Microbial Competition

A. G. Fredrickson and Gregory Stephanopoulos

Microorganisms may be most well known as agents that cause disease and spoil food. They also perform many functions that are beneficial to man. They decompose sewage and solid and industrial wastes. They make antibiotics, vitamins, and plant growth factors. They are essential in some food processing operations. They are important geochemical agents, and they were involved in the formation of coal, oil, and some mineral deposits. In fact, the biosphere could not function without microorganisms, and the higher organisms, man included, could not exist as we know them (*1*).

Summary. Populations of microorganisms inhabiting a common environment compete for nutrients and other resources of the environment. In some cases, the populations even excrete into the environment chemicals that are toxic or inhibitory to their competitors. Competition between two populations tends to eliminate one of the populations from their common habitat, especially when competition is focused on a single resource and when the populations do not otherwise interact. However, a number of factors mitigate the severity of competition and thus competitors often coexist.

Almost all these microbial activities involve the participation of several to many different populations of microorganisms. Pure, or single population, cultures are almost always the creations of microbiologists. In mixed culture situations the populations invariably interact with one another as well as with the abiotic part of their environment. In this article we deal with one of these interactions, competition, and with other interactions that often occur in conjunction with it.

Patterns of Competition in Microbial Ecosystems

Microbial populations must have chemicals and available energy (*2*) in order to grow and proliferate. The chemicals are needed to supply elements such as carbon, oxygen, nitrogen, sulfur, and phosphorus from which biological molecules are formed, and available energy is needed to synthesize these molecules and to maintain life. These common needs give rise to competition, which occurs in all but the simplest ecosystems.

Populations can satisfy their needs for chemicals and available energy in different ways and, because of this, some avoid and some turn to other kinds of interaction. In fact, satisfaction of needs in different ways by different populations is the origin of that profoundly important network of interactions that ecologists call the food web.

The need of microbial populations for chemicals from which to synthesize biological molecules or to obtain available energy is met in two ways. Osmotrophic organisms—such as bacteria, yeasts, molds, and microalgae—obtain chemicals by molecule-by-molecule or ion-by-ion transfer of the chemicals across their cell membranes. Phagotrophic organisms, such as many protozoan populations, obtain chemicals by ingesting and digesting particulate matter and then absorbing the products of digestion. Evidently, phagotrophic microorganisms are more likely to prey on osmotrophic microorganisms than to compete with them. However, populations of phagotrophic microorganisms are likely to compete with one another for resources of particulate matter, and populations of osmotrophic microorganisms are likely to compete for resources of chemicals.

Additional important differences between organisms appear in the way they satisfy the needs for specific elements. The element most often considered here is carbon. Heterotrophic microorganisms obtain carbon by uptake of organic compounds, phagotrophically or osmotrophically, whereas autotrophic microorganisms obtain carbon by uptake of carbon dioxide. Synthesis of organic compounds from carbon dioxide requires a supply of available energy, and photoautotrophs obtain this by absorbing light, whereas chemoautotrophs or chemolithotrophs obtain it by oxidizing certain inorganic compounds. Yeasts, molds, most bacteria, and most protozoa are heterotrophs; microalgae and a few bacteria are photoautotrophs; and a few bacteria are chemolithotrophs. Evidently, heterotrophs that are also osmotrophs will compete for organic compounds present in their common environment, but they will not compete for such compounds with chemolithotrophs that are present. Photoautotrophs will compete similarly for light. Although heterotrophs do not have to compete with autotrophs for organic compounds, they may compete with autotrophs for other chemicals since, in the case of osmotrophic populations, there is no single chemical substance that can supply all requirements for chemicals of any population. Therefore, populations that do not compete for sources of carbon and available energy may still compete for sources, for example, of nitrogen or phosphorus.

Some microbial populations use several to many different chemical compounds to satisfy the same need in their subcellular economies, whereas other populations are obligately dependent on one compound or perhaps a few compounds to satisfy such need. Populations of the first kind are often called generalists and populations of the second kind specialists. Some things that a generalist population uses will likely be exempt from its competition with a specialist population, and this can have important effects on the result of the competition. We would expect generalists also to be gleaners in the sense that they can glean enough resources from a lean environment to grow, albeit slowly, and specialists to be exploiters in the sense that they can exploit an environment rich in the resources they can use to grow rapidly.

Thus many different patterns of competition between two populations can occur, even when populations are of the same general class—for example, when both are osmotrophic and heterotrophic. Some means of classifying these patterns is needed. Also, the usual meaning of

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A. G. Fredrickson is a professor in the Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis 55455, and G. Stephanopoulos is an assistant professor in the Department of Chemical Engineering, California Institute of Technology, Pasadena 91125.

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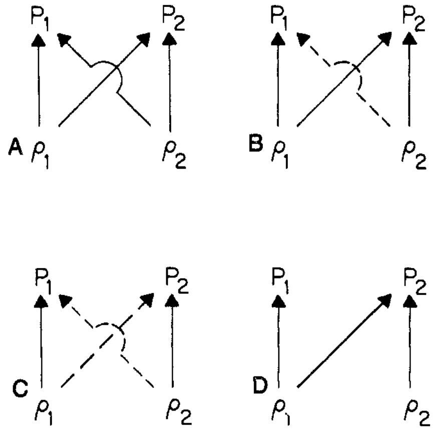
competition, a striving of two units of biological organization for a common object, is not precise enough when we discuss competition in detail. Hence, we must consider some matters of definition as well.



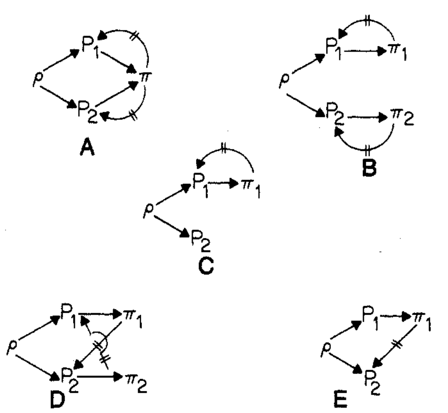


Fig. 1 (left). Four cases of pure competition involving two populations (P_1 and P_2) and two resources (ρ_1 and ρ_2) that exert dynamical effects. A solid arrow indicates that the resource is used by and exerts a dynamical effect on the population. A dashed arrow indicates that the resource is used by but exerts no dynamical effect on the population. (A through C) Cases of total and double competition. The situation shown in (B) can arise when ρ_1 and ρ_2 satisfy different needs in the economies of the populations: P_2 is an obligate user of both ρ_1 and ρ_2, but P_1 is a facultative user of ρ_2 (that is, P_1 uses ρ_2 when this substance is freely available but switches to some other resource, assumed here to be freely available and not shown, when ρ_2 becomes scarce); resource ρ_2 and its substitute will not exert dynamical effects on P_1 so long as at least one of these resources is freely available. (D) A case of partial and single competition that can arise when ρ_1 and ρ_2 satisfy the same need in the economies of P_1 and P_2 and when P_1 is an obligate user of ρ_1 and P_2 is a facultative user of ρ_1 and ρ_2. Fig. 2 (right). Five cases of single competition complicated by the production of autoinhibitors or inhibitors. An arrow from the nutrient (ρ) to a population (P) indicates that the nutrient is used by that population, whereas an arrow from a population to an inhibitor (π) indicates that the inhibitor is produced by the population. An arrow with crossbars indicates that the inhibitor at the tail of the arrow inhibits the population at its tip.

Some Definitions and a Classification

The common meaning of competition is broad enough to be applied to two quite different types of population interactions. In the first type, chemicals or particulate matter and perhaps light are extracted from the common environment, and the consequent reductions of the resources reduce the net growth rates of the populations. The words resource, exploitation, or indirect competition are often applied to this type. In the second type, microbial populations release chemical substances that have toxic or inhibitory effects on other microbial populations. The words interference or direct competition are among those applied to this type. Since the mechanisms involved are quite different, we shall reserve the term competition for the first type of interaction. The second type of interaction between two populations will be called "antagonism" if the toxic or inhibitory effects of substances excreted are mutual, and "amensalism" if only one population produces a toxin or inhibitor. Antagonism and amensalism are important interactions in some microbial ecosystems (*1*, *3*), but they will not be treated further, except incidentally, in this article.

The existence of antagonistic and amensal interactions shows that population growth and proliferation rates can be affected by chemical substances other than those used for growth. In fact, the presence of such substances can alter the outcome of competition. We must therefore make a distinction between substances that are resources—those used for growth and proliferation—and those that are not resources. Of course, some chemicals used for growth by certain populations may also be inhibitory if their concentrations are sufficiently high. Phenol, a well-known antiseptic, exerts such effects on some bacterial populations, for example. Substances that act in this dual way may be classified as resources.

In order to classify the pattern of competition between two populations for all resources in their common environment, we must first define exactly what is meant by competition between them for any one of the resources. Thus, we say that populations P_1 and P_2 compete for resource ρ if and only if (i) both P_1 and P_2 use, but do not necessarily require, ρ and (ii) resource ρ has a dynamical effect on at least one of the populations, and possibly on both of them. Resource ρ has a dynamical effect on a population if its availability at any time has a significant effect on the net growth rate of that population (*4*). In terms of mathematical models of systems, the availability of a resource that has a dynamical effect on one or more populations of a system must be one of the dependent variables of the system. It is emphasized that populations P_1 and P_2 are not said to compete for ρ when neither is dynamically affected by it, even though both use it (*5*).

Consider the pattern of competition of two populations for the whole set of resources in their common environment. One way to classify this pattern is by counting the number of resources competed for: if one resource is competed for the competition is single, and if two are competed for it is double, and so on. Here, a rather subtle point arises and must be mentioned.

Single and double competition are appropriate concepts when the resources involved are discrete entities, such as different chemical compounds. The available energy carried by light, however, is distributed over a continuous spectrum of frequencies, and microalgae use light of a range of frequencies to satisfy their requirements for available energy. Competition of populations of microalgae for light is therefore somewhat analogous to competition of bacteria for many different chemical compounds. Thus we apply the terms single or double only to competition for discrete resources (*6*).

Another way to classify the pattern of resource use by two populations is the following. We say that competition between two populations is total if every substance that has a dynamical effect on one or on both populations is a resource, and a resource that is competed for. Competition will be partial rather than total if there is at least one resource that is used by and exerts a dynamical effect on one population and not on the other. Partial competition can arise when a generalist (P_2) and a specialist (P_1) compete (Fig. 1D). It has been studied experimentally [see (*7*) and discussion below]. But competition can fail to be total if some substance not used for growth by either population and not, therefore, a resource, nevertheless exerts a dynamical effect on one or on both populations. Competition is the only interaction between P_1 and P_2 in Fig. 2, B and C, but the competition is partial (*8*).

Other classifications of patterns of resource use by two populations are possible, but we will mention only one more special case. We shall say that total competition which is also single is simple competition. In simple competition, only one substance has dynamical effects on the competitors, this substance is a resource, and it exerts dynamical effects on both competitors.

Finally, it is desirable to have a word to classify the pattern of interactions between two populations that compete with one another, because in most real situations it is quite likely that interactions in addition to competition will occur. Thus we say that competition is pure if it is the only interaction between two populations.

We turn next to competition of osmotrophic populations for chemical resources or nutrients. We shall not consider competition of phagotrophic populations for biotic resources because little is known about such competition. Conclusions about competition for chemical resources do not necessarily apply to competition for biotic resources, which is complicated by growth of the resources consumed.

Pure and Simple Competition

In pure and simple competition there is only one nutrient whose availability affects the growth rates of the populations, and competition for this nutrient is the only interaction between the populations. We shall make this highly idealized situation even more idealized by considering situations where the system in which the competition occurs is spatially homogeneous at all times and where all external circumstances that affect the system—hereafter called inputs to the system—do not change with time.

This idealized situation can be approximated in the laboratory in a chemostat device (*9*). This is a vessel fed at a constant rate with sterile nutrient medium of constant composition and from which culture is removed at the same volumetric rate at which fresh medium is added. The culture is kept spatially homogeneous by mixing. In addition, the ratio of culture volume to area of the vessel wetted by the culture should be very large (*10*). Such a device is a model, admittedly very crude, of natural microbial ecosystems (*11*). By employing several chemostats with flows between them, with feeds whose compositions or flow rates, or both, vary periodically, with imposed temperature that varies periodically, and so forth, we can make the model system mimic some of the disturbing nonidealities of natural ecosystems.

A classic analysis of pure and simple competition in a chemostat with constant inputs was made by Powell (*12*). He was interested in the ability of this apparatus to select one population over several initially present and the basis of its selective power. The questions that his analysis answered were: Can the chemostat be operated with constant inputs so that two pure and simple competitors coexist? If the chemostat is operated in such a way that one competitor is excluded, what is it that determines which population is excluded?

Populations can coexist in two fundamentally different ways. The coexistence may involve a perpetually transient state in which the densities of the populations oscillate continually. If the same cycles of oscillations are obtained regardless of the initial conditions imposed on the system, and if these cycles are periodic, the system is said to exhibit limit cycle oscillations or simply a limit cycle. Limit cycles often arise in systems with periodically varying inputs, but it is well known that they also arise in systems with constant inputs. The other kind of coexistence is that in which the population densities become constant and the state of the system does not vary with time. This is the steady-state situation. Evidently, steady-state coexistence cannot occur if the inputs to the system are not steady. Steady-state coexistence appears to be the more significant kind of coexistence in the sense that competitors that coexist in a steady state can also be made to coexist in a limit cycle if appropriate time-varying inputs to the system are used, but the converse does not seem to be true. Powell's analysis of pure and simple competition was concerned with coexistence in a steady state.

If populations coexist in a steady state, then the intrinsic rates of increase of both populations must be zero. The intrinsic rate of increase of a population in a spatially homogeneous system is the difference between the rate at which new individuals are added to the population by reproduction and immigration and the rate at which individuals are lost by death and emigration, divided by the number of individuals in the population. The intrinsic rate of increase of a given microbial population in a steady-state situation is determined by the environmental circumstances, such as temperature, concentrations of nutrients and inhibitors, and perhaps light intensity, and also, in a chemostat, by the dilution rate (the volumetric flow rate of liquid through the vessel divided by the volume of culture in it). The dilution rate affects the intrinsic rate of increase since flow of culture from the vessel causes organisms to emigrate.

Populations distributed throughout a spatially uniform system are exposed to the same set of environmental circumstances. Thus the nutrient concentrations adjacent to the cells of one population are the same as those adjacent to the cells of the other population. In pure and simple competition of two populations, say 1 and 2, only the concentration of the single nutrient competed for (the limiting nutrient) effects the intrinsic rates of increase of the populations. If we denote this concentration by s, then the steady-state intrinsic rates of increase of the populations are functions only of s and the dilution rate D, if other external factors, such as temperature, are fixed. Necessary conditions for coexistence of pure and simple competitors 1 and 2 in a chemostat steady state are that $r_1(s,D) = r_2(s,D) = 0$, where r_1 and r_2 are the steady-state intrinsic rates of increase of populations 1 and 2. In general, there may be several solutions for s and D, but only those solutions for which s is positive and less than the concentration of the competed for nutrient in the feed to the chemostat, and D is positive, have physical and biological meaning. Vanishing of r_1 and vanishing of r_2 are not a sufficient condition for coexistence in a steady state for, among other reasons, the steady state or states so determined may not be stable with respect to perturbations.

The equations $r_1(s,D) = r_2(s,D) = 0$ define two relations between s and D that can be determined experimentally from measurements on pure cultures of the populations. In many cases, the results obtained will be like one of the situations shown in Fig. 3. More complicated cases, in which the curves do not pass through the origin or in which they intersect at several points in the first quadrant sometimes arise (*13*) but, as these do not alter our conclusions, they will not be considered.

For the curves shown in Fig. 3A there are no meaningful values of s and D that satisfy the necessary conditions for coexistence in a steady state, and Powell's analysis showed that population 2 would always be excluded from the chemostat regardless of the dilution rate. In Fig. 3B the curves cross at $s = s_c$ and $D = D_c$, and the necessary conditions for coexistence can be satisfied if D has exactly the value D_c. At all other conditions of operation one of the populations would necessarily be excluded (*14*). Powell's analysis showed that population 1 would be excluded if D was constant and less than D_c but that population 2 would be excluded if D was constant and greater than D_c.

It can be shown that the coexistence steady state allowed for $D = D_c$ (Fig. 3B) is stable, although in a restricted sense. In practice, however, a physical parameter such as a chemostat dilution rate will always exhibit random variations with time, and the variations will even be biased. Stephanopoulos *et al.* (*15*) modeled the random fluctuations in the dilution rate as white noise and showed that one competitor will be excluded from the chemostat if the intensity of the noise in D and the bias of the mean of D away from D_c are not both zero. Moreover, they showed that there is a finite probability that either population may be excluded. If the intensity of the noise and the magnitude of the bias are both small, then the drift toward exclusion of a population will be slow but it will always occur.

Many experiments in which the population interactions should approach pure and simple competition have been performed (*16*). For example, Jannasch (*17*) studied competition between *Escherichia coli* and a marine *Spirillum* sp. in a chemostat fed with lactate-supplemented seawater. He found that the density of *E. coli* declined toward zero if the dilution rate was low, whereas the density of the *Spirillum* sp. declined if the dilution rate was high. These results agreed with predictions made from Powell's analysis and data from the pure cultures. Harder and Veldkamp (*18*) studied competition for lactate by two species of marine psychrophilic bacteria in a situation where chemostat dilution rate and temperature were varied. At −2°C population O, an obligate psychrophile, excluded population F, a facultative psychrophile, at all dilution rates, and at 16°C population F excluded population O at all dilution rates. At 4° and 10°C, however, the outcome of competition was dependent on the dilution rate; population O was excluded at low dilution rates and population F at high rates. These results, predicted in part from data on pure cultures, are important because they show that whether or not a certain level of an externally imposed parameter confers a competitive advantage on a population depends on the levels of the other parameters imposed. Hansen and Hubbell (*19*) studied competition of bacteria for tryptophan in a chemostat and were able to predict from pure culture data the outcomes of competition and, semiquantitatively, the time courses of population density changes.

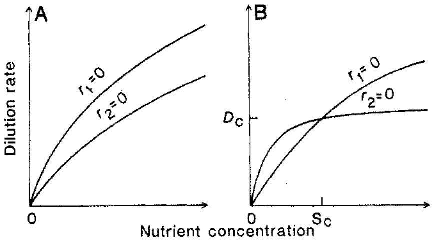


Fig. 3. Graphs of the steady-state relations between the concentration of a limiting nutrient (s) and the dilution rate (D) for pure and simple competition in a chemostat; the intrinsic rates of increase r_1 and r_2 for populations P_1 and P_2 equal 0 along the curves shown. Since dilution rate just balances growth rate in a steady-state chemostat, the graphs really show how steady-state growth rates depend on the concentration of the limiting nutrient. In (A), P_1 grows faster than P_2 at all positive nutrient concentrations. In (B), this is so only when $s > s_c$; when $s < s_c$, P_2 grows faster than P_1. The behaviors shown in (B) are typical of a gleaner (P_2) and an exploiter (P_1) population.

We conclude that pure and simple competition of two populations in a spatially homogeneous environment leads to exclusion of one of the competitors if all inputs to the competitive system are time-invariant. It is important to emphasize the reason why pure and simple competition leads to exclusion. In order for steady-state coexistence to occur when all inputs to a chemostat are constant, the concentration of the single dynamically significant nutrient must satisfy two conditions: $r_1(s,D) = 0$ and $r_2(s,D) = 0$. If D is set, it will not be possible for s to satisfy both conditions, the intrinsic rates of increase cannot both be zero, and no steady state of coexistence is possible (*20*). The analysis does not say what the mechanism of exclusion is and, therefore, excluded populations are not always starved to death. In fact, it is possible to set up a chemostat competition experiment in which a population will be excluded even though it is growing as fast as its genetic constitution will allow. In other situations, even the population that persists may be in a partially starved state.

Our conclusion is a highly qualified version of the competitive exclusion principle proposed several decades ago by Hardin (*21*), whose statement of the principle was that "Complete competitors cannot coexist." He admitted that every word in his statement was ambiguous and had been chosen so deliberately, because he did not want "to hide the fact that we still do not comprehend the exact limits of the principle." Although we still have much to learn about competition, we now have reasons to believe that there are competitive situations, some of which can be described as "complete," where populations can coexist, and sometimes coexist in a steady state; these are discussed below.

Time-Varying Inputs and Environmental Heterogeneity

If it is true that complete competitors cannot coexist, the question arises how is it that so many species of phytoplankton, obviously competing for limited supplies of nutrients, can persist in the relatively homogeneous environments of large bodies of water—the so-called paradox of the plankton raised by Hutchinson (*22*). Hutchinson accepted the idea that "one species alone would out compete all the others so that in a final equilibrium situation the assemblage would reduce to a population of a single species." However, he thought that "equilibrium would never be expected in nature whenever organisms had reproductive rates of such a kind that under constant conditions virtually complete competitive replacement of one species by another occurred in a time . . . of the same order, as the time. . . taken for a significant seasonal change in the environment." In the language of our article, his suggestion was that competitive exclusion might not occur in a spatially homogeneous ecosystem that has periodic, time-varying inputs, and pure and simple competitors therein might coexist in a limit cycle. This possibility has been analyzed mathematically by Stewart and Levin (*23*), Grenney *et al.* (*24*), Stephanopoulos *et al.* (*25*), and Hsu (*26*).

Stewart and Levin considered pure and simple competition in a homogeneous system from which, at fixed intervals of time, a constant fraction of the culture was removed and replaced by an equal volume of fresh nutrient medium. The interval of time between successive harvests of the culture was assumed to be so long that all the limiting nutrient added was consumed. This was their model of a "seasonal" growth situation. The other workers (*24–26*) considered pure and simple competition in a chemostat when the inputs were varied periodically and investigated four patterns of variation, one a generalization of the seasonal growth situation. Stewart and Levin showed that pure and simple competitors could coexist in their seasonal growth situation, and the other workers showed that periodic inputs could sometimes allow competitors to coexist (*27*). A necessary condition for coexistence was that the steady-state growth rate dependences of the two populations on the concentration of the limiting nutrient be of the type shown in Fig. 3B. In addition, coexistence in specific cases could occur only if certain quantitative conditions on the operation of the competitive system were satisfied. For example, Stewart and Levin found that coexistence could occur in their seasonal situation only if the concentration of limiting nutrient in the system immediately after dilution and the fraction of the culture left in the vessel at harvest fell within a rather restricted domain [figure 6 in (*23*)]. Similar constraints were found in the other studies. Thus, some kinds of periodic disturbances of a spatially homogeneous system can allow pure and simple competitors to coexist in a limit cycle, provided that the growth rate properties of the competitors are of the right kind and operating parameters are kept within certain bounds.

These analyses raised further questions such as: Can three populations of organisms that compete (in pairs) purely and simply for a single dynamically significant resource coexist in a spatially homogeneous system with periodic inputs? If so, what growth rate properties must the populations have? And what constraints on the values of operating parameters must be imposed?

Although the mathematics of three-population systems is complex, numerical calculations can provide some answers. Stewart and Levin, for instance, found that three populations could coexist on a single limiting nutrient in their seasonal growth situation if the growth rate relations (the three-population generalization of Fig. 3) were of a special kind and the domain of operating parameters was restricted. The conclusion they drew was that periodic disturbance of inputs to a spatially homogeneous system cannot "by itself . . . explain the coexistence of large numbers of species on a few limiting resources" (*23*). We have no reason to dispute this conclusion at present.

Heterogeneity of environmental conditions is often invoked to resolve the paradox of the plankton.

A laboratory model of an environmentally heterogeneous system can be constructed with two chemostats (Fig. 4). The feed of fresh, sterile medium is split between the two vessels, and flows carry cultures between the vessels. In the absence of the intervessel flows of culture, two competing populations could still coexist if their growth rate characteristics were of the type shown in Fig. 3B. All that would be required would be to adjust the split of the feed so that the dilution rate in one vessel exceeded D_c (Fig. 3B) and was less than D_c in the other. One population would then be excluded from each vessel, but the mixed overflow could contain both populations. With intervessel flows, however, a population must be present in both vessels if it is present in one, and coexistence means that both competitors are present in both vessels.

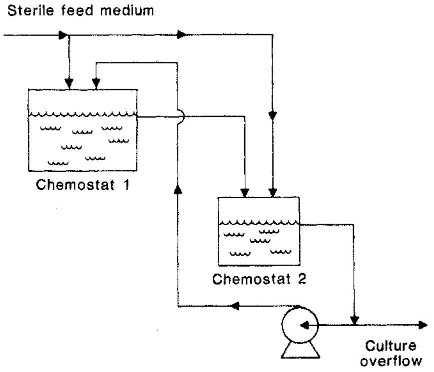


Fig. 4. A laboratory chemostat situation in which environmental heterogeneity occurs. All flow rates are time-independent.

In general, the activities of organisms present will cause concentrations of nutrients in the two vessels of a chemostat (Fig. 4) to adjust differently and the concentrations will depend on the flow rates and the volumes of the vessels as well as on the properties of the organisms. If the competition is pure and simple, coexistence might be expected to occur even with time-independent inputs, if the levels of the limiting nutrient in the vessels are such that one population grows faster in one vessel and the second in the other vessel. This would require that the growth rates of the two populations obey the relations shown in Fig. 3B. The two-vessel system was investigated mathematically by Stephanopoulos and Fredrickson (*28*), and their analysis confirmed the expectation.

In the model system, a heterogeneous environment divisible into two homogeneous subenvironments, with migrations between them, is predicted to be capable of allowing coexistence of two pure and simple competitors even when all inputs to the system are time-independent. It appears, therefore, that a necessary condition for heterogeneity of environmental circumstances to allow coexistence of *n* populations that compete purely and simply is that there be at least *n* different subenvironments in the system (*29*). Other conditions must also be satisfied, however. The case *n* equals 2 showed that one population must be favored in the one subenvironment and the other population in the other subenvironment. This suggests that *n* competitors can coexist in a system with *n* subenvironments only if a different population is favored in each subenvironment. This can be proved for the case where there are no migrations between subenvironments, for then there will be but one population per subsystem. However, we have been unable to prove that the statement is true when there are migrations between the subenvironments, except when *n* is 2.

The kind of environmental heterogeneity we have been discussing involves discontinuous changes in environmental conditions at the boundaries between homogeneous subenvironments. Another kind of environmental heterogeneity involves continuous changes within a system that is not divided into discrete subsystems. If such a system is not agitated, diffusion of nutrients and organisms will be important processes within it. If the organisms are motile, chemotaxis will be an additional important process in the system's dynamics. Lauffenburger (*30*) found that a coexistence steady state is possible in such a system, but he has not shown whether this steady state is stable.

Pure but Not Simple Competition

Studies of two population situations in which competition is pure but not simple show that such competitors can, under some conditions, coexist in a steady state in a spatially homogeneous system with constant inputs. In fact, up to *n* populations can coexist in such a system if *n* nutrients exert dynamical effects on the system, because the steady-state intrinsic rate of increase of a population will depend on the concentrations of these *n* nutrients. Necessary conditions for coexistence of *m* populations in a steady state are that intrinsic rates of increase of all *m* populations must be zero. These conditions impose *m* constraints on the *n* nutrient concentrations, and if *m* is greater than *n*, these constraints cannot usually be satisfied (*31*). Evidently, existence of numerous resources that exert dynamical effects in a system is another circumstance, in addition to time-varying inputs and spatial heterogeneity of the environment, that can be advanced to resolve the paradox of the plankton.

Many different competitive situations are possible when two or more nutrients exert dynamical effects in a competitive system. For example, when there are two populations (P_1 and P_2) and two nutrients (ρ_1 and ρ_2) that exert dynamical effects, competition may be total and double (Fig. 1, A to C) or partial and single (Fig. 1D). There are two subcases of Fig. 1A: in one, ρ_1 and ρ_2 fill the same need in the cellular economies, and P_1 and P_2 are facultative users of these resources; in the other, they fill different needs, and P_1 and P_2 are obligate users of the resources. These two subcases have been described (*23*, *32–34*). Titman (*33*) and Tilman (*34*) reported experimental data on total competition of two species of diatoms for silica and phosphate in a periodically diluted culture. Coexistence occurred at some conditions of operation, and Tilman was able to make fair predictions of the conditions that resulted in coexistence from data taken on the pure cultures (*35*). Gottschal *et al.* (*7*) studied partial competition in a chemostat. One of the competitors was a strain of *Thiobacillus* that could grow as a heterotroph on an organic compound such as acetate, as a chemolithotroph on thiosulfate, or as a mixotroph on both acetate and thiosulfate. Competition of this *Thiobacillus* with an obligately heterotrophic species of *Spirillum* led to exclusion of the *Thiobacillus* when the feed medium contained acetate and no thiosulfate and when the dilution rate was not too low; this was pure and simple competition. When the medium contained thiosulfate as well as acetate, the populations coexisted in a steady state with the fraction of *Thiobacillus* in the mixed population being an increasing function of the concentration of thiosulfate in the feed medium. This was partial competition for the acetate, as the *Spirillum* does not use thiosulfate.

Single Competition and Other Interactions

Microbial populations change their environment by extracting nutrients and also by excreting metabolic by-products and other substances. Such materials can effect the growth rates of the populations that produce them as well as those of other populations. Excretion of metabolic by-products can easily give rise to population interactions additional to competition for nutrients. We consider some examples of this beginning with situations where a by-product has no effect on the population that produces it and a stimulatory effect on another population that uses it. In the examples we discuss, competition for a single nutrient that has dynamical effects is assumed to occur.

Megee *et al.* (*36*) grew yeast and *Lactobacillus casei* in a chemostat fed with a glucose-minimal medium; the organisms competed for glucose. The *L. casei* used was an obligate consumer of riboflavin, which was produced and excreted by the yeast. In addition to competition, another interaction, usually called commensalism (*37*), therefore occurred. By adding riboflavin to the chemostat feed, the commensal dependence of *L. casei* on yeast could be destroyed, and simple competition was the only interaction. With high concentrations of riboflavin in the feed, the yeast density continually declined, and it was clear that the bacteria were excluding the yeast. When no riboflavin was in the feed, the two populations coexisted in a steady state at all dilution rates below an upper limiting value. It was possible to predict these results quantitatively from data on pure cultures of the two populations.

A similar result was reported by Gottschal *et al.* (*7*) on two species of *Thiobacilli*, an obligate chemolithotroph and a mixotroph, which competed for thiosulfate in a chemostat. The obligate chemolithotroph excreted glycolate that the mixotroph used to supply part of its requirement for carbon and available energy. The populations coexisted; the addition of glycolate to the feed, however, caused the fraction of mixotroph cells in the mixed population to increase.

Meyer *et al.* (*38*) considered a hypothetical situation of single competition in which each population also produces a metabolic by-product essential to the growth of the other. If the two growth factors are not supplied in the medium, neither population will grow in the absence of the other; thus the interactions involved are competition and mutualism (*37*). A simple mathematical model of these interactions showed that the competing populations could coexist in a steady state when grown together in a chemostat. Meyer also studied these interactions in a *Streptococcus-Lactobacillus* system described earlier by Nurmikko (*39*). The organisms competed for glucose and the growth factors involved in the mutualism appear to have been phenylalanine and folic acid. The populations were apparently sensitive to factors not accounted for in the model, however, and their behavior did not follow the predictions of the model except in the most general way, although the two populations did coexist in a steady state in a chemostat fed with medium lacking both phenylalanine and folic acid. Such experiments and analyses demonstrate that production by competing populations of metabolic by-products with stimulatory effects on growth rates can prevent competitive exclusion and allow competitors to coexist in a steady state (*40*).

The production of metabolic by-products in a system was also studied by Lee *et al.* (*41*); they grew *Lactobacillus plantarum* and *Propionibacterium shermanii* in a chemostat fed with glucose-minimal medium. Although glucose was the only carbon source provided in the medium, *L. plantarum* produced lactic acid as a by-product of its metabolism of glucose, and *P. shermanii* can use either glucose or lactic acid as a carbon source. It has been shown that *P. shermanii* uses lactic acid in preference to glucose when both are available. Thus, *P. shermanii* avoids competition with *L. plantarum* for glucose, but it does so at the expense of becoming commensally dependent on the latter organism for lactic acid. Experimentally, the two organisms coexisted over a wide range of chemostat dilution rates, and it was possible to predict system dynamics rather well with a simple mathematical model. Such avoidance of competition through the ability to select one substrate over others is probably an important feature of many biological waste treatment processes, particularly those in which the water contains many different carbon energy sources (*42*).

Cases in which by-products excreted into the medium have negative—that is, inhibitory or toxic—effects on one or more of the populations that compete for a single dynamically significant nutrient have been analyzed by mathematical models (*43*); these analyses assumed that two populations only interact and that the interactions occur in a chemostat (Fig. 2). In situations that involve the production of autoinhibitory substances, the autoinhibitor can be general in its action (Fig. 2A) or specific (Fig. 2, B and C). Steady states of coexistence of the populations can occur in all three of these cases. The domain of chemostat operating conditions for which coexistence is possible is much broader in the case illustrated in Fig. 2B than it is in case of Fig. 2A; thus production of autoinhibitors that are specific has a greater mitigating effect on competition than does production of autoinhibitors that are general. The cases in Fig. 2, B and C, represent pure and single competition, but they are not simple competition because the competition is partial rather than total.

One population may produce substances that inhibit another population.

The interactions of such a situation could be competition and antagonism (Fig. 2D). Possibly these two negative interactions could just balance one another, and the populations could coexist in a steady state. Analysis shows, however, that coexistence steady states are always unstable in this case, and one population is always excluded. In a case in which the two interactions are competition and amensalism (Fig. 2E), no steady state of coexistence is stable either. In both cases (Fig. 2, D and E), exclusion of a population will always occur, but the identity of the excluded population may depend on the conditions imposed on the system when it is started, such as the initial values of the population densities and the nutrient and inhibitor concentrations. For example, Adams *et al.* (*44*) grew two strains of *E. coli* on glucose-minimal medium in a chemostat. One strain produced colicin, a substance that inhibited the other. The colicin-producing strain grew more slowly and would have been excluded were it not for the amensal interaction. The two strains did not coexist in a steady state, but exclusion depended on the initial composition of the mixed population: A strain was excluded when it made up less than about half of the initial population.

Effects of Predation and Parasitism

Predation can affect the outcome of competition (*22*, *45*) in diverse ways. Predation can prevent a competitive exclusion as well as destroy a potential coexistence situation. Evidently, selectivity of predation will be a factor in determining what effects predation will have on competition. Selectivity occurs when the ratio of attack rates of a predator on two prey populations is not equal to the ratio of the densities of the prey populations. In some cases, selectivity may be essentially perfect so that one of the prey populations is immune from the attacks of the predators.

Competition between two species of bacteria, *E. coli* and *Azotobacter vinelandii*, for glucose was studied by Jost *et al.* (*46*). In the absence of predators, *E. coli* excluded *A. vinelandii* from the chemostat. With the addition of the protozoan predator *Tetrahymena pyriformis* it appeared that the three populations would coexist in a state of sustained oscillations of their densities, but the experiment had to be terminated prematurely because the organisms grew on the walls of the chemostat.

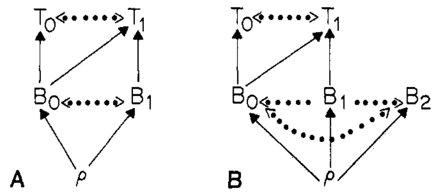


Fig. 5. Two patterns of competition regulated by parasitism (*48*). Here, *T* denotes a parasite (bacteriophage), *B* a bacterium, and ρ sugar. Subscript 0 denotes a wild population, subscript 1 denotes a first-order mutant, and subscript 2 denotes a second-order mutant. Solid arrows indicate transfers of material, and dotted arrows indicate possible mutations.

The regulatory effects of parasitism on competition are quite analogous to those of predation. For example, Levin *et al.* (*47*) reported that the presence of the virulent bacteriophage T2 stabilized the competition of two strains of *E. coli*, strains B and K12, for sugar; strain K12 was resistant to the phage, but strain B was not. This is analagous to selection of prey by a predator. The same workers (*48*) also studied a more complex system. They introduced into a chemostat a wild population of *E. coli*, B_0, and a wild population of bacteriophage, T_0, which attacked the bacteria. Mutation of the bacteria produced a strain, B_1, that was resistant to the original phage population. However, a mutant of this phage, T_1, also appeared, and the mutant was able to infect both the wild and the mutant bacterial populations. A second order mutant of the bacteria, B_2, proved to be resistant to both the wild and mutant populations of phage. The two, or three, strains of bacteria competed for sugar and interacted in two different ways (Fig. 5). In one situation (Fig. 5A), the densities of bacteria and phage were low but the sugar concentration was high, and no strain of bacteria immune from parasitism was present; this was called the predator-limited case. In the other situation (Fig. 5B), the densities of bacteria and phage were high but the sugar concentration was low, and the B_2 strain of bacteria, which was resistant to both strains of phage, was present; this was called the resource-limited case. Steady states of coexistence were observed in both cases. The version of the competitive exclusion principle put forward by Chao *et al.* (*48*) on the basis of these results and mathematical analysis is: the number of competing populations that can coexist in a steady state in a spatially homogeneous system with time-invariant inputs is equal to or less than the sum of the number of resources competed for and the number of parasite, or predator, populations that infect, or prey upon, the competitors. It should be noted that the two parasite populations shown in Fig. 5 also compete with one another. This competition is partial and is for the wild-type population of bacteria B_0

Miscellaneous Factors That Can Allow Competitors to Coexist

Competition for a distributed resource. Competition of two populations of microalgae for light is an example of competition for a distributed resource, since such organisms use light of a range of frequencies in their photosynthetic activities. This type of competition should be similar to competition for a number of discrete resources. We would predict, therefore, that two light-limited algal populations might be able to coexist in a chemostat if they have the appropriate biological properties (*49*). Apparently this prediction has not been tested experimentally.

Vegetative and dormant cells. Vegetative or metabolically active cells of certain microbial populations form dormant, nonproliferating cells when environmental conditions become adverse; for example, some bacteria and molds form spores, and some protozoans form cysts. The metabolically inactive spores and cysts, which can survive adverse conditions for long periods of time, may possess other adaptations that favor their retention in a habitat. The ciliated protozoan *Colpoda steinii*, for instance, forms sticky resting cysts that tend to settle out of aqueous suspension and attach to solid surfaces where they cannot be carried away by currents of water (*50*).

Formation of such dormant cells under conditions of resource scarcity is evidently a means of dropping out of competition, and reversion to vegetative cells under favorable conditions is the means of getting back into competition. Hence, these morphological transformations may be the thing that allows some competitors to persist in some ecosystems.

Discussion and Conclusions

Laboratory experiments with idealized sytems and analyses of mathematical models of such systems have revealed some of the principles of microbial competition.

Experimental work has been aided greatly by the Coulter particle counter (*51*), but new counting techniques are needed to distinguish between populations whose cell size distributions overlap significantly. Flow microfluorometry (*52*) offers some promise here. In addition, rapid means of analyzing water for biologically important chemicals present in low concentrations and in many combinations are needed, as are new techniques of stability analysis of complex systems to aid those investigators concerned with the mathematics of population interactions (*53*).

Time-varying inputs to ecosystems, spatial heterogeneity of environments, multiplicity of dynamically significant resources competed for, occurrence of commensal and mutual interactions, avoidance of competition by facultative switching to alternate substrates, effects of autoinhibitors, regulation by predators and parasites, and transformations between vegetative and dormant cells provide an inventory of circumstances that mitigate the severity of competition. We might think that this inventory is sufficient to explain the coexistences of many competing populations in real-world situations; if so, there would no longer be any paradox of the plankton. Successful application of the principles of competition to natural situations, however, is not so easy and, in fact, such application remains as a major task of microbial ecology.

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5. Evidently, competition for resource ρ can occur in two different ways: in one, ρ has a dynamical effect on both competitors, but in the other it has a dynamical effect on only one of them. Terms, such as strong and weak competition for ρ, could be used to distinguish between these two situations.
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