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A Continuous Culture Model To Examine Factors That Affect Transduction among *Pseudomonas aeruginosa* Strains in Freshwater Environments

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Transduction among *Pseudomonas aeruginosa* strains was observed in continuous cultures operated under environmentally relevant generation times, cell densities, and phage-to-bacterium ratios, suggesting its importance as a natural mechanism of gene transfer. Transduction was quantified by the transfer of the Tra$^-$ Mob$^-$ plasmid Rms149 from a plasmid-bearing strain to an F116 lysogen that served as both the recipient and source of transducing phages. In control experiments in which transduction was prevented, there was a reduction in the phenotype of the mock transductant over time. However, in experiments in which transduction was permitted, the proportion of transductants in the population increased over time. These data suggest that transduction can maintain a phenotype for an extended period of time in a population from which it would otherwise be lost. Changes in the numbers of transductants were analyzed by a two-part mathematical model, which consisted of terms for the selection of the transductant's phenotype and for the formation of new transductants. Transduction rates ranged from 10^{-9} to 10^{-6} per total viable cell count per ml per generation and increased with both the recipient concentration and the phage-to-bacterium ratio. These observations indicate an increased opportunity for transduction to occur when the interacting components are in greater abundance.

The application of genetically engineered microorganisms (GEMs) to environmental uses has led to questions regarding the persistence of these organisms and their genes in the environment. If genes from an introduced organism were passed to members of the indigenous population by horizontal gene transfer, these genetic elements might persist in a population, even after the GEM dies out.

Current information about the prevalence of phage-bacterium associations (16, 18) and the results of numerous microcosm experiments (15, 17, 21, 22, 24) clearly show the potential for transduction to occur in natural environments. However, the potential for transduction to affect allele frequencies and gene-pool diversity in these environments has not been explored. The study reported here was designed to address our lack of knowledge concerning the potential of transduction to affect the evolution of natural bacterial populations and species. The objectives were (i) to create a mathematical model to describe how the processes of selection and transduction affect the establishment of a new phenotype in a bacterial population and (ii) to apply this model to continuous-culture populations of *Pseudomonas aeruginosa* undergoing transduction. Populations in which transduction was either permitted or inhibited were compared to discern the contribution of horizontal gene transfer to the evolution of the gene pool. Experiments were conducted over a range of environmentally relevant generation times, cell densities, and phage-to-bacterium ratios (PBR) to identify factors that influence transduction frequency. Without transduction, the relative number of cells with the transductant's phenotype declined. When transduction occurred, the relative number of cells displaying the transductant's phenotype increased despite their selective disadvantage. These data indicate that transduction can act to stabilize an otherwise less-fit phenotype in a genetically heterogeneous population of bacteria.

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MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. All bacterial strains used in these studies were derivatives of *P. aeruginosa* PAO and are listed in Table 1. The *P. aeruginosa* plasmid Rms149 (21) is nonconjugative (Tra$^-$) and nonmobilizable (Mob$^-$). It encodes resistance to carbenicillin (Cb^r), streptomycin (Sm^r), gentamicin (Gm^r), and sulfonamide (Su^r). This plasmid is highly stable and is not curable by various techniques including exposure to acridine orange. Bacteriophage F116 (7) is a generalized transducing phage and has been shown to efficiently transduce plasmid Rms149 DNA (21).

Media and cell growth. Chemostat and batch experiments were performed by using a variation of *Pseudomonas* minimal medium (13) from which the sodium citrate was omitted (PMM-c). This medium was supplemented with various concentrations of yeast extract, which served as the sole source of carbon and nitrogen.

Routine laboratory procedures were performed on Luria broth or on L-agar plates (13), which were incubated at 37°C. When required for selection, antibiotics were provided at final concentrations (per milliliter of medium) of 75 μg of rifampin, 500 μg of carbenicillin, 500 μg of nalidixic acid, 1,000 μg of streptomycin, and 200 μg of chloramphenicol. Transductants were selected on L-agar containing higher concentrations of rifampin (600 μg/ml) and carbenicillin (1,000 μg/ml) to discourage growth of spontaneous chromosomal drug resistance mutants. Selection for the ability to use acetamide as the sole carbon and nitrogen source (Ami$^+$) was performed on PMM-c agar containing 0.01 M acetamide.

Lysate preparation and bacteriophage titration. Cell-free phage lysates were prepared by the method described by Miller and Ku (13). Titers of bacteriophages were determined by mixing a dilution of the phage-containing sample with the indicator strain into a lambda top agar overlay (14). PFU were enumerated.

Determination of transducing particle-to-total phage ratios. Shaker flasks containing 50 ml of PMM-c and 0.5% yeast extract were inoculated with exponential-phase cells at donor-to-recipient ratios of 42:1 to 1:244 at a total con-

TABLE 1. Bacterial strains used

Strain	Role in model	Relevant characteristic(s)[a]	Plasmid	Source or reference
PAO1	Indicator	Prototroph	None	13
RM2141	Precursor of donor	*met-9011 nalA5 amiE200*	Rms149	22
RM2235	Donor	*nalA5 amiE200*	Rms149	This study
RM300	Recipient	*rif-901 chl-901* F116 lysogen	None	This study
RM4412	Transductant	*rif-901 chl-901* F116 lysogen	Rms149	This study
RM287	Mock recipient	*rif-901 chl-901*	None	This study
RM289	Mock transductant	*rif-901 chl-901*	Rms149	This study

[a] Genotype symbols are as recommended by Demerec et al. (3). *met*, methionine auxotrophy; *nal*, nalidixic acid resistance; *ami*, unable to utilize acetamide as the sole carbon and energy source; *rif*, rifampin resistance; *chl*, chloramphenicol resistance.

centration of 3×10^6 CFU/ml. After 16 and 25 h of incubation at room temperature, the flasks were assayed for concentrations of recipients, donors, bacteriophages, and transducing particles. To estimate the concentration of transducing particles, 10-ml samples were centrifuged for 20 min at $3,450 \times g$. The supernatant fluid was filtered through a 0.45-mm-pore-size cellulose-nitrate filter and was used to transduce exponential-phase recipient cells at a multiplicity of infection of 0.1 as described by Miller and Ku (13). The number of transductant colonies that appeared was quantified to determine the number of transducing particles present.

Continuous-culture conditions. A Bioflo C32 fermentor (New Brunswick Scientific, Edison, N.J.) was operated with PMM-c supplemented with various concentrations of yeast extract. The chemostat was inoculated with cells in mid-exponential phase, which were grown in PMM-c with 0.5% yeast extract and washed in the chemostat medium. All trials were conducted aerobically at room temperature with an agitation rate of 200 rpm.

Procedure for chemostat experiments. (i) With transduction. Plasmid transduction was quantified by the transfer of the Tra^- Mob^- plasmid, Rms149, from the donor strain (RM2235) to the plasmid-free, lysogenic recipient strain (RM300). We used lysogens in this system for two reasons that were based on our in situ studies of transduction (21). First, the spontaneous induction of lysogens to lytic production provided a continual source of phage particles to the system. Second, lysogeny imparts immunity from further phage infection, thus allowing for increased survival of the recipient (21).

When donor and recipient bacteria were coincubated, plasmid transduction could occur by the following series of events. First, F116 phage particles are released from the spontaneous induction of recipients. Second, these free phage particles adsorb to and lyse donor cells, occasionally producing plasmid-containing transducing particles. Third, the transducing particles infect the recipient, leading to the introduction of the transduced plasmid DNA and expression of the transduced phenotype.

To allow for the accumulation of transductants to a detectable level, batch growth was permitted for 1 to 2 days before the pump was activated at the desired dilution rate. When transductants had reached this level, continuous-culture conditions were initiated at the desired dilution rate. The population was sampled to determine concentrations of donors, plasmid-bearing cells, recipients, transductants, and bacteriophages. Stable maintenance of plasmid Rms149 by the donor strain was confirmed by similarity in concentrations of cells exhibiting plasmid-encoded carbenicillin resistance to those exhibiting chromosomally encoded nalidixic acid resistance.

Bacterial concentrations on L-agar selection plates were determined as follows: concentrations of recipients, donors, Rms149-containing cells, and transductants were determined on rifampin, nalidixic acid, and carbenicillin-rifampin carbenicillin plates, respectively. To obtain significant numbers of transductants, it was necessary to centrifuge 15 to 200 ml of the chemostat culture fluid for 10 min at $3,450 \times g$ before performing plate counts. The number of bacteriophages was determined by determining the titer of supernatant fluid with *P. aeruginosa* PAO1 as the indicator strain.

(ii) Control experiments without transduction. Control experiments were performed at donor and mock recipient concentrations of 10^5 to 10^6 CFU/ml. The mock transductant strain was inoculated to approximately 10 CFU/ml. The nutrient pump was activated at the time of inoculation. The chemostat was sampled for concentrations of mock recipient, donor, and mock transductant in the manner described above.

Verification of transductants. Transductants were confirmed by their ability to grow in the presence of chloramphenicol and streptomycin, their ability to use acetamide as the sole carbon source, and their inability to grow in the presence of nalidixic acid. To further verify the presence of Rms149 in selected transductants, plasmid DNA was isolated by rapid alkaline lysis (11), digested with *Eco*RI (Boehringer Mannheim, Indianapolis, Ind.) as recommended by the manufacturer, and electrophoresed on 0.7% agarose gels (11). Digestion patterns were compared with those obtained from the digestion of Rms149 DNA from the donor strain.

Statistical methods. Correlations between experimental parameters were determined by linear regression analysis by using the statistical program SYSTAT (23) and were considered significant at $P \leq 0.05$. Bacterial growth rates and their standard errors were determined from coefficients of linear-regression analysis. Multiple linear-regression analyses were performed to determine the significance between experimental parameters and the rate of transduction. Squared multiple correlations (r^2) and P values were computed for each multiple linear-regression model.

Values for the selection coefficient and transduction rate were determined by using experimental data for the variables in equations 2 and 6 (see below) and by solving for the best-fit values of s and $\Delta(T/N)_{trans}$. These values were calculated by using the nonlinear modeling function of SYSTAT, which minimizes the squared deviation of the dependent variable values from values estimated by the function at the same independent variable datum points.

THEORY

Model description. In order to understand the dynamics of the transductant population in a continuous-culture model, a two-part mathematical expression was created to separate and identify the effects of selection and transduction. It contains a term for the change in the relative concentration of transductants due to negative or positive selection and a term for the rate at which transduction events add new transductants to the population.

Selection. As described by Dykhuizen and Hartl (5), competition between two bacterial strains in continuous culture results in a decrease in the relative concentration of the less-competitive strain over time. The ratio between the two strains changes as follows:

$$\ln(p_t/q_t) = \ln(p_0/q_0) + st \quad (1a)$$

or

$$(p_t/q_t) = (p_0/q_0)\, e^{st} \quad (1b)$$

where p_t and q_t represent the genotypic frequency of the two competing strains at time t and s is a measure of the differential growth rate per unit time (5). The strain with gene frequency p is favored over the strain with gene frequency q when $s > 0$, is disfavored when $s < 0$, and is neutral when $s = 0$ (5). Values for the selection coefficient may be empirically determined from changes in the relative concentrations of competing populations over time. Values for the selection coefficient, s, are expressed as selection per hour or selection per generation.

In this study, cells expressing the transductant phenotype were a minor component of the total population and, therefore, did not contribute significantly to total cell density. Therefore, the ratio between the transductant and nontransductant populations is approximated as the number of transductants per total. Changes in the transductant-to-total population ratio are described as follows:

$$\ln(T/N) = \ln(T_0/N_0)st \quad (2a)$$

or

$$(T_t/N_t) = (T_0/N_0)\, e^{st} \quad (2b)$$

where T represents the concentration of transductants, N is the total cell concentration and s is the selection coefficient. After a single generation ($t = 1\ g$) of selection, the transductant-to-total cell ratio would be reduced or increased by a factor equal to the selection factor, $f\ (=e^s)$, or

$$(\mathrm{T/N})_1 = (\mathrm{T/N})_0 f \tag{3}$$

Transduction. Equation 3 can be modified to include a term for the addition of new transductants to populations undergoing transduction as follows:

$$(T/N)_1 = (T/N)_0 f + \Delta(T/N)_{trans} \tag{4}$$

where $\Delta(T/N)_{\mathrm{trans}}$, the transduction rate, represents the increase in the frequency of transductants per total CFU per generation due to new transduction events. This rate is constant when the donor and recipient populations are stable and present in much greater numbers than are the transductants. Under these conditions, the transducing particle density is constant and transduction does not significantly reduce the number of recipients in the population.

Solving equation 4 for the generations 1 to g gives the following set of equations:

$$(T/N)_1 = f(T/N)_0 + \Delta(T/N)_{trans} \tag{5a}$$

$$\begin{aligned}(T/N)_2 &= f(T/N)_1 + \Delta(T/N)_{trans} \\ &= f\{f(T/N)_0 + \Delta(T/N)_{trans}\} + \Delta(T/N)_{trans} \\ &= f^2(T/N)_0 + f\{\Delta(T/N)_{trans}\} + \Delta(T/N)_{trans}\end{aligned} \tag{5b}$$

$$\begin{aligned}(T/N)_3 &= f(T/N)_2 + \Delta(T/N)_{trans} \\ &= f^3(T/N)_0 + f^2\{\Delta(T/N)_{trans}\} + f\{\Delta(T/N)_{trans}\} + \Delta(T/N)_{trans} \\ &= f^3(T/N)_0 + \{f^2 + f + 1\}\{\Delta(T/N)_{trans}\}\end{aligned} \tag{5c}$$

$$\cdots$$

$$(T/N)_g = f^g(T/N)_0 + \{f^{g-1} + f^{g-2} + \ldots + f^2 + f + 1\}\{\Delta(T/N)_{trans}\} \tag{5d}$$

In equation 5d, the finite geometric progression

$$f^{g-1} + f^{-2} + \ldots + f^2 + f + 1$$

takes the general form

$$\frac{f^g - 1}{f - 1}$$

Substituting in equation 5d, we obtain

$$(T/N)_g = f^g(T/N)_0 + \Delta(T/N)_{trans}\left(\frac{f^g - 1}{f - 1}\right) \tag{6}$$

When the transduct phenotype is at a selective advantage ($f > 1$), we obtain

$$(T/N)_g = f^g\left\{(T/N)_0 + \frac{\Delta(T/N)_{trans}}{f - 1}\right\} - \frac{\Delta(T/N)_{trans}}{f - 1} \tag{7a}$$

and the number of transductants increases exponentially. When the transductant has a selective disadvantage ($f < 1$), we obtain

$$(T/N)_g = \frac{\Delta(T/N)_{trans}}{1 - f} + f^g\left\{(T/N)_0 - \frac{\Delta(T/N)_{trans}}{1 - f}\right\} \tag{7b}$$

and the transductant-to-total cell ratio approaches a limiting value where the loss due to selection cancels the contribution from transduction. When no selection is present ($f = 1$), the computed transductant-to-total cell ratios increase linearly at a rate equivalent to the relative rate of transduction, $\Delta(T/N)_{trans}$. Finite solutions to equation 6 were determined for the first 50 generations by using different values of f (Fig. 1) to illustrate these predictions. Similar predictions for the dependence on selection of the accumulation of spontaneous mutants in a continuous culture environment have been made by Kubitschek (9).

Given experimental values for transductant densities, total concentrations of CFUs, numbers of generations passed, and the selection factor, f, equation 6 may be solved for $\Delta(T/N)_{trans}$, the transduction rate.

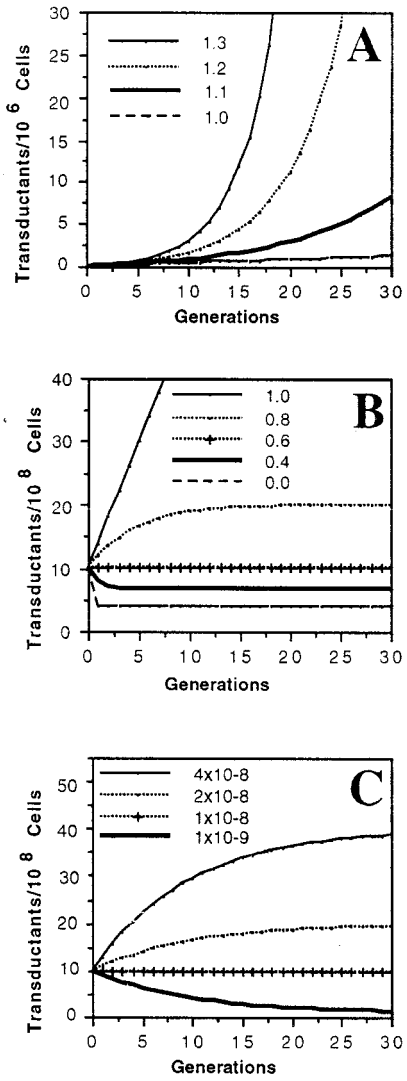

FIG. 1. Calculated changes in the transductant-to-total cell ratios predicted by equation 4. (A) Changes predicted for transductants under neutral and positive selection. Transductants consisted of 10^{-7} of the total population initially. The selection factor (f) was varied from 1.0 to 1.3, while the transduction rate was held constant at 4×10^{-8} transductants formed per total CFU per generation. (B) Changes predicted under conditions of neutral and negative selection. Here the initial concentrations and transduction rates are the same as those but the selection factor was varied from 1.0 to 0.0. (C) Predictions for negatively selected ($f = 0.9$) transductant populations. Transductants comprised 10^{-7} of the total population initially and the transduction rate was varied from 1×10^{-9} to 4×10^{-8} transductants formed total^{-1} generation^{-1}.

TABLE 2. Selection coefficients (s) calculated from changes in the density of mock transductants with respect to the total population[a]

Expt	Generation time (h)	s (± SE) (generation^{-1})	s (± SE) (h^{-1})
1	1.9	−0.02 ± 0.01	−0.011 ± 0.006
2	2.4	−0.03 ± 0.02	−0.012 ± 0.008
3	8.3	−0.04 ± 0.03	−0.005 ± 0.004
4	10.1	−0.14 ± 0.03	−0.014 ± 0.003
5	13.4	−0.28 ± 0.13	−0.021 ± 0.010

[a] Selection coefficients and standard errors were obtained for each experiment by solving s in equation 2 by using the nonlinear modeling function of SYSTAT.

RESULTS

Selection in mock transduction experiments. Control experiments were performed under conditions in which transduction could not occur with nonlysogenic strains. This allowed the identification of the effects of selection alone on the frequency of the transductant's phenotype. Inocula from mock transductant (RM289), donor (RM2235), and mock recipient (RM287) cultures were added to chemostat vessels containing PMM-c supplemented with 4×10^{-5}% yeast extract. Preliminary experiments demonstrated that this level of nutrient would support a total cell density of 10^5 to 10^6 CFU/ml. Mock transductants were introduced as a minority population ($<10^{-3}$% of the total cell concentration) to mimic an emerging transductant population. Various generation times of 1.9 to 13.4 h were investigated. The reservoir was sampled at least once daily for concentrations of donors, mock recipients, and mock transductants.

The relative number of mock transductants decreased exponentially with time over the range of generations tested. For each experiment, the selection coefficient, s, was determined with the aid of equation 2b by using the ratio of mock transductants per total bacteria, T_t/N_t, observed at various times, t. All values for the selection coefficient were negative, indicating that selection was acting against the mock transductants (Table 2). The rate of selection per hour was independent of generation time, with an average value of -0.013 ± 0.006, or 1.3% loss of the phenotype per hour. However, negative selection per generation increased as the length of the generation time increased (Table 2). Dykhuizen (4) observed a similar phenomenon in mixed populations of tryptophan auxotrophs and wild-type *Escherichia coli.*

The transduction system. Plasmid transduction was quantified by the transfer of the Tra$^-$ Mob$^-$ plasmid, Rms149, from the donor strain (RM2235) to the plasmid-free, lysogenic recipient strain (RM300). Putative transductants were tested for the presence of genetic markers from the recipient chromosome and plasmid Rms149 and also for the absence of markers from the donor chromosome. Selected transductants were verified by performing plasmid minipreparations. Plasmid DNA was digested with *Eco*RI and analyzed by agarose gel electrophoresis (Fig. 2). Plasmid Rms149 was found in all transduced isolates tested.

Transduction experiments were performed over a range of environmentally relevant cell concentrations, generation times, and donor-to-recipient ratios as summarized in Table 3. Companion trials and control experiments were performed under similar experimental conditions (Fig. 3). In the absence of transduction (Fig. 3A and C), there was a decline in the proportion of transductants in the population. Trials permitting transduction resulted in increased numbers of transductants per total over the same periods of growth (Fig. 3B and D). Under all conditions tested, permitting transduction to occur caused the transductant-to-total cell ratio to increase (Table 4).

Applying the mathematical model. Data obtained from the transduction experiments were analyzed to determine how well the derived model could describe the observed changes in the transductant populations. Mock transduction experiments suggested that transductants experience negative selection with respect to the donor and recipient strains. Hypothetical curves generated from equation 6 for conditions of negative selection indicated that transductant populations approach a limiting value, which depends on the rate of transduction and the degree of selection (Fig. 1B).

For each experiment, equation 6 was solved for the best-fit value of the transduction rate, $\Delta(T/N)_{trans}$, by using the nonlinear modeling function of SYSTAT. Concentrations of transductants, T, and total bacteria, N, observed at cell generations, g, were entered as variables into the equation. Values for the selection factor, f, were based on the selection coefficients, s, determined in control experiments (Table 2; Fig. 3A and C) performed at comparable generation times by using the simple relation, $f = e^s$.

Table 4 gives the values for the relative transduction rate, $\Delta(T/N)_{trans}$, computed from equation 6. All experiments had positive transduction rates and an overall increase in the transductant-to-total cell ratio. The relative number of transductants were found to fluctuate randomly along a path of increase predicted from equation 6 (Fig. 3). Fluctuations in the transductant populations may have been due to the random nature of the processes of transductant formation and cell washout.

The relative transduction rate, $\Delta(T/N)_{trans}$, is the rate of increase in transductants with respect to the total cell density (transductants per total CFU per milliliter per generation). Table 4 also gives the rate at which transductants themselves accumulate, or the apparent transduction rate, ΔT_{trans} (transductants per milliliter per generation).

Factors that affect transduction. Transduction experiments were conducted over a range of experimental conditions, so that factors affecting the transduction rate could be identified (Table 3). Concentrations of donors, recipients, and phages, the donor-to-recipient ratio, the PBR, and generation times

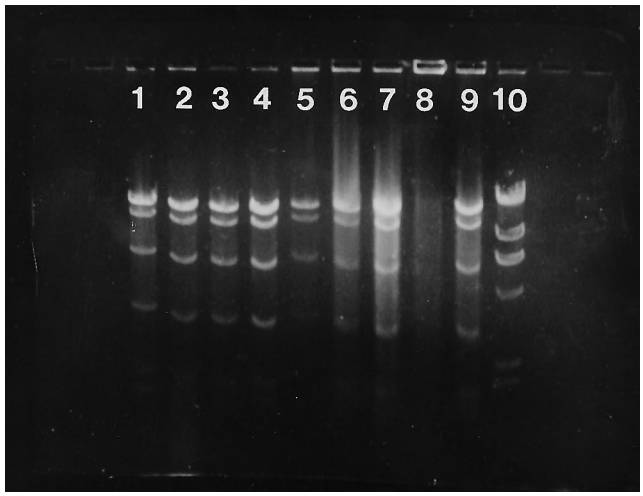

FIG. 2. Restriction patterns of plasmid DNA isolated from transductants. Lanes: 1 to 6, *Eco*RI digests of transductants from experiments 8, 11, 10, 5, 9, and 7 (see Table 5), respectively; 7 to 9, *Eco*RI digests of RM2235 (donor), RM300 (recipient), and RM289 (mock transductant), respectively; 10, *Hin*dIII digests of lambda DNA.

TABLE 3. Transduction experiments: a summary of conditions and equilibrium concentrations

Expt and condition	Yeast extract concn (%)	Generation time (h)	Total no. of cells (ml^{-1})[a]	PBR	No. of donor cells (ml^{-1})[a]	No. of recipient cells (ml^{-1})[a]	No. of phage F116 (ml^{-1})[a]
High cell concn							
1	5.0×10^{-1}	2.8	7.3×10^{8}	0.01	2.8×10^{8}	4.5×10^{8}	1.1×10^{7}
2	1.6×10^{-1}	9.8	6.4×10^{8}	1.66	9.7×10^{7}	6.4×10^{8}	8.3×10^{8}
Low cell concn and low PBR							
3	4.0×10^{-5}	2.1	3.8×10^{5}	0.25	5.7×10^{4}	3.2×10^{5}	2.7×10^{4}
4	4.0×10^{-5}	3.0	1.6×10^{6}	0.15	2.7×10^{5}	1.3×10^{6}	7.6×10^{4}
5	4.0×10^{-5}	3.4	4.6×10^{5}	0.09	1.1×10^{5}	3.5×10^{5}	9.8×10^{4}
6	4.0×10^{-5}	6.9	3.8×10^{6}	0.22	6.1×10^{5}	3.2×10^{6}	5.9×10^{5}
7	4.0×10^{-5}	8.4	2.7×10^{6}	0.14	1.6×10^{5}	2.5×10^{6}	5.9×10^{5}
8	4.0×10^{-5}	9.2	3.2×10^{6}	0.12	6.6×10^{5}	2.6×10^{6}	2.1×10^{5}
9	4.0×10^{-5}	10.3	1.1×10^{7}	0.03	7.3×10^{5}	1.1×10^{7}	2.1×10^{5}
10	4.0×10^{-5}	13.6	1.4×10^{7}	0.02	2.8×10^{6}	1.1×10^{7}	1.7×10^{5}
Low cell concn and high PBR							
11	9.0×10^{-9}	8.9	7.5×10^{5}	3.60	5.2×10^{5}	2.3×10^{5}	6.5×10^{5}
12	4.0×10^{-5}	9.6	2.5×10^{6}	125.0	2.5×10^{6}	8.0×10^{4}	1.2×10^{7}
13	4.0×10^{-5}	7.8	4.0×10^{5}	66.0	3.3×10^{5}	6.3×10^{4}	6.2×10^{6}

[a] Equilibrium concentrations.

were all examined to determine their potential influence on the apparent transduction rate, ΔT_{trans}. Variables were analyzed after transformation into logarithmic values on account of their large range. By using the SYSTAT statistical analysis program, a multiple linear regression was performed with the apparent transduction rate as the dependent variable and the factors listed above as independent variables.

This analysis revealed that only the recipient concentration and PBR showed significant correlations to the apparent rate of transduction (Fig. 4). The dependence on recipient concentration reflects the increased opportunity for transduction to occur when the recipients are in abundance. The dependence on PBR is most likely due to the direct proportionality of the PBR to the transducing particle-to-bacterium ratio (TBR).

The production of transducing particles is a relatively infrequent event (12). Since it was possible that the transducing-particle frequency might depend on the ratio of plasmid-bearing donors to lysogenic recipients present, these strains were combined in varying ratios (Table 5) in experiments designed to examine the frequencies at which plasmid-bearing transducing particles and infective phage virions appeared.

We found that the density of phage virions increased with the donor-to-recipient ratio. However, the ratio of plasmid-containing transducing particles per phage virion remained constant over the range of donor-to-recipient ratios explored. These observations indicated that phage particles were produced predominantly from the lysis of plasmid-bearing donor cells. There was an average of $(4.0 \pm 2.5) \times 10^{-7}$ transducing particles per infective phage virion. Because transducing particles make up a constant proportion of all phage-specific particles produced in an infection, the likelihood of recipient-transducing particle interaction increases with the total number of bacteriophages in the habitat.

When the donor-to-recipient ratio was increased (Fig. 5),

TABLE 4. Transduction rates calculated by equation 7

Expt and condition	Sample size (n)	Relative rate, $\Delta(T/N)_{trans} \pm SE$[a]	Equilibrium total concn (N)	Apparent rate, ΔT_{trans}[b]
High cell concn				
1	8	$(7 \pm 3) \times 10^{-9}$	7.3×10^{8}	5.1×10^{0}
2	3	$(10 \pm 11) \times 10^{-7}$	7.4×10^{8}	7.2×10^{2}
Low cell concn low PBR				
3	3	$(3 \pm 0) \times 10^{-8}$	1.2×10^{6}	3.1×10^{-2}
4	5	$(4 \pm 1) \times 10^{-8}$	1.8×10^{6}	7.8×10^{-2}
5	12	$(9 \pm 1) \times 10^{-9}$	8.2×10^{5}	7.3×10^{-3}
6	10	$(3 \pm 1) \times 10^{-9}$	4.1×10^{6}	1.3×10^{-2}
7	3	$(1 \pm 0) \times 10^{-8}$	2.6×10^{6}	2.9×10^{-2}
8	6	$(8 \pm 3) \times 10^{-9}$	3.2×10^{6}	2.6×10^{-2}
9	7	$(7 \pm 0) \times 10^{-8}$	1.1×10^{7}	7.2×10^{-1}
10	6	$(2 \pm 1) \times 10^{-8}$	1.8×10^{7}	3.0×10^{-1}
Low cell concn high PBR				
11	7	$(8 \pm 5) \times 10^{-8}$	1.2×10^{6}	9.9×10^{-2}
12	6	$(3 \pm 1) \times 10^{-7}$	7.4×10^{6}	2.1×10^{0}
13	10	$(8 \pm 1) \times 10^{-8}$	4.5×10^{5}	3.7×10^{-2}

[a] Change in the number of transductants per total number of CFU per milliliter per generation.
[b] Change in the number of transductants per milliliter per generation.

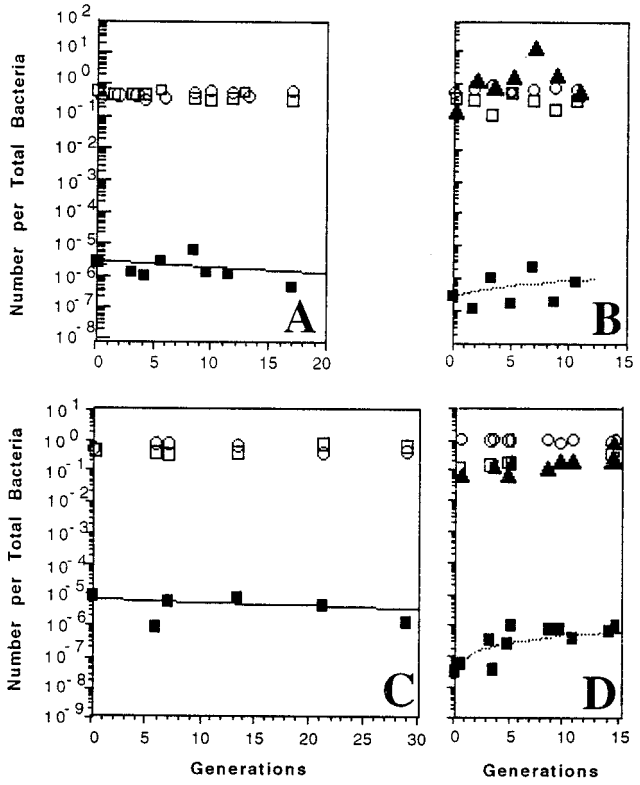

FIG. 3. Changes in the relative numbers of recipients (○), donors (□), transductants (■), and phage F116 (▲) in companion experiments. The data shown in panels A and B are from experiments that were performed at comparable generation times (8.3 and 8.9 h). (A) Without transduction (the relative numbers of mock transductants declined 4.1% per generation [solid line]); (B) with transduction (experiment 11; Table 5) (the transductants increased). The data shown in panels C and D are from experiments that were performed at shorter generation times (2.4 and 3.4 h). (C) Without transduction (the relative numbers of mock transductants showed a decline of 2.8% per generation); (D) with transduction (experiment 5; Table 5) (an increase in the relative numbers of transductants was observed). The slopes depicted in panels A and C show the rates of transductant selection described by equation 2, with the selection coefficient, s, and initial transductant-to-total cell ratio, $(T/N)_0$ determined from the individual experiments. The dashed lines in panels B and D represent the predicted kinetics of transductant accumulation determined from equation 6 for these individual experiments.

there was a significant increase in the PBR. This effect was due to a proportionately greater number of nonlysogens (i.e., donors) available for lytic infection by bacteriophage F116. The relationship between nonlysogenic bacteria and phage concentration was previously noted by Ogunseitan et al. (16). Increasing the donor-to-recipient ratio in the chemostat experiments affected the frequency of transduction inasmuch as it altered the PBR and thus the TBR.

DISCUSSION

The results presented in this study indicate that transduction can act to maintain a novel phenotype that would otherwise be eliminated from a bacterial population if horizontal gene transfer did not occur. Continuous-culture experiments were conducted in low-nutrient medium at dilution rates (generation times) that may be experienced in natural aquatic habitats. These conditions resulted in bacterial and bacteriophage densities representative of natural levels of aquatic bacteria (2, 10) and phage (1, 6).

The continuous-culture experiments showed plasmid transduction rates of 10^{-6} to 10^{-9} per total CFU per ml per generation between the plasmid-bearing donor and the lysogenic recipient. These frequencies were comparable to those observed for F116-mediated transduction (10^{-6} to 10^{-8}) between *P. aeruginosa* strains in in situ-incubated microcosm studies conducted in a freshwater lake (15, 22).

The apparent rate of transduction (i.e., the rate at which transductants accumulate in the population) ranged from 10^{-2} to 10^{3} transductants formed per ml per generation (Table 4). This rate increased significantly with both the recipient density and the PBR (Fig. 4). The dependence on recipient concentration simply indicates an increased opportunity for transduction to occur when the bacterial strain receiving the transduced DNA is in greater abundance. The correlation between increased transduction rate and increased PBR reflects a concomitant increase in the TBR. Simply put, transduction proceeds more rapidly when there are greater numbers of phages, and therefore transducing particles, per bacterium.

In our system, both donor and recipient strains could intercept transducing particles. Hence, transduction rate would be expected to depend upon the ratio of transducing particles to total bacteria, not just recipients, because both donor and recipient strains are able to adsorb transducing particles. However, transduction of the donor strain with plasmid-bearing

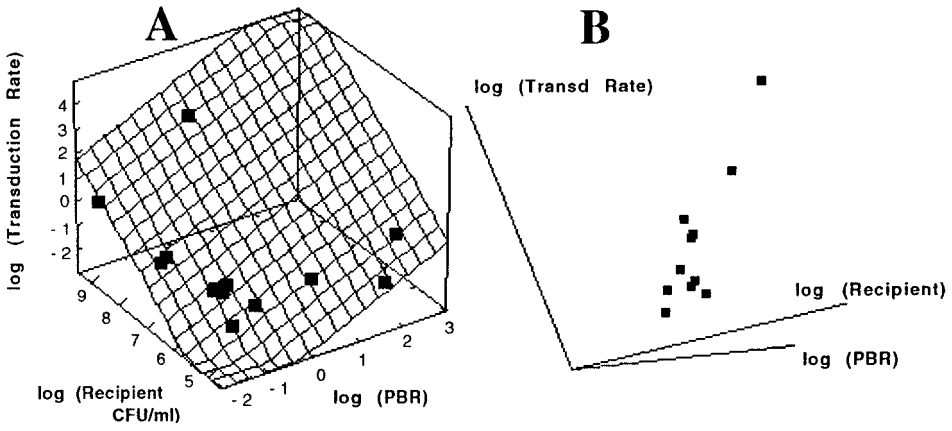

FIG. 4. Two views of a three-dimensional logarithmic graph showing the rise in apparent transduction rate with recipient concentration and PBR. Each point represents the average value observed in transduction experiments 1 to 13 (see Table 5). Panel A also shows a best-fit x-y plane, with the following formula: log (transduction rate) = 1.23 (± 0.17) log (recipient) + 0.84 (± 0.17) log (PBR).

TABLE 5. Concentrations of transducing particle and phage F116 and their ratios observed in batch culture experiments

Trial	Incubation time (h)	Donor/recipient[a]	Transducing particle density[b]	Phage density	TPR[c]
1	25	42.0	4.4×10^3	4.0×10^{10}	1.1×10^{-7}
2	16	5.0	7.0×10^3	1.0×10^{10}	7.0×10^{-7}
	25	3.1	4.8×10^3	2.5×10^{10}	1.9×10^{-7}
3	16	0.10	5.6×10^3	6.8×10^9	8.3×10^{-7}
	25	0.17	2.3×10^3	9.1×10^9	2.5×10^{-7}
4	16	0.024	1.2×10^3	2.0×10^9	6.0×10^{-7}
	25	0.012	4.8×10^2	1.1×10^9	4.4×10^{-7}
5	16	0.010	2.0×10^1	7.0×10^7	2.9×10^{-7}
	25	0.004	5.1×10^0	2.3×10^7	2.2×10^{-7}

[a] Donor-to-recipient ratio at total cell concentrations of 10^8 to 10^9 CFU/ml.
[b] Values for transducing particles were obtained by selecting for plasmid-bearing transductants on rifampin and carbenicillin selection plates.
[c] TPR, transducing particle-to-phage virion ratio.

transducing particles would not be detected because the donor strain already contained Rms149. Therefore, many of the transducing particles were lost to donor bacteria and did not contribute to the measurable transduction rate.

Information obtained from continuous-culture experiments and the mathematical model developed here demonstrates the importance of transduction as a mechanism of gene transfer and stabilization in the environment. In our model system, the transductant phenotype was at a selective disadvantage with respect to the total population. Under all experimental conditions, transduction served to counteract negative selection and act to stabilize an otherwise less-fit phenotype. Under circumstances of positive selection, our model predicts a continuous increase in the relative proportion of transductants, at a rate which depends on both the amount of selective advantage and the rate of transductant formation.

Because of its concentration-dependent nature, transduction would be predicted to be especially prevalent in habitats in which bacteria are present at very high densities, such as sewage, or waters polluted with excessive nutrients. Transduction would also be predicted to occur more frequently in habitats in which bacteria and bacteriophages congregate, such as on suspended particles (19) or on the surfaces of water bodies, at the liquid-gas interface (20).

Kokjohn et al. (8) found that bacteria and bacteriophages can still undergo adsorption and reproduction at very low host-cell densities. The present study suggests that although the rate of transduction may be reduced at low cell densities, transduction will occur at a rate proportional to the recipient concentration and the TBR.

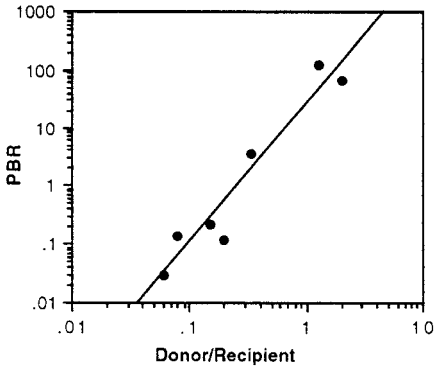

FIG. 5. Raising the donor-to-recipient ratio caused an increase in the PBR. The points represent average values from transduction experiments performed under otherwise similar conditions (experiments 6 to 9 and 11 to 13; Table 5).

When assessing the potential for transduction of genes from a genetically heterologous immigrant bacterium into an established gene pool, it is important to consider the circumstances of its introduction into the breeding population. This is of particular importance when the potential affects of introducing a GEM into a specific habitat are assessed. If the species carrying the new genetic material is represented in the indigenous population, the organism's ability to successfully participate in transduction is increased because many bacteriophages have very narrow host ranges. However, numerous examples of broad-host-range bacterial viruses now exist (12). The location of an engineered gene in the chromosome of the invading organism does not significantly reduce its potential to be transduced, because, unlike conjugation, transduction may transfer plasmid or chromosomal DNA with similar frequencies (21, 22). Increased fitness of the released organism would increase both its survival and its potential for transferring genes to the indigenous population. However, even if the immigrating organism is at a selective disadvantage, it does not eliminate its potential to introduce its genetic material into the gene pool of the indigenous microbiota, thereby increasing the richness and genetic diversity of the habitat. This introduction, may be positive, negative, or neutral to the environment, depending on the genetic marker introduced and the ecosystem into which it is introduced. Therefore, it is of primary importance to risk assessment that the potential benefit or harm of the genetic character itself, and not simply the genetically engineered organism, be thoroughly evaluated.

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