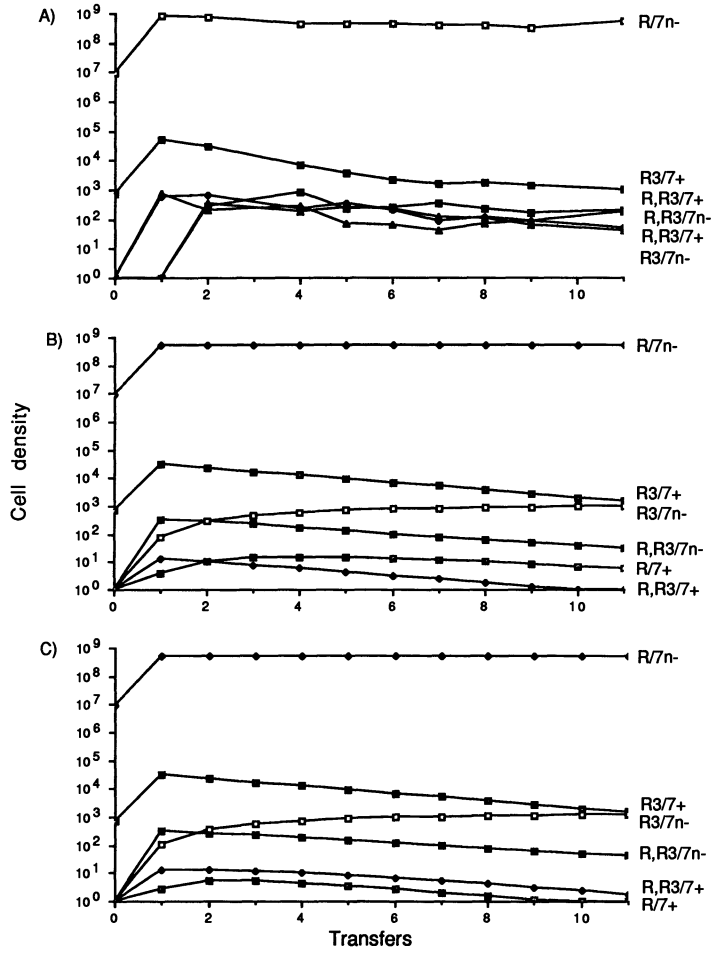

FIG. 3. R3/7$^+$ invading R/7n$^-$ in minimal medium. There are 6.6 cell generations per transfer. A) Experimental results; B) simulation with transposition rate set at the estimate given in the text (a simulation with transposition rate set to zero looked indistinguishable and is not shown); C) simulation with transposition rate set very high (0.05) but all other parameters identical to those in B.

transconjugants rose in abundance when the invading strain proliferated and waned when the invading strain declined.

There was no consistent fitness difference between the 7n$^-$ and 7$^+$ hosts in chemostat experiments, and neither Tn5 nor Tn3 increased in frequency after being introduced on 7$^+$ into a population of R/7n$^-$ (Fig. 4). Transconjugants were rare throughout the experiments and showed no tendency to rise in frequency.

Simulations.—The fitness difference between 7n$^-$ and 7$^+$ and variations in plasmid transfer created "noise" which obscured the impact of transposition. Simulations with the same fitness and plasmid parameters can be used to isolate effects of fitness, plasmid exchange, and transposition on population changes.

In three of four cases, simulations were similar to experiments. The simulation of R3/7n$^-$ invading R/7$^+$ illustrates this best (Fig. 1b): the rate of increase and absolute densities of all six strains in simulations paralleled experimental results closely. Runs with the transposition rate set to zero, rather than the measured value of 6.3×10^{-6}, were indistinguishable from the one shown in Figure 1b. Simulations with high transposition rates (Fig. 1c) did not account for the experimental data as well as those with no transposition or low transposition. For the

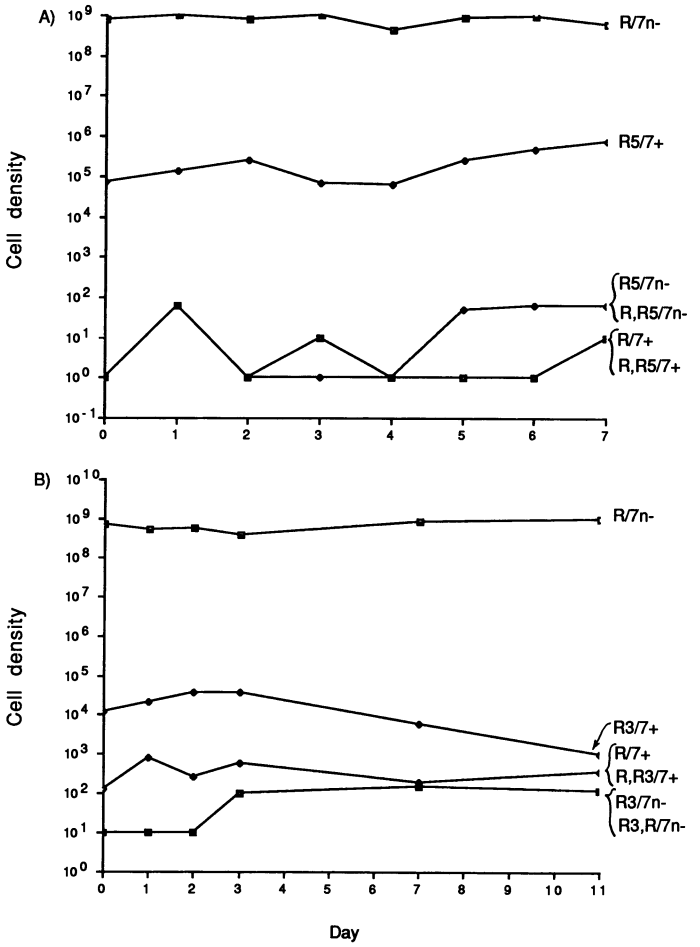

FIG. 4. Invasion experiments in a chemostat: A) Tn5; B) Tn3. Each day includes 4.5 generations. The two lowest lines in each case are transconjugant populations; each one includes heteroplasmid and homoplasmid cells as labelled. Transconjugant densities were so low that the two types could not be separated, as indicated; in fact, in the R5 experiment transconjugant densities were not reliably different from zero. When cell density (N) was less than 10 cells ml^{-1}, which was the minimum detectable density, N was set to 10^0.

two experiments in which the transposon was introduced on the low-fitness cell line, simulations predicted quite closely the declines in density of the invading strain and all transconjugants, as well as their relative abundances (only one experiment illustrated; Fig. 3b). A simulation with a high transposition rate (Fig. 3c) did not improve the fit; in fact, it was almost indistinguishable from the runs with low transposition rate. Again, a simulation with transposition rate set at zero was indistinguishable from the low transposition run.

In the fourth serial transfer case (Fig. 2b), there was a sizable discrepancy between model and data. The observed density of R5 cells in Figure 2a is about two orders of magnitude above expected (Fig. 2b), and the abundance rankings of the cell lines in simulations do not match those in the experiment. Simulations with high transposition rate (Fig. 2c) improved the fit to experimental results somewhat, but differences remained.

In chemostats, simulations with low transposition or no transposition matched the experimental results closely, predicting transconjugant densities less than 10^2 cell ml^{-1}. Moreover, due to low transconjugant densities, transposition had no impact on the computer results; if the transposition rate was high (0.1 hr^{-1}), transconjugant den-

sities remained less than 10^2 throughout the experiment. Similar results were obtained even if the segregation rate was varied by an order of magnitude around the approximate value used.

Discussion

Previous experimental studies of the population dynamics of transposons considered only two populations, one with the transposon on the chromosome and one without any transposon (Hartl et al., 1983; Chao et al., 1983; Chao and McBroom, 1985). By avoiding the inconvenience of conjugative plasmids, these studies were able to detect small differences in fitness due to the carriage of a transposon; however, they were unable to evaluate the role of infectious transfer of transposons with plasmids or phage as vehicles. By including conjugative plasmids as vectors for intercellular movement of transposons, my experiments were capable of testing the hypothesis that transposons can be maintained as parasitic DNA. As is often the case, however, added realism restricted the sensitivity of the experiments, and incorporating plasmid transfer made it necessary to use a complex model to interpret the results.

The experimental results presented here are in more or less qualitative agreement with predictions of the model. Detailed appraisal of three of the four serial-transfer experiments and the two chemostat experiments reveals close fit between simulations and experiments. Transposition appeared to make no contribution to the dynamics of the transposable elements in these cultures. Simulations without transposition or with a very low transposition rate account for the observed dynamics as well as or better than simulations with the transposition rate set at a high value. Therefore, although Tn3 and Tn5 were capable of transposing, transposition had no detectable effect on experimental outcomes. Plasmid transfer and differences in fitness between the host-cell lines accounted for the observed dynamics.

In one serial-transfer experiment, the fit of the model's predictions was not close, demonstrating that evidence counter to the model could have been obtained. In this experiment, the density of Tn5 rose at a higher rate than anticipated. Figure 2c suggests that a high transposition rate alone cannot account for the discrepancy. Another possible explanation is that plasmid transfer parameters, which were measured at cell densities below 10^9 cell ml^{-1}, were not applicable at the high cell densities achieved in Luria Broth (up to 5×10^9). Simulations using lower transfer parameters did improve fit to the experimental population trajectories, favoring this hypothesis, but the evidence is not conclusive. The significant point is that in five out of six experiments, the model worked well.

The major shortcoming of the experiments was their lack of resolution. Changes in frequency of any cell type below about 10^{-3} hr^{-1} would not have been detected, so transposition rates may have been as high as 10^{-3} between plasmids, or the transposons may have been reducing fitness slightly, and experiments would not have revealed it. The experiments were also consistent with a system in which transposition never occurred or was a conservative process (as it might be for Tn5 [Berg, 1977]). I could have pursued experiments for longer periods, but fitness and conjugation differences would still have overwhelmed the effects of transposition. Because transposition rate in experimental populations could not be set to a value high enough to detect its effect, validation of the model must be considered incomplete. Ideally, one would find an element whose rate of transposition between chromosome and plasmid was 10^{-2} or higher and then examine the contention that its invasion rate would be on the order of 1% hr^{-1}, which would probably be detectable even against a background of plasmid transfer and fitness differences.

In sum, certain conclusions of the model for the population dynamics of transposable elements were confirmed by experiments with laboratory populations. Predictions about the exchange of plasmids were borne out, and the statement that transposition plays a minor role in the dynamics of parasitic transposable elements was confirmed, at least over the range of conditions studied. I conclude that transposition will seldom have an important impact on the rearrangement of bacterial genomes—at least when there is no selection favoring the elements—

and that most bacterial transposons did not evolve as parasitic DNA nor do they persist as parasitic DNA. Transposons are quite different from plasmids, some of which can readily invade novel populations even with no selection (Lundquist and Levin, 1986) and which seem to be quite able bacterial parasites.

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APPENDIX

The parameters used in the model of Condit et al. (1988) were:

S = resource concentration in the culture (μg ml^{-1})
v = maximum growth rate of the most fit cell line (hr^{-1})
k = resource concentration at which growth rate is half-maximum; assumed to be constant for all cell lines
W_{N_i} = relative fitness of cell type N_i (dimensionless)
h = flow rate of chemostat (hr^{-1})
Ψ = growth rate of most fit cell line (hr^{-1}); Ψ is a function of S
e = amount of resource (in μg) used during one cell cycle; assumed to be constant for all cell types and independent of growth rate
c = resource concentration in inflow of chemostat (μg ml^{-1})
γ_{00} = conjugation rate of transposon-free plasmid into plasmid-free cells (ml hr^{-1} cell^{-1})
γ_{t0} = conjugation rate of transposon-bearing plasmid into plasmid-free cells (ml hr^{-1} cell^{-1})
γ_{op} = conjugation rate of transposon-free plasmid into plasmid-bearing cells (ml hr^{-1} cell^{-1})
γ_{tp} = conjugation rate of transposon-bearing plasmid into plasmid-bearing cells (ml hr^{-1} cell^{-1})
τ_0 = segregation rate to plasmid-free cells (hr^{-1})
τ_p = segregation rate for loss of one plasmid type from heteroplasmid cells (hr^{-1}); equal segregation rates for the two plasmid types in the same cell are assumed
δ = transposition rate (hr^{-1})
ϵ = excision rate of transposon (hr^{-1}).

There are 16 cell types, designated N_i and M_i ($i = 0, \ldots 7$), with each set of eight identical to those pictured in Condit et al. (1988 fig. 2); the N's and M's are different only at an unchanging chromosomal marker (one represents CSH7$^+$, the other CSH7n$^-$). The equations for rate of change of each cell type N_i are derived in detail in the appendix of Condit et al. (1988) and are identical here except for two minor changes: switching from chemostat to serial transfer and incorporating the second set of eight cells.

The differential equation for resource concentration in a chemostat is

$$\frac{dS}{dt} = h(c - S) - \Psi(S)e\Sigma(W_{N_i}N_i) \tag{A1}$$

(Stewart and Levin, 1973; Levin, 1981). To change this to a serial-transfer model, the $h(c - S)$ term, which represents continuous input and washout of resources in a chemostat, is dropped. The second term represents decline of the resource as cells consume it, and it remains in a serial-transfer model. Hence, $dS/dt < 0$ at all times, and when S declines below some small value L ($L = 0.001$ μg ml^{-1} in simulations), the culture is "transferred" by resetting S to its initial value, S_0. The equations for each cell population in a chemostat (Condit et al., 1988 eq. 3) also include a term for washout, $-hN_i$, which is removed from the serial-transfer model. Each population grows steadily until $S < L$ and Ψ approaches zero; then, N_i is divided by a constant dilution term (100 in these runs).

In order to incorporate a second cell type, one need only note that each M_i carries the same plasmids as N_i and that the only interaction between N's and M's is the exchange of plasmids. Let N_d be the sum of the density of cell types N carrying any plasmid, define M_d similarly for types M carrying the same plasmid, and let N_r be any recipient. In a model of single cell types, N_d conjugates with N_r at a rate $\gamma N_d N_r$. Incorporating a second cell type simply requires replacing this term with $\gamma(N_d + M_d)N_r$.

Growth rate is related to resource concentration by a Monod equation (Monod, 1949):

$$\Psi = vX \tag{A2}$$

with $X = S/(k + S)$. Assume also that conjugation and segregation rates decline with S in the same way, so each γ and τ are multiplied by X. For brevity, define

$$\begin{aligned} F &= N_1 + M_1 + N_5 + M_5 + 0.5(N_2 + M_2 + N_6 + M_6) \\ T &= N_3 + M_3 + N_7 + M_7 + 0.5(N_2 + M_2 + N_6 + M_6). \end{aligned} \tag{A3}$$

F is the sum of donors of the transposon-free plasmid, and T is the sum of donors of the transposon-bearing plasmid. The 0.5 term is based on the assumption that heteroplasmid cells transfer each plasmid at an equal rate. In the model, every transposition into the plasmid R inactivates the chloramphenicol gene, thus converting it to R3 or R5 (which are chloramphenicol-sensitive) and obviating the need for further plasmid types.

Below are the differential equations describing the resource concentration and the cell populations in a serial-transfer model.

$$\begin{aligned}
\frac{dS}{dt} &= -\Psi e \Sigma (N_i W_{N_i} + M_i W_{M_i}) \\
\frac{dN_0}{dt} &= W_{N_0}\Psi N_0 + \tau_0(N_1 + N_2 + N_3) \\
&\quad + \epsilon N_4 - \gamma_{00} N_0 F - \gamma_{t0} N_0 T \\
\frac{dN_1}{dt} &= W_{N_1}\Psi N_1 + 0.5X\tau_p N_2 - X\gamma_{tp} N_1 T \\
&\quad + \epsilon(N_2 + N_5) + \gamma_{00} N_7 F - \tau_0 N_1 \\
\frac{dN_2}{dt} &= W_{N_2}\Psi N_2 - X\tau_p N_2 + \epsilon(2N_3 + N_6 - N_2) \\
&\quad - 2\delta N_2 + X\gamma_{tp} N_1 T + X\gamma_{0p} N_3 F - \tau_0 N_2 \\
\frac{dN_3}{dt} &= W_{N_3}\Psi N_3 + 0.5X\tau_p N_2 - X\gamma_{0p} N_3 F \\
&\quad + \epsilon(N_7 - 2N_3) - 2\delta N_3 + \gamma_{t0} N_0 T \\
&\quad - \tau_0 N_3 + \delta N_2 \\
\frac{dN_4}{dt} &= W_{N_4}\Psi N_4 + \tau_0(N_5 + N_6 + N_7) \\
&\quad - \epsilon N_4 - \gamma_{00} N_4 F - \gamma_{t0} N_4 T \\
\frac{dN_5}{dt} &= W_{N_5}\Psi N_5 + 0.5X\tau_p N_6 - X\gamma_{tp} N_5 T \\
&\quad + \epsilon(N_6 - N_5) - 2\delta N_5 - \tau_0 N_5 + \gamma_{00} N_4 F \\
\frac{dN_6}{dt} &= W_{N_6}\Psi N_6 - X\tau_p N_6 + \epsilon(2N_7 - 2N_6) \\
&\quad + \delta(N_2 + 2N_5 - 2N_6) + X\gamma_{tp} N_5 T \\
&\quad + X\gamma_{0p} N_7 F - \tau_0 N_6 \\
\frac{dN_7}{dt} &= W_{N_7}\Psi N_7 + 0.5X\tau_p N_6 - X\gamma_{0p} N_7 F - 3\epsilon N_7 \\
&\quad + 2\delta(N_3 + N_6) - \tau_0 N_7 + \gamma_{t0} N_4 T
\end{aligned} \tag{A4}$$

The equations are repeated exactly for cell types M_0–M_7, but each N_i is replaced by M_i; the F's and T's remain.

Several of the cell types could not be distinguished experimentally; only eight strains were distinctive on plates (see text). In the output of simulations, populations of cell types that could not be separated were combined for comparison with the experimental results: types N_5 and N_6 combined with type N_2; types N_4 and N_7 were combined with type N_3; and likewise for types M with the same subscripts.

Values for S_0 and e were chosen in each simulation so that the maximum total cell density matched that from a particular experiment. Generally, for minimal medium, where $v = 0.72$ (Table 1), $S_0 = 200$ μg ml^{-1} and $e = 5 \times 10^{-7}$ μg gave the appropriate cell density. In broth, $v = 1.59$ and S_0 was set at about 1,800 μg ml^{-1}, with the same e. K was taken from Monod (1949): 4 μg ml^{-1}.