Protecting Haploid Polymorphisms in Temporally Variable Environments

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ABSTRACT

Analysis of a continuous-time model shows that a protected polymorphism can arise in a haploid population subject to temporal fluctuations in selection. The requirements are that population size is regulated in a density-dependent manner and that an allele’s arithmetic mean relative growth rate is greater than one when rare and that its harmonic mean relative growth rate is less than one when common. There is no requirement that relative growth rate be frequency dependent. Comparisons with discrete-time models show that the standard formalism used by population genetics ignores forced changes in generation time as rare advantageous alleles sweep into a population. In temporally variable environments, frequency-dependent changes in generation times tend to counteract these invasions. Such changes can prevent fixation and protect polymorphisms.

THE means by which temporal fluctuations in selection might maintain polymorphisms were first explored by Dempster (1955). He showed that a rare allele in an infinite randomly mating diploid population will increase in frequency if the geometric mean fitness of the heterozygote exceeds that of the common homozygote. Neither allele can fix if the geometric mean fitness of the heterozygote is greater than those of both homozygotes, and so the polymorphism persists indefinitely. In a haploid population there are only two genotypes and the allele with the larger geometric mean fitness inevitably sweeps to fixation—there is no third genotype to counteract this invasion. Dempster concluded that temporal fluctuations in selection can protect polymorphisms in diploids but not in haploids. Later, Haldane and Jayakar (1963), and then Gillespie (1972, 1973a,b), analyzed similar models and came to exactly the same conclusion.

Dempster’s (1955) model is widely cited as one of several mechanisms to maintain polymorphisms in diploids. Haploid monomorphism barely gets a mention. Nevertheless, the notion that temporal fluctuations in selection cannot protect polymorphisms in haploids is deeply ingrained (Felsenstein 1976; Hedrick et al. 1976; Hedrick 1986; Gillespie 1991; Maynard Smith 1998). All recent investigations invoke alternative selection schemes: fitness fluctuations in space (either implicitly, e.g., Dean 1995, or explicitly, e.g., Rainey and Travisano 1998), cooperativity (Rosenzweig et al. 1994; Rainey and Travisano 1998), stabilizing frequency-dependent selection (Bohannan and Lenski 2000; Lunzer et al. 2002), and even nontransitive fitness relations that allow clones to endlessly pursue one another across uniform surfaces (Kerr et al. 2002). None invoke temporal fluctuations in selection for haploids.

Competition between haploid clones is conceptually no different from competition between species. Ecologists long ago showed that temporal variability in the environment can promote coexistence (Stewart and Levin 1973; Levins 1979; Armstrong and McGehee 1980; Chesson 1985, 2000), as when a resource fluctuates either side of the intersection of two nonlinear growth curves (Figure 1). Realistic models tend to be particular, making generalizations difficult. Other models, perhaps unrealistic, are concocted to illustrate principles. Some general criteria leading to coexistence have been examined, but lack intuitive appeal.

Using a continuous-time model of chemostat competition, I derive the conditions under which temporal fluctuations in selection can maintain a genetic polymorphism in a haploid species subject to density-dependent population regulation.

BACKGROUND

Chemostat competition: The model of competition between two haploid clonal populations for a single growth-limiting resource inhabiting a chemostat is

\[
\frac{dN_1}{dt} = \left( \mu_1(S) - D \right) N_1
\] (1)

\[
\frac{dN_2}{dt} = \left( \mu_2(S) - D \right) N_2
\] (2)

\[
\frac{dS}{dt} = D(S_0 - S) - \frac{\mu_1(S)}{Y_1} N_1 - \frac{\mu_2(S)}{Y_2} N_2,
\] (3)
where $N_1$ and $N_2$ are the densities of the competing clones, $\mu_i(S)$ and $\mu_2(S)$ are their rates of growth, and $D$ is the chemostat dilution rate (the fractional rate of replacement of medium in the growth chamber). $S_s$ and $S$ are, respectively, the concentrations of the growth-limiting resource entering the growth chamber and in the growth chamber. $Y_1$ and $Y_2$ are yield coefficients that determine the biomass produced per amount of resource consumed.

To connect growth rates to resource levels assume that the former are concave monotonic functions of the latter. For example, let

$$\mu_i(S) = \frac{\mu_{max,i} S}{K_i + S}, \quad i = 1, 2,$$  \hspace{1cm} (4)

where $\mu_{max,i}$ is the maximum rate of growth of clone $i$ when the resource is in excess, and $K_i$ is a half-saturation constant, the concentration of resource sufficient to allow growth at exactly half the maximum rate.

Cell generation times are defined as the average time for one cell to become two (Kubitschek 1970),

$$g_i(S) = \frac{\log_{10} 2}{\mu_i(S)} \quad i = 1, 2,$$  \hspace{1cm} (5)

with the number of cell generations elapsed during time $t$ given simply as

$$G_i(t) = \frac{\mu_i(S)}{\log_{10} 2} t, \quad i = 1, 2.$$  \hspace{1cm} (6)

**A chemostat population at steady state:** Imagine a chemostat inoculated with a single clone ($N_i > 0$ and $N_i = 0$). After inoculation, the density of clone 2 grows as it consumes resources. Eventually, the resource concentration becomes sufficiently reduced that growth rate slows and a stable steady state is approached where $dN_i/dt = 0$, $dS/dt = 0$, and

$$\mu_2(S_{ss,2}) = D \quad (7)$$

$$N_i = N_{i,ss} = Y_i(S_0 - S_{ss,2}). \quad (8)$$

Here, $S = S_{ss,2}$ is the resource concentration in the chemostat growth chamber when clone 2 is at its steady-state carrying capacity $N_i = N_{i,ss}$.

This population is subject to density-dependent population regulation. The mechanism of regulation is resource depletion. In the absence of mutation or externally imposed change, the population will remain at $N_i = N_{i,ss}$ and will continue to grow at $\mu_2(S_{ss,2}) = D$ with generation time $g_2(S_{ss,2}) = \log_{10} 2/D$, indefinitely.

**A simple clonal sweep:** Now imagine that a second, fitter clone is introduced at very low density to this steady-state chemostat population. At the beginning of the sweep, when $t = 0$, the growth rate of the invading type 1 must exceed that of the resident clone 2 and

$$\mu_1(S_{ss,1}) > \mu_2(S_{ss,2}) = D \quad \text{for} \quad N_i \approx 0, N_2 = N_{i,ss}. \quad (9)$$

otherwise invasion is not feasible. Toward the end of the sweep, when $t \to \infty$, the growth rate of clone 1 must slow to the chemostat dilution rate and

$$\mu_2(S) < \mu_1(S) = D \quad \text{as} \quad N_i \to N_{i,ss}, N_2 \to 0; \quad (10)$$

otherwise the population grows without bound. Loss of clone 2 brings the population to a new steady state characterized by

$$\mu_1(S_{ss,1}) = D \quad (11)$$

$$N_i = N_{i,ss} = Y_i(S_0 - S_{ss,1}), \quad (12)$$

where $S = S_{ss,1}$ is the resource concentration in the chemostat growth chamber with clone 1 alone at steady state. As with clone 2, clone 1 is subject to density-dependent population regulation generated by resource depletion.

In simple models of resource competition such as this, the superior competitor reduces the resource concentration to a point where the inferior competitor can no longer sustain itself. With $S_{ss,1} < S_{ss,2}$ the inferior competitor is washed from the chemostat growth chamber. This is true for any simple concave monotonic growth function such as Equation 4 (Tilman 1982).

**Relative fitness:** The fitness of clone 1 relative to clone 2 is defined as a ratio of growth rates (Lunzer et al. 2002),

$$w_i(S) = \frac{\mu_i(S)}{\mu_2(S)} \quad (13)$$

The decline in resource concentration during a clonal sweep may affect growth rates differentially, generating
the appearance of frequency dependence in relative fitness. This does nothing to affect the outcome of competition, however, and the favored clone sweeps inexorably to fixation.

Changes in growth rates do not necessarily imply changes in relative fitness. Following inoculation of a chemostat the concentration of the limiting resource is commonly reduced far below the half-saturation constant (\(S < K_i, K_2\), Lunzer et al. 2002) and

\[
w^I(S) = \frac{\mu_{\text{max}}/K_i}{\mu_{\text{max}}/K_2},
\]

This case illustrates why, during clonal sweeps, relative fitness will often remain independent of the slowdown in growth rates produced by the decline in resource abundance.

**Estimating relative fitness:** A small change in the density of an exceedingly rare clone has a negligible impact on resource abundance. With a second clone at its carrying capacity the system enters a quasi-steady state, characterized by \(dS/dt = 0\). Over short periods of time the growth rates remain virtually constant. Equations 1 and 2 can be integrated:

\[
N_i(t) = N_i(0) e^{\mu_i(S) - D} t,
\]

\[
N_2(t) = N_2(0) e^{\mu_2(S) - D} t.
\]

Taking Log, ratios yields

\[
\log_2 \left( \frac{N_i(t)}{N_2(t)} \right) \approx \log_2 \left( \frac{N_i(0)}{N_2(0)} \right) + s(S) t.
\]

The slope of a plot of the Log, ratio of clone densities against time is commonly used to estimate the selection coefficient \(s(S) = (\mu_1(S) - \mu_2(S))\) per hour. The fitness of clone 1 relative to clone 2 is simply

\[
w^I(S) = 1 + \frac{s(S)}{\mu_2(S)}.
\]

**Relative fitness and generation times:** Relative fitness can also be described as a ratio of cell generation times or as a ratio of the number of generations per unit time

\[
w^I(S) = \frac{g_2(S)}{g_1(S)} = \frac{G_2(S)}{G_1(S)}.
\]

The average growth rate at quasi-steady state is approximately

\[
\mu(S) = \frac{\mu_1(S)p + \mu_2(S)q}{q} \approx D,
\]

where \(p = 1 - q = N_1/(N_1 + N_2)\) is the frequency of clone 1. In practice this approximation is excellent because the difference in steady-state resource levels is but a tiny fraction of the total resource entering the chemostat growth chamber \([i.e., (S_{a2} - S_{a1})/S_0 \approx 0]\). Thus, the population neither increases nor decreases during the clonal sweep and consequently \(\mu(S) = D\).

The **population generation time** is proportional to the reciprocal of the average growth rate

\[
\frac{\log_2}{\mu(S)} = \frac{\log_2}{D}.
\]

Being a function of the dilution rate, the population generation time is under the direct control of the experimenter. Whereas the population generation time is a reciprocal of the average growth rate, the **average cell generation time**, \(g_1(S)p + g_2(S)q \approx \log_2/D\) and is an average of the reciprocals of growth rates.

**CHEMOSTAT THEORY FOR VARIABLE ENVIRONMENTS**

**Clonal competition in a variable environment:** Now imagine the same two clones competing in a chemostat, but allow the environment to vary from time to time. As before, imagine that clone 1 is exceedingly rare and attempting to invade a numerically dominant clone 2 at steady state. Let the growth rate of clone 1 in environment \(j\) be \(\mu_{1j}(S_{a2})\), the dilution rate in environment \(j\) be \(D_j\), and the time spent in environment \(j\) be \(\Delta t_j\). As before, assume \(\mu_{1j}(S_{a2})\) is constant over each interval \(\Delta t_j\). After \(n\) environmental changes the density of clone 1, initially \(N_1(0)\) and very rare, is

\[
N_1(T) = N_1(0) \cdot e^{\sum_{1}^{n}(\mu_{1j}(S_{a2}) - D_j) - 0 \Delta t_j} \quad \text{for } N_{1j} \approx 0, N_{2j} = N_{2ss}.
\]

At time \(T = \sum_{1}^{n} \Delta t_j\) and with \(N_1(T)\) remaining very small. Similarly, when clone 2 is very rare and clone 1 is at steady state

\[
N_2(T) = N_2(0) \cdot e^{\sum_{1}^{n}(\mu_{2j}(S_{a1}) - D_j) - 0 \Delta t_j} \quad \text{for } N_{1j} = N_{1ss}, N_{2j} \approx 0.
\]

This model is approximate. It assumes that tiny changes in the densities of rare clones have negligible impacts on growth rates. It also assumes that selection during the transitions between environments is insignificant compared to the selection at quasi-steady state within environments where the dominant clone, growing at rate \(D_j\), remains close to its carrying capacity, \(N_{2ss}\). Finally, Equations 22 and 23 are concerned only with the fates of very rare clones. The interior dynamics when both clones have comparable frequencies and dynamics far away from quasi-steady state shall not concern us.

**Coexistence in a variable environment:** Coexistence is assured if the densities of each clone increase when rare. This requires

\[
\sum_{j=1}^{n} (\mu_{1j}(S_{a2j}) - D_j) \cdot \Delta t_j > 0 \quad \text{for } N_{1j} \approx 0, N_{2j} = N_{2ss}.
\]

\[
\sum_{j=1}^{n} (\mu_{2j}(S_{a1j}) - D_j) \cdot \Delta t_j > 0 \quad \text{for } N_{1j} = N_{1ss}, N_{2j} \approx 0.
\]
A further simplification uses \( \overline{w}_j = \mu_j(S_{u,j}) \Delta t_j / \sum_{j=1}^n \mu_j(S_{u,j}) \Delta t_j \), \( \overline{w}_j = \mu_j(S_{u,j}) \Delta t_j / \sum_{j=1}^n \mu_j(S_{u,j}) \Delta t_j \), and \( \overline{D} = \sum_{j=1}^n D_j \Delta t_j / \sum_{j=1}^n \mu_j(S_{u,j}) \Delta t_j \), to produce

\[
\overline{w}_j > \overline{D} \quad \text{for} \quad N_{i,j} = 0, \quad N_{2,j} = N_{2,u,j} \tag{26}
\]

\[
\overline{w}_j > \overline{D} \quad \text{for} \quad N_{i,j} = N_{1,u,j}, \quad N_{2,j} = 0. \tag{27}
\]

Although they turn out to be less useful than (24) and (25), (26) and (27) state the obvious: that the average growth rate of a rare type must exceed the average dilution rate if it is to persist.

Define \( \gamma_j = D_j \Delta t_j / \sum_{j=1}^n D_j \Delta t_j \) as the proportion of population generations spent in environment \( k \) and simply rewrite (24) and (25) as

\[
\sum_{j=1}^n \gamma_j w_j(S_{u,j}) > 1 \quad \text{for} \quad N_{i,j} = 0, \quad N_{2,j} = N_{2,u,j} \tag{28}
\]

\[
\sum_{j=1}^n \gamma_j w_j(S_{u,j}) > 1 \quad \text{for} \quad N_{i,j} = N_{1,u,j}, \quad N_{2,j} = 0. \tag{29}
\]

We see immediately that two clones can coexist whenever their weighted arithmetic mean relative fitnesses, when rare, are \( > 1 \). Since fitness is defined as a ratio of growth rates, so \( w_j^1(S_{a,j}) = 1/w_j^2(S_{a,j}) \) and Equation 29 can be rewritten as

\[
\sum_{j=1}^n \gamma_j / w_j(S_{u,j}) < 1 \quad \text{for} \quad N_{i,j} = N_{1,u,j}, \quad N_{2,j} = 0. \tag{30}
\]

This makes plain that the reciprocal of the arithmetic mean relative fitness of the invading type is the harmonic mean relative fitness of the resident clone.

In the special case where relative fitness is constant, and \( w_j^1(S_{u,j}) = w_j^2(S_{u,j}) \) regardless of clone frequency, Equations 28 and 30 provide the conditions necessary to protect both clones from loss (Figure 2). Temporal fluctuations in selection can protect genetic polymorphisms in haploid clones in the absence of frequency-dependent selection. All that is needed is for each allele, when rare, to have a weighted arithmetic mean relative fitness \( > 1 \).

**The generation time effect:** Using \( \gamma_k = D_k \Delta t_k / \sum_{j=1}^n D_j \Delta t_j \), and \( \gamma_{2,k} = \mu_2(S_{u,k}) \Delta t_k / \sum_{j=1}^n \mu_j(S_{u,k}) \Delta t_j \) as the proportion of cell generations clone 2 spends in each environment when common and when rare, rewrite (24) and (25) as

\[
\sum_{j=1}^n \gamma_j w_j^1(S_{u,j}) > 1 \quad \text{for} \quad N_{i,j} = 0, \quad N_{2,j} = N_{2,u,j}, \tag{31}
\]

\[
\sum_{j=1}^n \gamma_j w_j^2(S_{u,j}) < 1 \quad \text{for} \quad N_{i,j} = N_{1,u,j}, \quad N_{2,j} = 0. \tag{32}
\]

We see that when relative fitness is constant \( w_j^1(S_{u,j}) = w_j^2(S_{u,j}) \) frequency-dependent changes in growth rates, reflected as changes in the proportion of cell generations clone 2 spends in each environment when common and when rare \( (\gamma_j \neq \gamma_{2,j}) \), promote coexistence.

![Figure 2.—A protected polymorphism (shaded region) arises in a haploid population subject to density-dependent regulation when the arithmetic mean fitness (relative growth rate) of an allele is greater than one when rare and its harmonic mean fitness is less than one when common. The population is assumed to spend half its time in each environment.](image)

The generation time effect is explained as follows. Without loss of generality, assume relative fitness remains constant with \( w_j^1 = w_j^1(S_{a,j}) = w_j^2(S_{a,j}) \). The growth rate of clone 2 when common is \( \mu_2(S_{a,j}) = D_j \) and when rare is \( \mu_2(S_{a,j}) = D_j/w_j^2 \). The number of cell generations that clone 2 spends in an environment when common is \( (\mu_2(S_{a,j})/\log 2) \Delta t_j = (D_j/\log 2) \Delta t_j \) and when rare is \( (\mu_2(S_{a,j})/\log 2) \Delta t_j = (D_j/w_j^2 \log 2) \Delta t_j \). As clone 1 invades so clone 2 spends fewer and fewer generations in environments where clone 1 is favored (since \( D_j \Delta t_j/w_j^2 < D_j \Delta t_j \) when \( w_j^2 > 1 \)), yet ever more generations in environments where clone 1 is disfavored (where \( D_j \Delta t_j/w_j^2 > D_j \Delta t_j \) when \( w_j^2 < 1 \)). Frequency-dependent changes in cell generation times, driven by changes in resource levels, counteract the invasion. If sufficient they can prevent clone 1 reaching fixation.

A haploid polymorphism can be protected in a temporally variable environment when relative fitness and selection coefficients remain independent of allele frequencies so long as the population is subject to density-dependent regulation.

**DISCRETE-TIME MODELS**

The conclusion that a haploid polymorphism can be protected in a temporally variable environment when fitness is independent of allele frequency is seemingly at variance with the conclusions drawn from analyses of fluctuating selection using discrete-time models. Here, the two approaches are reconciled.
A simple clonal sweep revisited: The continuous-time model of a simple clonal sweep can be recast in discrete time with

\[
p(t) = \frac{p(0) W(S, t)}{p(0) W(S, t) + q(0)},
\]

where \( p(t) = 1 - q(t) = N_i(t)/(N_i(t) + N_j(t)) \) is the frequency of clone 1 and

\[
W(S, t) = \exp[\mu_1(S) - \mu_2(S)t]
\]

is fitness at resource concentration \( S \) over the interval \( t \) (Hartl and Clark 1997). Just as with Equations 15 and 16, this approximation is very accurate whenever \( t \) is sufficiently small that changes in \( S \), and hence changes in the growth rates \( \mu_i(S) \), are negligible. Nevertheless, as the clonal sweep proceeds \( W(S, t) \) must vary with \( S \) as it declines from \( S_{nz} \) to \( S_1 \). If variation in \( W(S, t) \) with clone frequency is interpreted as frequency dependence then the chemostat model of a clonal sweep is, by definition, a model of frequency-dependent selection.

Measuring time: The fitness of a clone is commonly measured relative to another, with time measured on a per generation basis (Hartl and Clark 1997). Just as with Equations 15 and 16, this approximation is very accurate whenever \( t \) is sufficiently small that changes in \( S \), and hence changes in the growth rates \( \mu_i(S) \), are negligible. Nevertheless, as the clonal sweep proceeds \( W(S, t) \) must vary with \( S \) as it declines from \( S_{nz} \) to \( S_1 \). If variation in \( W(S, t) \) with clone frequency is interpreted as frequency dependence then the chemostat model of a clonal sweep is, by definition, a model of frequency-dependent selection.

At the beginning of the sweep, when \( N_j = N_{nza}, N_i = 0, \mu_z(S_{nza}) = D, \) and \( w^2_1(S_{nza}) = \mu_1(S_{nza})/D, \) Equation 35 becomes

\[
\log(W(S_{nza}, 1)) = (w^2_1(S_{nza}) - 1)\log D.
\]

At the beginning of the sweep, when \( N_j = N_{nza}, N_i = 0, \mu_z(S_{nza}) = D, \) and \( w^2_1(S_{nza}) = \mu_1(S_{nza})/D, \) Equation 35 becomes

\[
\log(W(S_{nza}, 1)) = (w^2_1(S_{nza}) - 1)\log D.
\]

Returning to time measured per population generation, let \( G(S_{nza}) = (D/\log 2)t \) be one clone 2 cell generation when clone 2 is common. Then clone 2 spends \( G(S_{nza}) = (D/\log 2)(w^2_1(S_{nza}))t = 1/w^2_1(S_{nza}) \) cell generations per unit time when clone 2 is rare. Now rewrite Equations 36 and 37 as

\[
\log(W(S_{nza}, 1)) = G(S_{nza}) \cdot (w^2_1(S_{nza}) - 1)
\]

This formulation presents the two causes of frequency dependence in the discrete-time model. First is the passive decrease in the number of clone 2 cell generations per unit time, from \( G(S_{nza}) = 1 \) to \( G(S_{nza}) = 1/w^2_1(S_{nza}) \), attributable to density-dependent population regulation. Second are biological mechanisms that cause changes in relative fitness such that \( w^2_1(S_{nza}) \neq w^2_1(S_{nza}) \).

The 1955 Dempster classic: Comparing the geometric mean fitnesses of the haploid discrete-time model \( W(S_{nza}, t) = n^\sum_j(W(S_{nza}, t)_j) \) to the haploid continuous-time model (after being Log, transformed) reveals that

\[
\log(W(S_{nza}, t)) = \frac{1}{n} \sum_j (s(S_{nza}, t)_j) \cdot \Delta t_j
\]

for \( N_{ij} = 0, N_{ij} = N_{nza} \)

\[
\log(W(S_{nza}, t)) = \frac{1}{n} \sum_j (s(S_{nza}, t)_j) \cdot \Delta t_j
\]

for \( N_{ij} = N_{nza}, N_{ij} = 0, \) where \( s(S_{nza}, t)_j = \mu_j(S_{nza}) - \mu_j(S_{nza}) \) is the selection coefficient per hour. Dempster (1955) defined fitness as independent of...
allele frequency by setting \( W(S_{a_2}, \Delta t_j) = W(S_{a_1}, \Delta t_j) \). As a consequence the clone with the largest geometric mean fitness wins the competition.

Applying the same definition to the continuous-time chemostat model forces the selection coefficient per hour to be independent of allele frequency, \( s(S_{a_2}) = s(S_{a_1}) \). During a clonal sweep the growth rates slow with the decline in \( S \). As a consequence, the selection coefficients per clone 2 cell generation become frequency dependent: \( s(S_{a_2})/\mu_2(S_{a_2}) \neq s(S_{a_1})/\mu_2(S_{a_1}) \). Defining fitness per unit time as independent of allele frequency in the discrete-time model forces the selection per cell generation to become frequency dependent in the continuous-time chemostat model.

The discrete-time model can be modified to accommodate the slowdown in growth rates during a clonal sweep by defining \( W(S_{a_2}, \Delta t_j) = (V(S_{a_2}))^{\epsilon_2(S_{a_2})} \), where \( V(S_{a_2}) = e^{w_{a_2}(S_{a_2})} \) is relative fitness and \( G_2(S_{a_2}) = (\mu_2(S_{a_2})/\log(2)) \Delta t_j \) is the number of cell generations experienced by clone 2 in environment \( j \). The conditions necessary to protect a polymorphism can now be rewritten as

\[
\sum_{j=1}^{n} \log(W(S_{a_2}, \Delta t_j)) = \sum_{j=1}^{n} (G_2(S_{a_2}) \cdot \log(\log(S_{a_2}))
\]

\[
= \sum_{j=1}^{n} \left( \frac{D \Delta t_j}{\log(2)} \cdot (w_{a_2}(S_{a_2}) - 1) \right) > 0
\]

for \( N_i = 0, N_j = N_{a_2} \) (42')

\[
\sum_{j=1}^{n} \log(W(S_{a_1}, \Delta t_j)) = \sum_{j=1}^{n} (G_2(S_{a_1}) \cdot \log(\log(S_{a_1})))
\]

\[
= \sum_{j=1}^{n} \left( \frac{D \Delta t_j}{(w_{a_1}(S_{a_1}) \log(2)} \cdot (w_{a_1}(S_{a_1}) - 1) \right) < 0
\]

for \( N_i = N_{a_1}, N_j = 0 \). (43')

The right-hand sides of these two inequalities can be rearranged using \( \gamma_j = D \Delta t_j / \sum_{j=1}^{n} D \Delta t_j \) to produce Equations 28 and 29 that define the conditions for protecting the polymorphism. The discrete-time model has been reconciled with the continuous-time chemostat model by simply accounting for the change in number of cell generations per unit time generated by changes in clone frequencies.

The diploid model: The diploid model is a simple extension of the haploid model—we need consider only invasion of an equilibrium population of homoygotes by those few heterozygotes carrying an exceedingly rare allele. The conditions for invasion are

\[
\sum_{j=1}^{n} \gamma_j w^a_{a_2, j} > 1 \quad \text{for } p_a = 0
\]

\[
\sum_{j=1}^{n} \gamma_j w^a_{a_1, j} > 1 \quad \text{for } p_i = 0.
\]

We conclude that a protected polymorphism is established when the weighted arithmetic mean relative growth rate of a rare heterozygote exceeds those of the common homozygotes.

By convention (e.g., see Felsenstein 1976), the fitnesses of the homozygotes are usually presented relative to that of the heterozygote:

\[
\frac{1}{\sum_{j=1}^{n} \gamma_j w^a_{a_2, j}} < 1 \quad \text{for } p_a = 0
\]

\[
\frac{1}{\sum_{j=1}^{n} \gamma_j w^a_{a_1, j}} < 1 \quad \text{for } p_i = 0.
\]

Hence, a protected polymorphism can be established in a temporally variable environment when the weighted harmonic mean fitnesses of both homozygotes are each less than that of the heterozygote.

**EXPERIMENTAL EVIDENCE**

The above theory makes two predictions: (1) the number of cell generations varies with time and (2) both clones can increase in frequency when rare in a temporally variable environment. Neither is predicted by the classical population genetics theory.

Frequency-dependent changes in generation time have been observed directly. Equations 16 and 17 state that when relative growth rates are independent of frequency [i.e., when \( w^s_{a_2}(S_{a_2}) = w^s_{a_2}(S_{a_1}) \) and proportional to resource abundance, the selection coefficient per population generation will decline during a selective sweep, from \( s(S_{a_2}) = (p_{max}/K)S_{a_2} \) to \( s(S_{a_1}) = (p_{max}/K)S_{a_1} \). Chemostat competition experiments between *Escherichia coli* strains TD10 and TD2 for growth-limiting concentrations of the sugar methylgalactoside produce sufficiently strong selection that the change in the intensity of selection can be discerned (Figure 3). Whenever selection is very intense, frequency-dependent changes in cell generation time need to be taken into account when estimating relative fitness (Lunzer et al. 2002).

Studying selection in a temporally variable environment directly is immensely difficult, even in near idealized laboratory populations of *E. coli* (Sutter et al. 2003). Instead, our evidence is indirect, the argument taking an interesting, if circuitous, route. First, a model of authentic stabilizing frequency-dependent selection in a uniform environment is described and the evidence for it presented. Second, the model is extended to accommodate temporal variability. Third, it is shown that the stabilizing frequency dependence seen in a uniform environment, and the balancing selection generated in a temporally variable environment, are synonymous. Fourth, since the one implies the other, demonstrating stabilizing frequency dependence in a uniform environment implies that the selection generated in a temporally variable environment can protect a polymorphism.

First, differential consumption of mixed substitutable resources generates stabilizing frequency-dependent se-
It is instructive to introduce it to the model. Here, the super- and subscripts refer to concentrations (e.g., \( \mu(S_{LU}, S_{MG}) = \alpha_{LU} S_{LU} + \alpha_{MG} S_{MG} \)), coexistence is possible whenever the arithmetic mean fitness exceeds one and the harmonic mean fitness is less than one (Lunzer et al. 2002):

\[
lu \cdot w^{10}_{LU} + mg \cdot w^{10}_{MG} > 1 \quad \text{for} \quad p_{TD10} \approx 0 \quad (48)
\]

\[
\frac{1}{lu/w^{10}_{LU} + mg/w^{10}_{MG}} < 1 \quad \text{for} \quad p_{TD2} \approx 0. \quad (49)
\]

Here, the super- and subscripts refer to E. coli strains TD10 and TD2, \( w^{10}_{LU} \) and \( w^{10}_{MG} \) are the relative growth rates obtained during competition in chemostats for 100% lactulose (\( w^{10}_{LU} = \alpha_{10LU}/\alpha_{2LU} = 0.91 \)) or 100% methylgalactoside (\( w^{10}_{MG} = \alpha_{10MG}/\alpha_{2MG} = 1.32 \)), and \( lu \) and \( mg = 1 - lu \) are the proportions of each resource in the fresh medium supplied to the chemostats. Experiments confirm that a narrow zone of coexistence exists between 23 and 30.5% methylgalactoside (Figure 4). As long as the resource supply lies within this zone neither strain can fix.

Second, temporal environmental variability plays no role in producing this balanced polymorphism. Nevertheless, it is instructive to introduce it to the model. Assume, as before, that tiny changes in the frequencies of rare types have negligible impacts on growth rates, and that selection during the transitions between environments is insignificant compared to selection at steady state within environments. Define, as before, the number of population generations spent in each environment at one time (i.e., \( n \)).

Third, there is no requirement for a realized resource supply to ever reside within the zone of coexistence. Suppose only one resource is ever present in the environment at one time (i.e., \( lu = 1, mg = 0 \) or \( lu = 0, mg = 1 \)), and let \( \gamma_{LU} \) and \( \gamma_{MG} \) represent the time spent consuming each. Then rewrite (50) and (51) as

\[
\gamma_{LU} \cdot w^{10}_{LU} + \gamma_{MG} \cdot w^{10}_{MG} > 1 \quad \text{for} \quad p_{TD10} \approx 0 \quad (52)
\]

\[
\frac{1}{\gamma_{LU}/w^{10}_{LU} + \gamma_{MG}/w^{10}_{MG}} < 1 \quad \text{for} \quad p_{TD2} \approx 0. \quad (53)
\]
Figure 5.—A protected polymorphism of two types competing for a single variable resource. (A) Relationships between growth rates and resource abundance. As clone 1 sweeps through a population with dilution rate $D_a = 1.5$, the resource abundance drops from $S_{ss,2,A} = 45 \mu M$ to $S_{ss,1,A} = 30 \mu M$, forcing growth rates to slow and relative fitness to decline from $w_1(S_{ss,2,A}) = 1.5$ to $w_1(S_{ss,1,A}) = 1.125$. Despite evident frequency dependence, coexistence is not possible at a fixed dilution rate. (B) Regions of coexistence (shaded) calculated assuming the population spends equal time at $D_a = 1.5$ and $D_a = 0.5$. The regions were calculated assuming changes in death rates are infrequent. Individuals cannot respond to very rapid changes in dilution rates, which will be perceived to converge on a single death rate, $D = (D_a + D_b)/2$, where coexistence is no longer possible.

We have just transited from a balanced polymorphism attributable to stabilizing frequency-dependent selection in a uniform environment ([50] and [51]) to a protected polymorphism in a variable environment where relative growth rates, $w^{LU}_{10} = 0.91$ and $w^{MG}_{10} = 1.32$, are independent of frequency ([52] and [53]).

Fourth, the only difference between these equations is that the former use $lu$ and $mg$ as the proportions of each resource available for consumption in a uniform environment, whereas the latter use $\gamma_{lu}$ and $\gamma_{mg} = 1 - \gamma_{lu}$ to represent the proportion of time spent consuming each resource in a variable environment. In both cases the same proportions of lactulose and methylgalactoside are delivered into the environment, and in both cases the same quantities of lactulose and methylgalactoside are consumed. Hence, the same mechanism, the differential consumption of resources, promotes coexistence both in constant and in variable environments. The only difference is whether competitors consume resources concurrently or consecutively. These models are synonymous, despite selection being frequency dependent when resources are consumed concurrently and frequency independent when they are consumed consecutively.

DISCUSSION

Analyses of both continuous- and discrete-time models reveal that temporal fluctuations in the direction of selection can protect a polymorphism in a haploid species whose population size is subject to density-dependent regulation. The growth rate of an invading type must exceed the dilution rate when rare, else invasion fails, just as it must eventually slow to match the dilution rate, else the population grows without bound. Hence, density-dependent population regulation forces growth rates to slow as a selective sweep proceeds. Decelerating growth rates, which cause changes in generation times, can be attributed to a variety of ecological mechanisms. One of the most familiar, and the one modeled here, is resource depletion: an invading type consumes a limiting resource more efficiently, reducing its abundance, which in turn lowers the growth rates of all competitors. This causes a decline in the intensity of selection per population generation (but not per cell generation) as a selective sweep proceeds (Figure 3).

A direct demonstration that temporal fluctuations in selection can protect a polymorphism is most improbable. Measuring selection coefficients accurately in natural populations is extraordinarily difficult, and the generation times of most species are too long for sufficient data to be gathered. Laboratory populations of microorganisms inhabiting defined environments provide better opportunities but here again time is limited. With reproduction clonal, periodic selection of new mutants is likely to confound all long-term experiments.

Nevertheless, indirect evidence has been obtained. Competition for two simultaneously limiting substitut-
able resources promotes coexistence through stabilizing frequency-dependent selection generated by differential resource depletion (Lunzer et al. 2002). Readily transmogrified into a model of competition for two alternating substitutable resources, the frequency dependence dissipates (fitness on a single resource is not a weighted average) even as the ability to promote coexistence is retained. Hence, stabilizing frequency-dependent selection produced by concurrent consumption of resources is evident that temporal fluctuations in selection, generated by consecutive consumption of resources, can promote coexistence.

What is true for one mechanism of selection is not necessarily true for all. Consider the case where the growth rate of type 2 is a concave function of resource abundance \( [e.g., \mu_{2j} = \mu_{\text{max},2} S_{2j}/(K_{2j} + S_{2j})] \). Fitness now changes with clone frequency because the equilibrium resource levels (referred to as \( R^* \) by Tilman 1982) differ according to which clone is dominant \( [e.g., \text{when } D_i = 1.5 \text{ hr}^{-1}, S_{1,4} + 1 = 1.5/0.05 = 30 \mu \text{m and } S_{2,3} = 15 \cdot 1.5/(2 - 1.5) = 45 \mu \text{m}] \). This frequency dependence changes the intensity of selection, but with \( D_i \) held constant it cannot change the direction of selection and so is unable to promote coexistence. When the dilution rate moves across the intersection of the growth curves a change in the direction of selection is produced. Seasonal switches in the dilution rate between \( D_i \) and \( D_k \) are necessary, although not sufficient, for coexistence (Figure 5B). This example nicely illustrates two facts: (1) alone, frequency-dependent selection need not promote coexistence, and (2) fluctuations in the availability of a single limiting resource are necessary, although not sufficient, for two competitors to coexist.

Selection during the transitions between environments assumes greater importance as seasons are shortened. In the limit, when the length of each season is infinitesimally short, the death rates converge on \( (D_i + D_k)/2 \). Instead of protecting a polymorphism, selection now drives clone 2 to fixation as \( S_{1,4} \rightarrow 15 \cdot 1/(2 - 1) = 15 \mu \text{m} \). This outcome differs from the case where the seasonal consumption of different substitutable resources generates stabilizing frequency-dependent selection when seasons are infinitesimally short. These examples serve to demonstrate that the outcome of selection depends on the ecological context. Identifying the underlying ecological processes that produce selection is crucial to population genetics.

None of this should be taken to imply that Dempster’s 1955 discrete-time model is incorrect. Rather, it describes a situation where the selection coefficients and the number of generations per unit time are fixed. This might arise during selection for seed productivity among annual plants. We have to assume only that a fixed number of seeds germinate each year, that seeds germinate in proportion to genotype frequency, and that different genotypes are favored in different years.

The biological conclusion most often drawn from Dempster’s model is that temporal fluctuations in selection cannot protect polymorphisms in haploid species unless selection is frequency dependent (Dempster 1955; Haldane and Jayakar 1963; Gillespie 1972, 1973a,b, 1991; Felsenstein 1976; Hedrick et al. 1976; Hedrick 1986; Maynard Smith 1988). If variation in \( W \) with clone frequency is interpreted as frequency dependence, then all clonal sweeps in all populations subject to density-dependent regulation are, by definition, frequency dependent. We conclude that Dempster’s 1955 discrete-time model is irrelevant to the vast majority of haploid species subject to density-dependent population regulation.

In defining \( W \) as relative fitness the standard formalism used by population genetics amalgamates two phenomena: frequency-dependent changes in generation time and frequency-dependent changes in relative fitness. In ascribing all changes in \( W \) to frequency dependence in relative fitness, this formalism ignores the role played by changing generation times in populations regulated by density-dependent mechanisms. In confounding relative fitness and cell generation time an unnecessarily restrictive condition has been inadvertently imposed on the ability of temporal variations in selection to protect polymorphisms. Thus was born the myth that temporal fluctuations in selection cannot protect a polymorphism in a haploid species.

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LITERATURE CITED


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