eralized depression, profound exophthalmos, and pilomotor effects. A variety of effects on blood pressure, smooth muscle, and respiration are also exhibited by a large number of *O*- and *N*-alkyl derivatives of 6,7-dihydroxy-TIQ and by the corresponding 5,6-dihydroxy-TIQ analogs (*22, 23*). These actions indicate stimulation of sympathetic nerve function.

Our observation of uptake and storage of 6,7-dihydroxy-TIQ by sympathetic nerves suggests several mechanisms of action for this alkaloid. First, it may be capable of acting as a false transmitter. A number of other amines that are taken up and stored by adrenergic neurons in the periphery or in the brain are believed to function as false transmitters (*24*). Second, 6,7-dihydroxy-TIQ may produce sympathomimetic effects by displacement of catecholamines from storage sites. Release or displacement of catecholamines from rat brain synaptosomes in vitro has been observed for 1-methyl-6,7-dihydroxy-TIQ (salsolinol) (*20*). Lastly, the TIQ's may produce sympathomimetic effects by interfering with reuptake of normally released natural transmitters. In our experiments with the rat iris, there was indication that the alkaloid and NE shared the same uptake mechanisms since they were both blocked by low concentrations of DMI. In studies with rat brain synaptosomes, 1-methyl-6,7-dihydroxy-TIQ blocked the uptake of both DA and NE (*20*). These observations open a number of interesting possibilities for pharmacologic and therapeutic studies with naturally occurring and synthetic TIQ alkaloids.

Biosynthesis of TIQ alkaloids might occur in the catecholamine storage sites in the adrenals, sympathetic nerves, and brain of man during ingestion of alcoholic beverages (*1, 4*). The potential actions of TIQ alkaloids as false transmitters and as agents that release catecholamines and block their uptake are consistent with the hypothesis that these alkaloids may contribute to alterations in activity of the sympathetic or central nervous system during ingestion of alcoholic beverages.

GERALD COHEN
CATHERINE MYTILINEOU
ROBERT E. BARRETT

Clinical Research Center for Parkinson's and Allied Diseases, Columbia University College of Physicians and Surgeons, and *New York State Psychiatric Institute, New York 10032*

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Coexistence of Two Asexual Strains on a Single Resource

Abstract. *A stable equilibrium was obtained for two F⁻ strains of* Escherichia coli *in a glucose minimal medium. This equilibrium cannot readily be explained by traditional models of population genetics and apparently violates some forms of the ecological principle of competition exclusion. A mechanism involving an inverse relationship between the growth rates of these strains at the exponential and "stationary" phases is suggested as a possible explanation for the observed stable equilibrium.*

In the literature on population genetics considerable attention has been given to the conditions necessary for multiple genotypes to maintain stable equilibria in a single population. Ecological literature gives fair consideration to analogous equilibria, those for multiple species in the same environment. The pure frequency models of population genetics predict a number of situations by which the opposing forces of mutation, selection, or both mutation and selection lead to stable equilibria for multiple alleles at the same locus (*1*). Although ecological theory does not predict the precise condition under which stable equilibria will obtain for species at the same trophic level, it seems generally agreed that a necessary condition is a fair amount of environmental heterogeneity and ecologically divergent species (*2*). By combining ecological and genetic theory, additional mechanisms for stable genetic polymorphisms (*3*) and stable states of interspecific coexistence (*4*) may be derived.

While performing some experiments on the partitioning of resources by competing strains of the bacterium *Escherichia coli,* a stable equilibrium was obtained for two F⁻ strains in a glucose minimal medium. This equilibrium cannot be readily explained by traditional models of population genetics. In addition, a stable equilibrium by closely related forms in a very simple environment is unexpected on ecological grounds and is in apparent violation of the sometimes criticized principle of competitive exclusion (*5, 6*). Consequently, I felt this result and a possible explanation for it worthy of a special report.

The culture method used was essentially similar to that of Atwood, Schneider, and Ryan (*7*). An initial inoculum of 0.1 ml of stationary phase cells of two F⁻ strains of *E. coli* was placed in 50-ml Erlenmeyer flasks con-

taining 9.9 ml of glucose minimal medium. The first strain, a derivative of *E. coli* K12 marked with a galactose deletion (*8*), is denoted Δgal⁻; and the second strain, a derivative of *E. coli* B marked with an arabinose point mutation (*9*), is denoted ara⁻. The flasks were shaken at 200 rev/min at 37°C in B.O.D. incubators for a period of 48 hours. After this period, 0.1 ml of this stationary phase culture was transferred to a fresh flask containing a similar quantity of the medium. This process was continued for a number of transfers, with samples being taken at each. Estimates of the relative frequencies of the two genotypes were obtained by plating appropriate dilutions to obtain about 200 colonies on a galactose tetrazolium indicator medium. To insure the accuracy of estimates of low frequencies, lower dilutions were plated on minimal medium.

The relatively low concentration of glucose used, 300 mg/liter, is within the range in which there is a linear relationship between sugar concentration and the stationary phase population for both genotypes. Within this range a doubling of the glucose concentration leads to a doubling of the stationary phase population of ara⁻. The slope of the line for Δgal⁻ is less acute, and the linearity of the sugar-stationary phase level is lost at a lower glucose concentration. At 800 mg of glucose per liter the stationary phase population of Δgal⁻ was less than that which would be expected if the linear sugar-stationary phase relationship was maintained. This may result from a greater sensitivity of the Δgal⁻ cells to oxygen limitation. In any event, the direct relationship between glucose concentration and stationary phase level at the concentrations used suggests that glucose, which is the sole initial carbon source, represents the limiting resource.

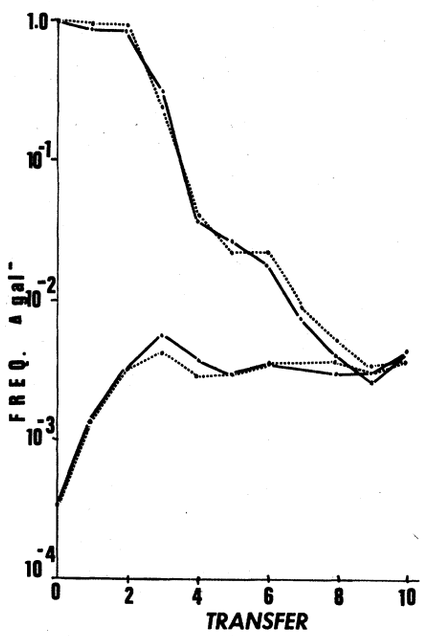

Fig. 1. Stable equilibrium between two asexual genotypes in a glucose minimal medium (300 mg of glucose per liter): the relative frequencies of Δgal⁻ at the beginning of each transfer. Each point represents the frequency in one of four independent populations.

The observation of a non-zero equilibrium was made during a period of seven transfers. Two cultures were set up with initial frequencies of Δgal⁻ of 0.50; at the end of the first transfer the frequency of Δgal⁻ was 0.019 in the first culture and 0.040 in the second. During the following six transfers the frequency of Δgal⁻ remained at about 1 percent (frequency estimates being made in this case only with the tetrazolium galactose plates). To determine whether this represented a stable equilibrium, four separate serial transfer populations were set up, two with initial frequencies of Δgal⁻ of 0.000338 and two with initial Δgal⁻ frequencies of 0.999739. After nine transfers the frequencies of Δgal⁻ in the different populations converged (see Fig. 1). Mean frequencies of Δgal⁻ of 0.003 and 0.0035 were obtained for the 9th and 10th transfers, respectively. If, at a concentration of 300 mg/liter, glucose represents the sole limiting resource, then it appears that the ara⁻ and Δgal⁻ strains are able to maintain a stable equilibrium when limited by a single resource.

Further experiments indicated that both the level of the equilibrium and its very existence are dependent upon the sugar concentration. An increase in the concentration of glucose leads to an increase in the equilibrium frequency of Δgal⁻. At 600 mg of glucose per liter the equilibrium appears to be at about 1 percent Δgal⁻. At low concentrations of glucose Δgal⁻ may be lost. At 30 mg of glucose the frequency of Δgal⁻ continues to decline. In two cultures initiated with a frequency of 0.000338 Δgal⁻, no Δgal⁻ cells were observed in the 10th and the 11th transfers.

The classical frequency models of population genetics are really unable to explain these results. Given a situation of two asexual haploid genotypes in a homogeneous environment, a stable equilibrium can be maintained in three basic ways: (i) a balance between forward and back mutation; (ii) a balance between mutation and selection; and (iii) some form of frequency-dependent selection. The first two mechanisms can be ruled out by the rapid rate at which the equilibrium was achieved (30 to 35 cell generations for cultures starting at low frequencies of Δgal⁻). At reasonable mutation rates this would not be anticipated. In addition, mutational hypotheses could not account for the similarity of behavior of all four separate populations. I could describe the equilibrium by some form of frequency-dependent model; however, such models are generally not explicit about the mechanisms which lead to frequency dependence and consequently do not provide an adequate explanation of our observations.

I can think of no way existing ecological models explain these data. In fact, the coexistence of two asexual strains on a single resource is in apparent violation of the form of the principle of competitive exclusion which asserts that the number of competing species must be less than the number of resources (*10*). However, the preceding statement represents an empirically un-

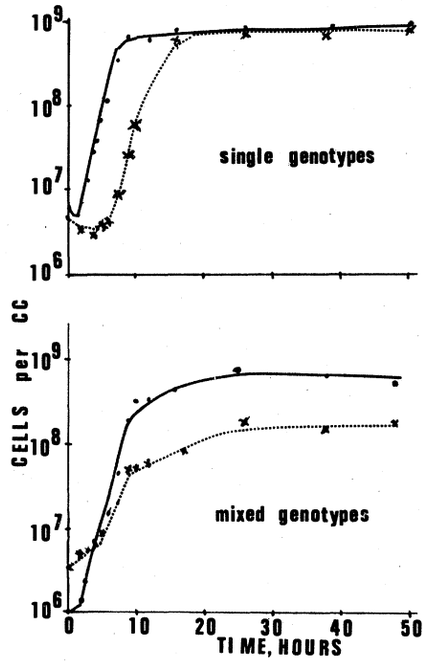

Fig. 2. Growth of Δgal⁻ and ara⁻ in single genotype and high-frequency, mixed genotype culture (300 mg of glucose per liter): number of cells per cubic centimeter as a function of time in hours. Solid line, numbers of ara⁻; broken line, numbers of Δgal⁻.

testable hypothesis. That is, even in this, which is perhaps the most simple of experimental systems, it would not be possible to demonstrate that there is only a single resource in the medium. In fact, it is reasonable to assume that as a result of cell metabolism and mortality an array of potentially utilizable carbon sources may be excreted into the medium. However, the only initial carbon source, and what is the primary and appears to be the limiting resource, is glucose. Whether the array of potentially utilizable carbon sources that may be produced as a result of glucose metabolism may lead one to consider this a situation of "multiple resources" is an unresolvable semantic issue.

Some theoretical work which F. M. Stewart and I have recently completed suggests that under a resource utilization scheme similar to the serial transfer technique a stable equilibrium may be obtained for two species even when there is only a single resource. In the model examined this result obtains when one species grows faster at high concentrations of the single resource while the other grows faster at low concentrations. Unfortunately the experimental system appears somewhat more complex than the model, and the relation between the equilibrium level and the sugar concentration is different from that predicted by the model. Consequently, that work only suggests that such an equilibrium is theoretically possible but does not serve to explain the observed equilibrium.

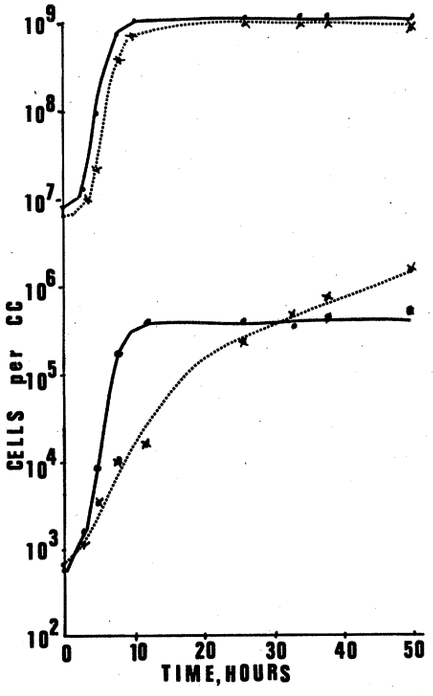

Fig. 3 Growth of Δgal⁻ and ara⁻ in mixed culture initiated with very low frequencies of one genotype (in 300 mg of glucose per liter): number of cells per cubic centimeter as a function of time in hours. Solid line, numbers of ara⁻; broken line, numbers of Δgal⁻.

Additional experimental work suggests that an explanation for the observed equilibrium lies in an understanding of what is occurring at or near stationary phase. The concentration of glucose in filtrates of stationary-phase single or mixed strain cultures of Δgal− and ara− is less than that which can be estimated by enzymatic means (*11*) (less than 0.1 mg/liter). However, if a small inoculum of stationary phase cells of either ara− or Δgal− is introduced into Millipore filtrates of these stationary-phase cultures, additional growth occurs. For a 1 percent inoculum there is a 7- to 10-fold increase in cell numbers in a 48-hour period. This result is consistent with the hypothesis that the stationary phase is not a static situation, but rather a dynamic one in which there is both cell division and cell mortality. Presumably these cells would be using glucose which may be in very low concentration or more likely products of cell metabolism and breakdown of dead cells. When both genotypes are in relatively high frequencies or when they are the only genotypes, there is little change in population size between 24 and 48 hours (see Fig. 2). However, when Δgal− is in very low frequency its numbers continue to show considerable increase after those of ara− have pretty much stabilized (see Fig. 3). For the Δgal− initiated at low frequencies there is about a 50-fold increase in numbers between 12 and 50 hours. Even when initiated at these low frequencies in these mixed cultures, ara− shows little increase during this same period.

These growth curves suggest that the primary disadvantage of Δgal− when in competition with ara− may be explained by its initially longer lag period. A major portion of the glucose may be usurped by the ara− cells before the Δgal− cells start to grow. However, when Δgal− is in low frequency, its population continues to increase after ara− have stopped growing. Given a sufficiently low concentration of Δgal− in mixed culture with ara−, the capacity for growth in stationary phase may allow for an increase in the relative frequency of these cells between successive transfers. Thus, as a result of its shorter initial lag phase, ara− may increase in frequency when it is rare; and due to its capacity for growth in stationary phase, Δgal− may also increase in frequency when it is rare. These are the necessary conditions for a stable equilibrium.

Currently it is not at all clear why the Δgal− cells are able to increase at "stationary phase" when they are very rare, but do not appear to do so when they are more common, or why the equilibrium should depend on the glucose concentration. One possibility is that a resource substance which may only be utilized or may be utilized more efficiently by Δgal− is produced by a by-product of the ara− cell metabolism or breakdown of dead ara− cells. If this were the case, and the "by-product resource" could only be used when in sufficiently high concentration, then it would be possible to explain both the stationary phase growth of Δgal− when in low frequency and the decreasing equilibrium level of Δgal− with decreasing glucose concentration. That is, the amount of such a "resource" would be directly proportional to the total number of ara− cells, and the proportionate increase in Δgal− would be inversely proportional to the numbers of Δgal− cells present and utilizing this "resource." Experiments are currently under way to test this and other possible hypotheses for the observed stable equilibria.

BRUCE R. LEVIN*
Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island 02912

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* Present address: Department of Zoology, University of Massachusetts, Amherst 01002.

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