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A COMPLEX COMMUNITY IN A SIMPLE HABITAT: AN EXPERIMENTAL STUDY WITH BACTERIA AND PHAGE[1]

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Abstract. Continuous culture populations of the bacterium *Escherichia coli* and its virulent virus T7 have been studied as a model of a predator-prey system in a simple habitat. These organisms maintain apparently stable states of coexistence in: (1) a phage-limited situation where all of the bacteria are sensitive to the coexisting viruses and the sole, and potentially limiting carbon source, glucose, is present in excess; and (2) a resource-limited situation where the majority of the bacteria are resistant to these phage and in which there is little free glucose.

The composition of these interacting populations is examined in detail and evidence indicating that this simple experimental culture system can support relatively complex communities is presented. In the predator-limited situation, two populations at each of two trophic levels can be maintained; the wild-type bacterial and phage strains, denoted B_0 and $T7_0$, a mutant bacterial clone which is resistant to $T7_0$, denoted B_1 and a host range mutant phage, $T7_1$, which is capable of growth on both B_0 and B_1. In the resource-limited situation, three populations of bacteria and two populations of phage can coexist. They include the above described clones and a third bacterial strain, B_2, which is resistant to both $T7_0$ and $T7_1$. In phage-free competition, the wild-type B_0 bacterial clone has a marked advantage relative to both B_1 and B_2 while no difference is detected between B_1 and B_2. When competing for a B_0 host, the wild-type $T7_0$ phage clone has a marked advantage over $T7_1$.

The fit of these observations to some previously developed theory of resource-limited growth, competition and predation is discussed and a mechanism to account for the persistence of these communities is presented. The latter assumes that their stability can be attributed solely to intrinsic factors, i.e., the population growth and interaction properties of the organisms in this continuous culture habitat.

Key words: bacteria; bacteriophage; competition; continuous culture; genetic feedback; predator; prey; resources; selection.

INTRODUCTION

In recent years there has been a major shift in the interpretation of the factors necessary for the stable coexistence of predators and their prey. It had been assumed that the temporal and spatial heterogeneities of the real world were fundamental to the persistence of predator-prey associations, see for example Slobodkin (1961) and MacFadyen (1963). This "heterogeneity hypotheses" was based on (1) the mistrust of the "pathological" neutral stability of the classical Lotka-Volterra models upon which much of predator-prey theory had been founded and (2) the failure of Gause (1934) and others to obtain stable states of coexistence for predators and their prey in simple laboratory cultures. This interpretation was reinforced by Huffaker's (1958) demonstration that the term of maintenance of an arachnid predator-prey system could be markedly increased by an expansion of the spatial complexity of the experimental habitat.

Although it remains possible that temporal or spatial heterogeneities are essential to the maintenance of particular predator-prey systems, theoretical studies have suggested that such "patchiness" is not a necessary condition for stability. The majority of the predator-prey models developed by ecologists had demonstrated the existence of solutions specifying stable equilibrium points or limit cycles (see reviews by Scudo 1971; May 1972, 1973). Since these models assumed mass behavior in a featureless habitat, this theoretical result implied that stable predator-prey associations are possible without environmental heterogeneity.

Recent experimental studies reporting the coexistence of microbial predators and their prey in simple laboratory culture also question the justification for this heterogeneity hypothesis. The results of experiments by Luckinbill (1974) suggest that Gause's (1934) failure to obtain stable states of coexistence in his *Didinium-Paramecium* studies may have been due to stochastic factors rather than the absence of spatial heterogeneity. A number of workers, Paynter and Bungay (1969), Horne (1971) and Levin et al. (1977) have obtained stable states of coexistence for *Escherichia coli* and its virulent viruses of the T series (T1, T2, T3, and T4) in minimal medium continuous culture. Tsuchiya et al. (1972) and Jost et al. (1973) reported analogous results with the protozoan predator, *Tetrahymena pyriformis* and two species of bacteria, *E. coli* and *Azotobacter vinelandii*. The results of these latter continuous culture experiments are also consistent with the earlier theoretical prediction of

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Gause and Witt (1935). In predator-free competition between *E. coli* and *Azotobacter*, the latter species is excluded. With the predator, the two bacterial and one protozoan populations coexist in an apparently stable state.

In this report we present the results of a detailed investigation of continuous culture populations of *E. coli* B and its virulent virus T7. We demonstrate that these species can persist in either a predator-limited or a resource-limited state and that the interacting populations represent a community of bacterial clones with different phage sensitivities and phage with different host ranges. We consider the fit of these experimental results to the predictions made from our theoretical consideration of resource-limited growth, competition and predation (Levin et al. 1977), and present a mechanism to account for the maintenance of this community.

The Phage-Bacterial System

Both from the point of view of trophic relationships and modeling, the association between bacteria and their virulent viruses can be considered that of a prey and its predator. The interaction begins with the random collision of the phage and the bacterium and the adsorption of the virus to specific receptor sites on the host cell. The phage genome is injected into the bacterium. After a latent period, the host cell lyses and dies and an array of infective phage particles is released.

In the phage-bacterial system, single gene mutations can have profound effects on ecological associations. A mutation eliminating or altering the structure of the appropriate receptor site can afford a bacterium complete resistance to the virus that utilizes that site. This adaptation can be countered by host range mutations in the phage which alter the structure of their adsorption organ, enabling them to attack members of the resistant clone as well as those of the original phage-sensitive population. This process of mutational changes can proceed a number of times and, as we show, can yield a community of competing bacterial and competing phage clones with different resistance and host range relationships. In the T7-*E. coli* B situation considered here, we have observed three bacterial states, the original wild-type T7 sensitive clone, denoted B_0, two higher orders of T7 resistant mutants, B_1 and B_2, and two phage orders, the original wild type, T7 strain, $T7_0$, and a host range mutant, $T7_1$. This host range and resistance series is depicted in Fig. 1. For a more complete and general consideration of the biology of the bacterial phage interaction see Adams (1959) or Stent (1963).

Experimental Culture and Sampling Procedures

The culture methods and sampling procedures used here were similar to those employed by Levin et al.

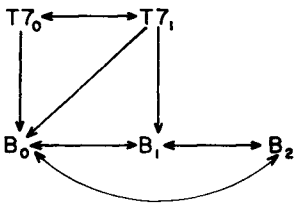

FIG. 1. Host range and resistance relationships for the T7-*Escherichia coli* B system. Horizontal arrows indicate possible directions of mutation. Vertical and diagonal arrows denote predator-prey associations. Subscripts indicate order of the various clones; "0" wild type, "1" the first order, and "2" the second order. B_2 is doubly resistant, B_1 resistant only to $T7_0$, and B_0 is sensitive to both phage.

(1977). The recipes for the media used are included in that earlier paper. All experimental cultures were maintained in continuous culture devices known as chemostats (Novick and Szilard 1950; Monod 1950). A minimal salt solution containing 100 μg/ml of glucose entered the culture vessels at a rate which was constant and equal to the rate at which unutilized resources, wastes and organisms were removed. At this concentration, glucose is the sole carbon and energy source, and in a phage-free system is the limiting resource. All culture vessels were maintained at 37°C and aerated and mixed by the rapid bubbling of sterile, cotton-filtered air. The *E. coli* B used were supplied by Dr. S. Lederberg and the T7 phage by Dr. C. Thorne. The chemostats were homemade, the plans for which are described in the Appendix.

At periodic intervals samples were taken from the effluent and serially diluted in sterile buffer. To estimate the number of phage, appropriate dilutions were mixed with a suspension of broth and agar containing a high concentration of sensitive bacteria (lawn) and poured on broth-agar plates. The product of the number of plaques and the dilution factor was the estimate of the number of phage in the culture vessel. The relative numbers of wild-type $T7_0$ and host range $T7_1$ phage were determined by comparing samples plated on lawns of B_0 and B_1 bacteria. The total number of bacteria in the cultures was estimated from colony counts on broth-agar plates.

Rough estimates of the relative frequencies of the different bacterial orders were obtained by plating appropriate dilutions with high concentrations of either $T7_0$ or $T7_1$ so that only resistant cells could produce colonies. Unfortunately, this technique is only useful to estimate frequencies of all cell types when the sensitive cells are common. To determine the composition of populations when the sensitive cells are rare, we initiated new cultures with bacteria labeled with different fermentation markers. For example, by using B_0 mal^+ and B_1 mal^- the two can be distinguished by the growth of the former on maltose minimal plates and their respective colony colors on tetrazolium maltose indicator plates (see section on "The Composition of the type A and type B communities").

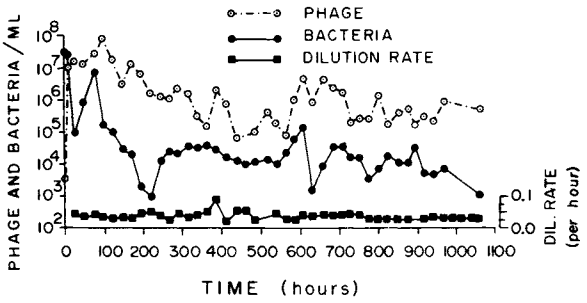

FIG. 2. A phage-limited (type A) T7-*Escherichia coli* B community in glucose minimal continuous culture. Mean dilution rate 0.036 ± 0.012/h.

These sampling procedures tend to underestimate the numbers of bacteria and phage in the culture vessel. Only bacteria capable of forming colonies and phage capable of growth after the shock of dilution and transfer are counted. Bacteria infected by the virus at the time of sampling or bacterial colonies growing into phage before reaching macroscopic size are not counted. Phage adsorbed to dead bacteria or bacterial fragments do not produce plaques. Bacteria adsorbed with more than one phage produce only one plaque. To minimize these errors inherent in the sampling procedures, only newly prepared medium and fresh lawn bacterial cultures were used.

LONG-TERM CULTURES

Mixtures of overnight, stationary phase, cultures of the sensitive bacterial clone B_0 and a diluted lysate of $T7_0$ were inoculated into the culture vessels of six independent chemostats. In all six, the bacteria and phage coexisted. These continuous culture populations were maintained for a minimum of 528 h to a maximum of 1160 h with termination occurring because of contamination with foreign bacteria or fungi or because of other needs for the equipment. These six populations displayed two distinct behaviors which we denote type A and type B. The type A cultures remained clear with relatively few bacteria and a higher concentration of phage. The type B cultures were initially clear, but became turbid and maintained a bacterial population density similar to that anticipated for a phage-free culture of similar bacteria. The concentration of phage in the type B cultures was similar to that of type A. Four of these six populations remained in the type A category.

In fig. 2, we present the census results of one of the type A cultures. The presence of phage in this system resulted in the maintenance of a bacterial population density three to four orders of magnitude lower tha that which would be obtained in a phage-free culture with the same concentration of glucose and a similar flow rate. This suggests that the bacterial population in this culture was limited by the virus rather than its primary resource, as would be the case in a classical (phage free) chemostat. This interpretation is supported by estimates of the effluent glucose concentration. Using a Glucostat® assay, we were unable to detect significant differences in the concentration of glucose in the reservoir and the concentration in the outflow in this or the other type A cultures. In similar cultures without phage, the free glucose in the effluent would be ≈5 μg/ml.

Throughout the course of this experiment, B_0 and $T7_0$ remained the predominant bacterial and phage types. However, these were not the only types present in the populations. After 500 h, members of a $T7_0$-resistant clone were observed. These B_1 cells were distinguished from B_0 by their ability to grow in the presence of high concentrations of $T7_0$ and their more irregular colony morphology. Cells of the B_1 type were observed in the four type A cultures where they remained the minority bacterial population with a relative frequency generally less than 10^{-2}. To account for the failure of the B_1 population to increase, it seemed reasonable to assume that a major portion of the phage population must be capable of growth on this clone. By using lawns of B_1 cells, the host range phage, $T7_1$, was detected in relatively high frequencies in all four type A cultures. $T7_1$ was capable of growth on B_0 and B_1.

Two of the other type A chemostats were maintained for 1105 h and the third for 528 h. The dilution rate in the type A chemostat depicted in Fig. 2 was relatively slow, 0.036 ± 0.012/h. The average dilution rates of the three additional type A chemostats were 0.123, 0.300 and 0.370/h. There did not appear to be any association between this rate of flow and the densities of bacteria and phage in these cultures.

The type B cultures are distinguished from the type A by the presence of a second order bacterial clone, B_2. The effects of the evolution of the B_2 clone are illustrated in Fig. 3. Here we present the census results of one of the two type B, long-term cultures. In this population, the B_0 bacteria were introduced and allowed to reach numerical equilibrium before the inoculation with phage. The introduction of the $T7_0$ lysate resulted in a marked decline in the bacterial population and a period of behavior similar to that of the type A cultures. At 400 h, the second order B_2 bacteria were detected. Their evolution resulted in an increase in the density of the bacterial population and its continued maintenance at a level similar to that of the phage-free B_0 culture with which this population was initiated. As is the case for the phage-free population, the effluent concentration of glucose after the evolution of B_2 was ≈5 μg/ml. The dilution rate of this chemostat was 0.040 ± 0.012/h.

In spite of the fact that $T7_0$ and $T7_1$ were incapable of growth on the B_2 bacterial population, the phage maintained a relatively high concentration in these type B cultures. There was no evidence of further host range changes in this viral population. A change in plaque morphology was, however, observed in the lat-

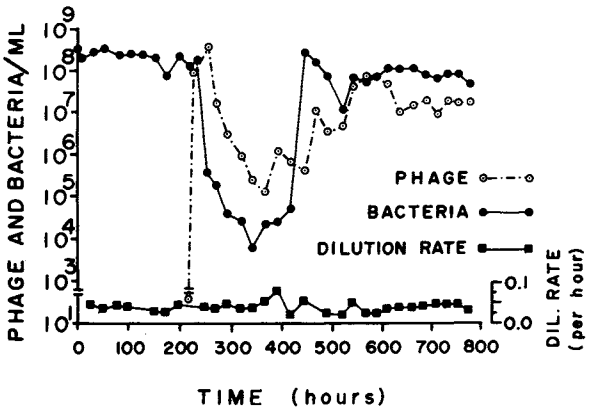

FIG. 3. A resource-limited (type B) T7-*Escherichia coli* B community in glucose minimal continuous culture. Mean dilution rate 0.040 ± 0.012/h.

ter samples of the depicted culture. After 655 h, phage producing distinctly smaller plaques on B_0 lawns than either $T7_0$ or the first detected $T7_1$ were noted. These small plaque phage, however, had the same host range as $T7_1$. Consequently, we cannot attribute the persistence of the viral population to their evolution. We therefore assumed, and we shall demonstrate, that the phage population in this type B culture was supported by coexisting minority populations of phage sensitive cells.

The second type B culture was initiated by simultaneously introducing $1 \times 10^8 B_0$ cells/ml and 4×10^6 $T7_0$ particles/ml. This culture was maintained for 800 h at an average dilution rate of 0.068/h. Unlike the above described type B culture, the plaque morphology of the predominant $T7_1$ phage population remained similar to that of the original $T7_0$ and the first order $T7_1$.

The Composition of the Type A and Type B Communities

The long-term continuous-culture results clearly indicate that the strains of T7 and *E. coli* B used in this investigation can coexist. However, the sampling procedure used offers only limited information about the relative frequencies of the various T7-sensitive and T7-resistant bacterial clones and of the first order and host range phage in these interacting populations. In this section we consider the composition of these cultures in more detail.

The chemostats described in the preceding section were initiated with B_0 and $T7_0$. The various resistant and host range clones then evolved naturally. The cultures described in the present section were initiated with clones of $T7_0$ and $T7_1$ phage and series of bacterial clones (B_0, B_1 and B_2) which were labeled with different genetic markers. The frequencies of these different bacterial clones were followed by sampling with combinations of tetrazolium indicator and minimal medium agar plates. Two fermentation systems were used, arabinose (ara) and maltose (mal). The original wild-type clone, B_0, was labeled ara$^-$ mal$^+$, the first order B_1, ara$^+$ mal$^-$ and the second order B_2, ara$^-$ mal$^-$. These genetically labeled clones were isolated as spontaneous revertants from cells isolated from a phage-bacterial chemostat initiated with an ara$^-$ mal$^-$ strain of B_0.

This approach has two potential pitfalls: (1) due to the selection in this system, the genetically marked sensitive, B_0, and first order resistant, B_1, clones could be replaced by similarly marked first or second order resistant mutants. To control for this problem, at periodic intervals bacteria isolated from these cultures were tested for sensitivity to $T7_0$ and $T7_1$. In all of the cultures considered here, the resistance relationships of the genetically marked strains isolated at the end of these experiments were similar to those of the clones used to originate these cultures; (2) the fermentation markers could affect the relative fitnesses of their host cells and thus the frequencies of these clones. Although we can't rule out this possibility, we don't believe that this effect would be sufficiently great to alter the qualitative conclusions drawn from these experiments. Our experience with mal markers suggests that, in general, the mal$^-$ clones have a slight selective disadvantage relative to mal$^+$. The ara marker doesn't seem to have any effect on clone fitness. This is, in fact, illustrated in the results reported in the next section where we consider the relative competitive performance of the different bacterial clones.

The $T7_1$ clone used in these experiments with marked bacterial stocks had normal plaque morphology and was isolated from the same chemostat employed for the isolation of the above-described bacteria. Although this and the other $T7_1$ clones produce plaques on both B_0 and B_1 lawns, for a given dilution the number of $T7_1$ plaques on a B_1 lawn was 0.260 ± 0.013 of that on the B_0 lawn. In presenting the relative densities of $T7_0$ and $T7_1$ clones, we correct for this difference in plating efficiency by multiplying the number of plaques on a B_1 lawn by the reciprocal of 0.26.

The results of a type A culture initiated with labeled B_0 and B_1 and $T7_0$ and $T7_1$ are presented in Fig. 4. Both bacterial and both phage clones remained in the population throughout the term of these experiments. Most interestingly, the first order B_1 population maintained a density of nearly three orders of magnitude less than the B_0 strain which was sensitive to both phage populations. The $T7_0$ and $T7_1$ populations persisted with approximately equal frequencies. This particular chemostat was maintained at an average dilution rate of 0.151 ± 0.010/h. A similar relative density relationship among clones was observed in the two other experiments with marked bacteria in type A chemostats.

The high frequency of B_2 in the long-term type B cultures precluded a determination of the composition of a minority bacterial population. However, the fact

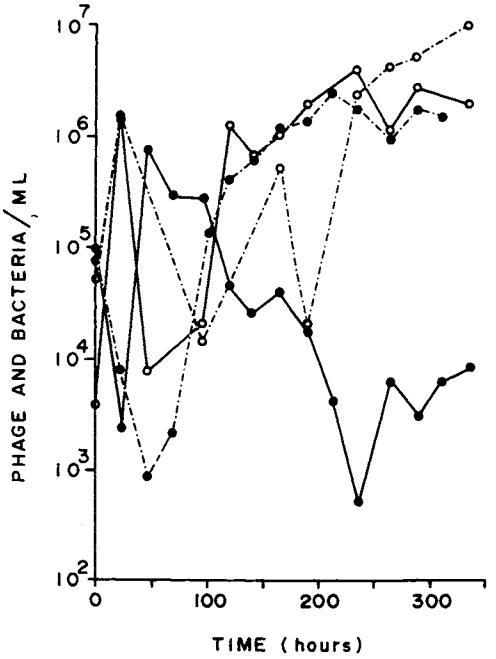

FIG. 4. A phage-limited (type A) T7-*Escherichia coli* B community in glucose minimal continuous culture with genetically labeled bacteria. Mean dilution rate 0.051 ± 0.010/h. B_0 (●—•—●), B_1 (●——●), $T7_0$ (○—•—○), and $T7_1$ (○——○).

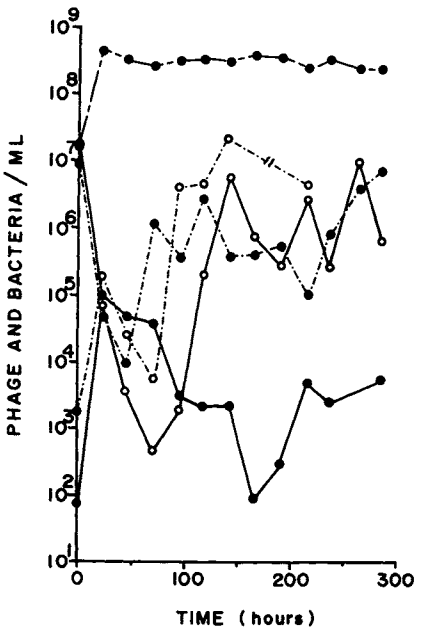

FIG. 5. A resource-limited (type B) T7-*Escherichia coli* B community in glucose minimal continuous culture with genetically labeled bacteria. Data points for the trajectory of the $T7_0$ population up to 141 h represent daily samples which indicated the presence of the wild-type phage. Following the 141-h sample, the presence of $T7_0$ was indicated by only one sample at 214 h. Mean dilution rate 0.139 ± 0.012/h. B_0 (●—•—●), B_1 (●——●), B_2 (●——●), $T7_0$ (○—•—○), and $T7_1$ (○——○).

that the phage population persisted after the evolution of the B_2 indicates that a population of T7-sensitive cells must exist. These sensitive bacteria could be (1) continually produced by recurrent mutation from B_2 or (2) a stable coexisting population of T7-sensitive cells. The results of our experiments with marked cells in type B cultures support the latter hypothesis.

In Fig. 5, we present the census results of one of the three type B culture experiments with marked cells. This population was inoculated with a mixture of marked B_0, B_1 and B_2 and $T7_0$ and $T7_1$. In the depicted culture, B_0 maintained a population of $\approx 10^6$ cells/ml. The first order clone B_1 initially dropped in frequency, but then continued to persist at a density of $\approx 10^3$ cells/ml. The dilution rate of this chemostat was 0.139 ± 0.012/h. A similar result was obtained in one of the two replicas of this experiment. In the other, the B_1 population failed to recover from the initial decline.

The relative density of $T7_0$ phage in this type B culture was generally less than that of $T7_1$ and, in some of the later samples taken from this culture, the estimated frequency of this phage clone was zero. However, the latter is, to a great extent, an artifact of the sampling procedures. We believe that a substantial population of $T7_0$ phage is maintained in these type B cultures. The estimate of the density of $T7_0$ is obtained from the differences in the plaque counts of permissive B_0 and nonpermissive B_1 lawns, and requires a correction for plating efficiency. Consequently, estimates of the density of these wild-type phage cannot be obtained when their relative frequencies are low. There are, however, a number of indications for the presence of a substantial $T7_0$ population in these cultures. First, in the last few contiguous samples in the culture depicted in Fig. 5, the density of $T7_0$ showed no indication of a rapid decline. Second, the sample taken after 214 h of this culture had a high frequency of $T7_0$. Finally, in the one replica of this experiment in which we did sample for the two phage types, the frequency of $T7_0$ reached very high levels. This was the culture in which the B_1 population declined in frequency. During this period of decline in B_1, the frequency of $T7_0$ continued to increase and achieved a relative frequency of 0.999 before the termination of the experiment.

Relative Competitive Performance of the Different Bacterial and Phage Clones

In our theoretical consideration of resource-limited population growth, competition and predation (Levin et al. [1977]), we demonstrated that if the predator and prey are able to coexist in a stable state, then this equilibrium can persist in the presence of a predator-resistant population of consumers. The condition being that the resistant population has a selective disadvantage in predator-free competition with the sensitive clones. Motivated in part by this theoretical conclusion, we have examined the relative competitive

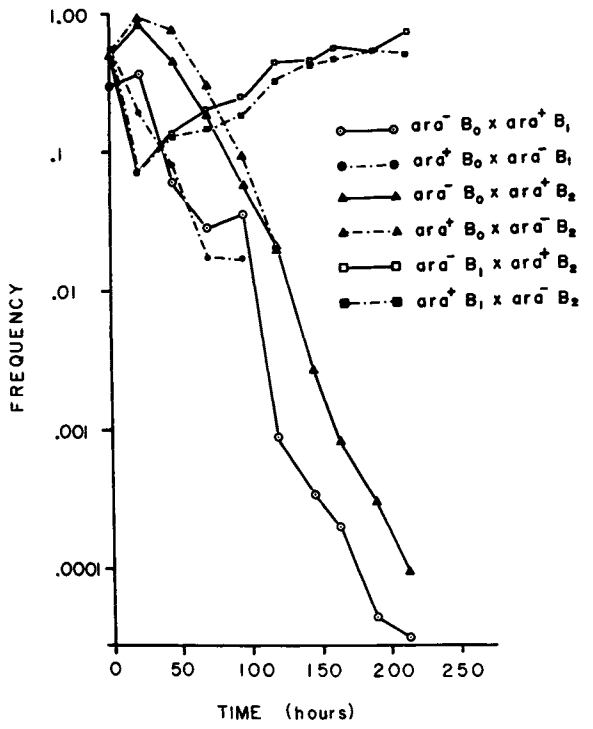

FIG. 6. Pairwise competition among the three orders of bacteria, B_0, B_1 and B_2, in phage-free, glucose minimal continuous culture. For the competition cultures containing B_0, the relative frequencies of B_1 and B_2 are plotted. For the cultures with B_1 and B_2, the relative frequency of the latter clone is plotted. The average dilution rates of these competition cultures were similar and had an overall mean of ≈0.25/h.

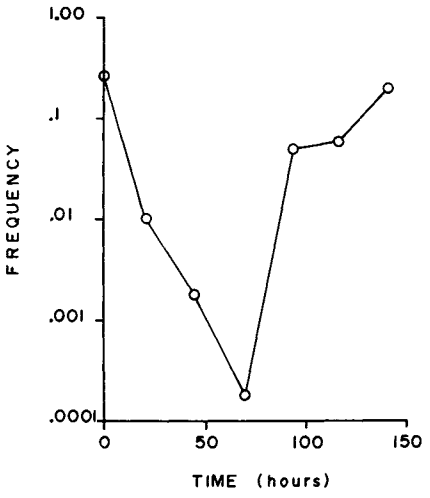

FIG. 7. Competition between $T7_0$ and $T7_1$ in glucose minimal continuous culture initiated with a wild-type, B_0, bacterial population. The relative frequency of $T7_1$ is plotted. Mean dilution rate 0.098 ± 0.005/h.

performance of the various bacterial clones in the absence of phage and of the phage clones on their common host B_0.

Pairwise competition experiments were performed by introducing genetically labeled B_0, B_1 and B_2 stocks into phage-free chemostats. Only one fermentation marker was used to distinguish between competitors. To control for the effects of this marker, reciprocal experiments were run, e.g., ara^+ B_0 with ara^- B_1 and ara^- B_0 with ara^+ B_1. The results of these continuous culture competition experiments are presented in Fig. 6. They clearly illustrate a marked disadvantage for both resistant strains B_1 and B_2 relative to the sensitive B_0 clone in phage-free competition and that this difference in clone performance cannot be attributed to the ara marker. We believe that this difference is best accounted for by a difference in the growth rates of the competitors. These results fail to demonstrate any difference in the performance of B_1 and B_2 in phage-free competition. The average dilution rate of these competition cultures were similar and had an overall mean of ≈0.250/h.

The relative competitive performance of $T7_0$ and $T7_1$ on the wild-type B_0 host was determined by continuous culture competition experiment. Lysates of the two phage types and a stationary phase population of B_0 were introduced into the culture vessel and samples were taken at periodic intervals. In Fig. 7, we present the plating efficiency corrected relative frequency of $T7_1$ in this experiment. During the first 69 h, the frequency of $T7_1$ declined, clearly indicating that this phage clone has a marked selective disadvantage relative to $T7_0$ on a B_0 host. We attribute this disadvantage to a difference between the growth rates of the two phage. The reversal in the relative frequency relationship of $T7_0$ and $T7_1$ can be accounted for by the evolution of B_1 bacteria. These first-order B_1 cells were first detected in the sample taken after 69 h. The average dilution rate of this chemostat was 0.098 ± 0.005/h.

Discussion and Conclusions

In our theoretical consideration of resource-limited growth, competition and predation (Levin et al. [1977]), we presented a form of the exclusion principle appropriate to three trophic levels: "The number of distinct predator populations cannot exceed the number of distinct prey populations and the number of distinct prey populations cannot exceed the sum of the number of primary resources plus the number of predator populations." Within these limits, $K \leq I \leq K + J$, it is possible to have solutions specifying stable equilibria with I primary consumer populations living on J resources and preyed upon by K predator populations. This principle is derived from the analysis of a model we had developed for an invariant continuous flow habitat such as that in an idealized chemostat. The model assumes that interactions among populations within a trophic level are only through the consumption of common resources and that there are no fluctuations in concentrations of the primary resources

or wastes. Furthermore, it should be emphasized that this principle defines a necessary but not sufficient set of conditions for coexistence. Within the set of communities defined by this principle, there are those where stable states of coexistence will occur and those where it will not. Whether or not the conditions for coexistence in a simple habitat exist in the real world remains an empirical question. We interpret the results of this and earlier studies with continuous culture populations of microorganisms (cited in Introduction) as affirmative answers to this question. We also see the present investigation as a significant extension of this earlier work. These previous studies demonstrated that communities consisting of (1) one population at each of two trophic levels and/or (2) two populations of primary consumers with one population of predators, can be maintained in a habitat supported by a single resource. The results of the present investigation indicate that this culture system, which is perhaps the simplest of experimental habitats, can also support (3) two populations at each of two trophic levels and (4) three populations of primary consumers with two populations of predators.

The trophic pattern described in the type A and type B T7-*E. coli* B communities conform to the requirements of the above exclusion principle. Considering glucose as a unique resource, B_0, B_1, and B_2 as distinct prey populations, and $T7_0$ and $T7_1$ as distinct predator populations, in the type A situation, $I = 2$, $J = 1$ and $K = 2$ and in the type B, $I = 3$, $J = 1$ and $K = 2$. However, we don't see this apparent conformity as proof of the quantitative fit of our model, or any other simple model of predator-prey associations. The concepts of unique resources, invariant flow and dimensionless habitats are only valid in a theoretical framework. Glucose is the only carbon and energy source entering the culture vessel and the unique resource driving the system. However, as a result of the metabolism of this sugar, a diverse array of secondary, by-product resources may be produced. The peristaltic pumps and vacuum system that maintain the flow through the culture vessels are, of course, less than perfect. There may be significant pulses in concentrations of resources and wastes. This combination of secondary resources and pulses in primary resource concentration can stabilize the association among the competing populations of bacteria, (B. Levin [1972]; Megee et al. 1972; Stewart and Levin 1973). A stabilization of the predator-prey association could be achieved by the existence of a physical refuge for the phage-sensitive bacteria, e.g., a wall population of sensitive cells which, due to their physical position, are immune to viral predation. A mechanism of this sort has, in fact, been suggested by van den Ende (1973) to account for coexistence between *Tetrahymena pyriformis* and *Klebsiella aerogenes* in continuous culture.

Although we cannot rule out the contribution of these environmental heterogeneities to the stability of the T7-*E. coli* B communities, we prefer a more parsimonious explanation. Namely, the stability of this system is intrinsic to the growth characteristics of the populations in the continuous flow habitat. The mechanism we postulate to account for this is an extension of that for which we present a formal mathematical treatment in Levin et al. (1977). Elements of this mechanism are included in Campbell's (1961) theoretical consideration of the population biology of the phage-bacterial association and in Paynter and Bungay's (1969) discussion of the results of their studies with continuous cultures of these organisms. There are three basic components: (1) A numerical refuge for phage-sensitive bacteria; (2) the continuous removal of organisms by flow through the culture vessel; and (3) the higher growth rates of wild-type bacteria and phage. The following is an intuitive description of this mechanism:

1) At any time the number of sensitive bacteria being killed by the virus varies with the product of the density of their population and that of the phage. When the phage density is low, the proportion of the bacterial population succumbing to viral predation will also be low. Consider, for example, the biologically realistic adsorption rate of 10^{-9} ml contacts per cell per phage per min (Schlesinger 1932) and a bacterial and phage population of 10^5/ml each. Assuming a product relationship for predatory contacts during a 1-min period, only 10 bacteria/ml would be killed by the virus. This fraction, 10^{-4} of the sensitive bacterial population, could be replaced readily by the production of new individuals by cell division. Thus, when the density of the sensitive bacterial population and/or that of the virus are relatively low, the number of bacteria lost to predation can be insignificantly small. This density-dependent response, in effect, protects the bacterial population from over-predation, a situation we shall refer to as a "numerical refuge."

This refuge, although a property of the form of the population interaction rather than the heterogeneity of the habitat, still acts as a true asylum. When the phage and bacteria are sparse, the prey population can increase with near impunity but support little growth of the predator population. However, when the density of this primary consumer population is great, the opposite is true. Now the phage thrive and, if they were not originally plentiful, they soon become so. This will halt the growth of the bacterial population. For one class of models with single predators living off single prey, Kolmogorov (1936) presented a mathematical formulation of this inverse density-growth rate relationship of the prey and predator populations and demonstrated that it will necessarily lead to their coexistence; for a review see Scudo (1971) or May (1973). Unfortunately Kolmogorov's theorem says nothing about models with more than one population at each of the two trophic levels. However, as long as

the population interaction takes the form described here, this numerical refuge, and resulting inverse density-population growth rate principle remains applicable.

2) The continuous flow of resources into the habitat and continuous removal of organisms from it play two very significant roles in the regulation of this community: (i) it ensures to the bacterial population the resources needed for its growth; and (ii) it prevents the unlimited increase in the phage population. When the density of the primary consumer populations is low, there will be plenty of resources available for each one and the rate of cell division will be high. However, no matter how favorable conditions may be, the outflow ensures that all populations will suffer attrition which cannot fall below some minimum. This is particularly critical for the persistence of the bacteria. In an appropriate medium, there is virtually no natural mortality for the almost inert, viral particles. In the absence of an outflow, their population would continue to increase and, in sufficient time, would eliminate all the bacteria.

3) The continued persistence of the phage-sensitive, wild-type bacterial population and the virus after the evolution of fully resistant bacterial clones can be accounted for by the relatively higher growth rate of the wild-type cells. The primary effect of a high-density phage-resistant bacterial population is a reduction in the resource concentration and a lower rate of cell division for all orders of bacteria. The resistant cell population would increase until the concentration of resources is sufficiently low and the rate of growth becomes equivalent to the dilution rate, the "chemostat equilibrium." If the sensitive bacterial population has an overall growth rate advantage, their population would be able to increase at the concentration of the resource which imposes the chemostat equilibrium on the resistant cells. They are, however, prevented from replacing these resistant bacteria due to viral predation. As a consequence of the numerical refuge and flow, the sensitive cells are not eliminated by the virus, but rather persist and support the viral population. In our theoretical consideration of this process, we demonstrated that if the parameter values are such that the phage-sensitive bacteria and the virus will coexist, then as long as the sensitive cells have a finite growth rate advantage, the presence of a resistant population will not preclude the phage-bacterial equilibrium.

We attribute the persistence of the wild-type phage after the evolution of the first-order resistant bacterial mutants to a mechanism analogous to that accounting for the polymorphism in the bacterial population. When the relative frequency of the wild-type (sensitive) bacterial population is sufficiently high, the disadvantage associated with the narrower host range of the wild-type viruses would be offset by their relatively higher growth rate on the sensitive host cells.

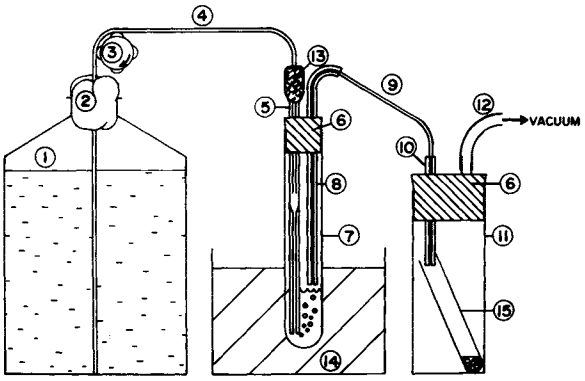

FIG. 8. Diagram of a chemostat. Reservoir with sterile media (1), cotton plug (2), peristaltic pump (3), silicone tubing (4), chemostat vessel inflow tube (5), rubber stoppers (6), chemostat vessel (7), chemostat vessel outflow tube (8), silicone or latex tubing (9), waste collection bottle inflow tube (10), waste collection bottle (11), waste collection bottle outflow tube (12), cotton (13), constant temperature water bath (14), and test tube for sample collection (15).

This interaction between the various orders of bacteria and phage balances the competitive differences which would, otherwise, drive one or more of these clones to extinction. Although the analogy is not exact, this situation contains a feedback resembling that in the models studied by Stewart (1971) and Levin and Udovic (1976).

This mechanism, which we postulate for maintaining the polymorphisms in the phage and bacterial populations, resembles "genetic feedback" (Pimentel 1961, 1968). In fact, a number of authors (Lomnicki 1971, 1974; S. Levin 1972; Levin and Udovic 1976; Maynard Smith 1974, p. 116–118), have used the term "genetic feedback" in their descriptions of analogous processes. However, we see little utility in employing this expression to describe the situation here. The phrase genetic feedback doesn't reveal very much about underlying processes and we tend to agree with Lomnicki's (1974) view that it can be misleading. It has been offered as a mechanism to maintain genetic polymorphisms, to stabilize associations between interacting species and in its most extreme, or perhaps most optimistic form, to regulate the densities of populations below the level imposed by environmental limitations.

In our interpretation of the T7-*E. coli* B situation, the stability of the association between the bacterial and viral populations is dependent upon the numerical refuge and the flow through the habitat. There is no call to invoke any other explanation. On the other hand, the polymorphism in the bacteria is made possible by the presence of the phage and host range polymorphism in the phage develops in response to the polymorphism in the bacteria. The relative fitness of the different bacterial genotypes depends on the densities of the different phage types and the relative fitness

of the different phage clones depends on the densities of the various orders of bacteria.

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Appendix

The chemostat depicted in Fig. 8 was developed by D. G. Searcy and B. R. Levin after a design suggested by T. Jones. Fresh sterile media is stored inside the reservoir (1), a Borosilicate glass carboy. Silicone rubber tubing (4) (Silichem [TM], 1 mm ID, New Brunswick Scientific International) leaves the reservoir through a cotton plug (2) and enters the culture vessel (7) via a modified Pasteur pipet (5), the inflow tube. A multichannel peristaltic pump (Polystaltic [TM] Buchler Instruments) (3) insures a uniform flow of media to the culture vessel. With this size tubing and pump, a minimum reliable flow rate of $\approx$5 ml/hr can be maintained. The lower half of the inflow tube consists of a thick wall capillary to reduce the likelihood of breakage. Thick wall capillary is also used for the outflow tube (8) of the culture vessel and the inflow tube (10) entering the waste collection bottle (11). We have used either 1 mm (ID) silicone tubing or larger diameter latex tubing (9) to connect the culture vessel with the collection bottle. For the latter we have been using 0.5 pint glass cream bottles (*Drosophila*) which give the apparatus a classical appearance. Two hole rubber stoppers (6) are used to seal the culture vessel and the collection bottle. A vacuum supply is connected to the outflow tube (12) from the collection bottle. The negative pressure created inside the culture vessel draws air down the inflow tube (5), aerating and mixing the media. Cotton (13) is packed around the silicone tubing at the top of the inflow tube to filter the air. The vacuum also removes excess media through the outflow (8) therefore maintaining a constant volume inside the culture vessel. Since the media inside the culture vessel is being continuously replenished, populations persisting inside a chemostat must reproduce at a rate equal to the dilution rate (dilution rate = flow rate of media through the chemostat $\div$ the volume of media inside the culture vessel). We normally adjust the dilution rate by raising or lowering the outflow capillary (8) in the culture vessel while maintaining the pumps at the minimum reliable flow rate. Borosilicate test tubes (25 $\times$ 200 mm) are used as culture vessels but Ehrlenmyer flasks can be used if larger volumes are desired. The culture vessels are kept in constant temperature water baths (14) adjusted to the desired temperature. Bacteria are inoculated directly into the culture vessel by removing the rubber stopper.

The flow rate through the chemostat is estimated by daily measurements of the volume of waste accumulated in the collection bottle. To minimize the likelihood of contamination, the tubing leaving the culture vessel (9) is clamped during the process. Population samples are collected by inserting a sterile 100 $\times$ 13 mm test tube (15) into the collection bottle after the latter has been alcohol-flame sterilized.

The reservoir, the culture vessels, and the collection bottles, assembled as a unit, are sterilized by autoclaving. To prevent caramelization, the sugar is added to the media in the reservoir after autoclaving, but while they are both hot. We have successfully refilled depleted reservoirs by directly pouring in hot sterilized media. With this design, various culture vessels can be fed by the same reservoir and can be regulated by a common peristaltic pump. For alternative chemostat designs and discussion of chemostat theory, see Kubitschek (1970).

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