Genetic analysis of a population of *Tribolium*. IX. Maximization of population size and the concept of a stochastic equilibrium

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Motivated by the genetic hypothesis that natural selection results in the maximization of the equilibrium population size, we quantified this latter equilibrium for laboratory populations of the flour beetle *Tribolium castaneum*, using the gamma probability density function. Gamma density functions were fitted to adult numbers for each of the experimental treatments that were started with frequencies of the corn oil sensitive (*cos*) allele in the range 0–1 at intervals of 0.1. The gamma density function adequately described all observed distributions. However, contrary to theory, statistical comparisons of the fitted distribution indicate that the polymorphic populations did not converge to the same identical distribution and that the polymorphic populations are intermediate in population size to the two homozygous groups. The need for a stochastic theory that combines both population size and genetic selection is discussed.

Key words: natural selection, *Tribolium*, gamma distribution, maximum population size, stationary distribution, stochastic differential equation, stochastic population model.

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Motivés par l'hypothèse génétique que la sélection naturelle conduit à une maximisation de l'équilibre de la densité d'une population, nous avons quantifié cet équilibre chez des populations d'élevage en laboratoire de *Tribolium castaneum*, cet insecte des farines, en utilisant la fonction gamma de probabilité de densité. Les fonctions gamma de densité ont été appliquées, dans chacun des traitements, aux nombres d'adultes qui ont débuté avec des fréquences de sensibilité à l'huile de maïs (*cos*) de 0 à 1, avec intervalles de 0,1. La fonction gamma de densité a décrit adéquatement toutes les distributions observées. Toutefois, contrairement à la théorie, les comparaisons statistiques des distributions concordantes indiquent que les populations polymorphes n'ont pas eu de convergence avec cette même distribution identique et, de plus, que ces populations polymorphes sont intermédiaires en densités de populations entre les deux groupes homozygotes. La nécessité de recourir à une théorie stochastique qui combinerait à la fois les densités de populations et la sélection génétique est discutée.

Mots clés : sélection naturelle, *Tribolium*, distribution gamma, densité maximale des populations, distribution stationnaire, équation différentielle stochastique, modèle stochastique de population.

[Traduit par la revue]

Introduction

A classical result in the population genetic theory of natural selection is that the Malthusian rate of growth is expected to increase monotonically until it reaches a local maximum at genetic equilibrium (Fisher 1930; Wright 1935). A density-dependent version of this Fisher–Wright theorem asserts that an equilibrium allele frequency corresponds to a local "maximum equilibrium population size" (MacArthur 1962; Anderson 1971; Charlesworth 1971; Roughgarden 1971; Leon and Charlesworth 1978). We recognize the limitations of the direct biological application of the latter result; nevertheless, one of the roles of a general theory is to stimulate new empirical research. In that spirit our objective is formulated: to evaluate experimentally the maximization of population size hypothesis.

In experiments with laboratory populations of the flour beetle *Tribolium*, adult abundances, even under controlled environmental conditions, fluctuate over time in an apparently random way (Leslie 1962). The equilibrium is stochastic; adult numbers are always changing. Recent work shows that the equilibrium can be quantified using a gamma probability density function for the stationary distribution of adult numbers (Costantino and Desharnais 1981; Dennis and Patil 1984; Desharnais and Costantino 1985; Dennis and Costantino 1988). Furthermore, statistical inferences for the gamma model from observed stationary distributions can be made, including estimation of parameters, testing goodness of fit, obtaining confidence intervals for parameters, and testing to compare two gamma distributions (Dennis and Costantino 1988).

In this paper, we examine the equilibrium size of populations in which the initial allele frequencies, $p(0)$, of a fitness-related physiological mutant are not equal to the globally stable equilibrium allele frequency, p^*. From the single-locus theory of natural selection, we expect the genetic disequilibium, $p(0) \neq p^*$, to be resolved as all populations initially segregating at this locus converge to the genetic equilibrium. Eventually, each of these populations will also reach

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a stochastic equilibrium of adult numbers, called the population size equilibrium (Costantino and Desharnais 1981). Our goal is to determine the effect of the nonequilibrium initial allele frequencies on the stationary distribution of adult numbers. Specifically, does convergence occur and is population size maximized at p^*?

Laboratory procedures

The adult census numbers were obtained from the experiment of Moffa and Costantino (1977); readers should consult that source for specific details. Here we briefly comment on the laboratory procedures. Two genetically related strains of *Tribolium castaneum* Herbst were used. One strain was genetically heterogeneous wild type (Purdue Foundation) and the other, derived from the first by laboratory selection (Yamada and Bell 1969), was homozygous for the corn oil sensitive (*cos*) mutant (Costantino et al. 1967). Experimental lines were labelled using initial frequency of the *cos* allele.

Genetic and demographic data were recorded for 11 experimental treatments in which the initial *cos* allele frequency ranged from 0 to 1 in increments of 0.1. The initial genotypic arrays were constructed from combinations of the +/+ and *cos/cos* homozygotes; each initial demographic array consisted of 10 newly emerged adults of each sex. Each population was maintained in a one-half-pint milk bottle with 20 g of corn oil media (90% flour, 5% dried brewer's yeast, and 5% corn oil by weight) in an unlighted incubator at 33 ± 1°C and 42 ± 6% relative humidity. The cultures were censused and placed in fresh media every 2 weeks for 68 weeks. At weeks 52 and 68, larvae were sampled and their genotypes determined with test crosses with *cos/cos* homozygotes. Additional genetic data were obtained from a separate but identically designed experiment (Moffa and Costantino 1977). In this second experiment, the frequency of the *cos* allele was determined from independent replicates at weeks 2, 6, 12, and 16.

In cultures segregating at the *cos* locus, allele frequencies quickly reached a mean (±SE) equilibrium of $p^* = 0.25 \pm 0.03$ (the median value was 0.28) and maintained this equilibrium throughout the experiment (Moffa and Costantino 1977). After a large initial increase followed by a decline, adult numbers settled into a stationary pattern of stochastic fluctuations by week 20 (Moffa and Costantino 1977). The biweekly census data for adult numbers from week 20 to week 68 provided 25 observations per replicate. There were three replicate populations for each of the treatment with $p(0) = 0.0$, 0.1, 0.2, 0.3, and 0.4 and five replicate populations for each of the remaining treatments. One observation was lost in each of the $p(0) = 0.0$ and $p(0) = 0.1$ treatments as a result of errors in the recording of data. Both of the $p(0) = 0.8$ and $p(0) = 0.9$ treatments lost two replicate populations to disease during the first half of the experiment. It is the adult census data from week 20 to week 68 that are examined in this report.

Stationary distribution model

Demographic time scales

Species of the genus *Tribolium* are cannibalistic: adults eat eggs, small larvae, pupae, and young adults; larvae eat eggs, pupae, and young adults (Park et al. 1965; Ho and Dawson 1966; Park et al. 1974; Craig 1986). These behavioral interactions can lead to stable demographic oscillations (Desharnais and Liu 1987). Indeed, in the experimental data, larval numbers generally display large amplitude oscillations, while for adult numbers it is often difficult to distinguish cyclic oscillations from random fluctuations. Recently, we suggested an analysis of this complex system of life-stage interactions using a separation of time scales approach, the dynamics of adult numbers occurring over a slow time scale and those of the immature age-classes over a fast time scale (Hastings 1987; Hastings and Costantino 1987; Costantino and Desharnais 1990). The main reason for the difference in time scales is that adults are so long-lived relative to the developmental interval. In this paper we make a slow time scale analysis of the adult population dynamics. The more general question of scaling as a modelling technique has recently been discussed by Segel (1988*a*, 1988*b*).

Deterministic model

An ordinary differential equation for the rate of change in adult numbers, $N(t)$, is

$$[1] \quad dN(t) = N(t)(b \exp[-cN(t)] - \mu)dt$$

where c is the per capita rate at which adults prevent a pupa or incompletely sclerotized adult from entering the adult population and b and μ are, respectively, the density-independent rates of reproduction and adult mortality (Costantino and Desharnais 1981; Desharnais and Costantino 1982*a*, 1982*b*, 1983, 1985; Dennis and Patil 1984; Dennis and Costantino 1988).

The dynamics of the adult recruitment model [1] are quite simple. If $b > \mu$, the equilibrium number of adults given by $N(\infty) = [\log(b/\mu)]/c$ is globally stable. If $b \leq \mu$, the population will go extinct. In the neighborhood of $N(\infty)$, the rate of approach to equilibrium is given by the eigenvalue $\lambda = \mu \log(\mu/b)$.

The familiar logistic model can serve as an approximation to the flour beetle model [1] by expanding the per capita growth rate, $b \exp[-cN(t)] - \mu$, in a Taylor series around the stable equilibrium $N(\infty)$, and discarding second-order and higher terms (Dennis and Patil 1984; Dennis and Costantino 1988). The logistic approximation is

$$[2] \quad dN(t) = N(t)[A - BN(t)]dt$$

where $A = \mu \log(b/\mu)$ and $B = c\mu$. The equilibrium, $N(\infty)$, in this logistic is a balance of recruitment, cannibalism, and mortality.

Stochastic model

The stochastic version of model [2] that we consider is

$$[3] \quad dN(t) = N(t)[A - BN(t)]dt + \sigma N(t)dW(t)$$

Here $dW(t)$ has a normal distribution with mean zero and variance dt, and σ is a positive constant. The model with its "white noise" represents the effects of unpredictable fluctuations in the per capita growth rate of the population. The differential $dN(t)$ is formally defined in terms of either an Ito or a Stratonovich stochastic integral (Horsthemke and Lefever 1984). We will use the Ito interpretation of [3].

If the constant σ in [3] is zero, we recover the logistic adult recruitment model [2]; however, with $0 < \sigma < (2A)^{1/2}$, as t becomes large the distribution for $N(t)$ approaches a limiting stationary gamma distribution with probability density function

$$[4] \quad f(x) = \left(\frac{\beta^\alpha}{\Gamma(\alpha)}\right) x^{\alpha-1} \exp(-\beta x)$$

where $0 < \chi < \infty$, $\alpha = (2A/\sigma^2) - 1$, and $\beta = 2B/\sigma^2$ (Leigh 1968; Dennis and Costantino 1988). The parameters α and β are positive. If $\sigma > (2A)^{1/2}$ (implying $\alpha < 0$), extinction occurs eventually.

Statistical inferences

We used the following statistical procedures for estimating

TABLE 1. Observed number of adults (y_j) and class interval boundaries (s_j) for the cultures segregating at the *cos* locus

Left (s_{j-1})	Right (s_j)	Observed (y_j)[a] 0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	7.5	0	0	0	0	0	0	0	0	0
7.5	12.5	0	0	0	0	0	1	1	1	1
12.5	17.5	0	3	1	1	0	6	4	2	4
17.5	22.5	1	4	1	1	2	14	9	3	1
22.5	27.5	4	2	8	3	7	9	6	5	9
27.5	32.5	4	11	9	2	10	10	15	8	12
32.5	37.5	3	3	6	2	9	18	16	7	8
37.5	42.5	7	1	5	5	8	12	13	8	13
42.5	47.5	4	3	7	6	8	14	10	11	15
47.5	52.5	7	6	4	3	10	16	15	7	9
52.5	57.5	5	5	6	9	15	9	6	8	6
57.5	62.5	13	5	4	10	8	4	7	6	9
62.5	67.5	5	4	5	7	9	7	10	4	4
67.5	72.5	10	7	4	10	10	3	3	7	1
72.5	77.5	7	4	5	4	11	0	2	5	0
77.5	82.5	0	3	3	5	7	1	2	4	0
82.5	87.5	1	5	3	2	1	0	4	2	0
87.5	92.5	1	2	0	0	4	0	0	4	0
92.5	97.5	0	3	0	3	1	1	1	0	1
97.5	102.5	2	0	2	1	3	0	0	1	1
102.5	107.5	0	2	1	1	1	0	0	0	0
107.5	112.5	0	1	0	0	1	0	1	0	0
112.5	117.5	0	1	0	0	0	0	0	0	0
117.5	∞	0	0	1	0	0	0	0	0	0
Total		74	75	75	125	125	125	125	93	94

[a]For $p(0) = 0.1$, 0.2, 0.3, and 0.4, there were three replicate cultures; for the remaining initial *cos* allele frequencies there were five replicate cultures. For $p(0) = 0.1$, one observation was lost as a result of a data recording error. For both the $p(0) = 0.8$ and 0.9 treatments, two cultures were lost to disease.

the parameters α and β and for conducting hypothesis tests concerning the stationary gamma distributions. Dennis and Costantino (1988) give further technical details, discuss the merits of using grouped data, and present a justification for using these procedures when the observations form an autocorrelated time series.

We computed maximum likelihood estimates of α and β using a multinomial likelihood function. Let q_j denote the probability that an observation falls within the population size interval (S_{j-1},S_j), where $0 = S_0 < S_1 < \ldots < S_{m-1} < S_m = \infty$, and let y_j denote the observed frequency count of observations in that interval. Under the gamma hypothesis,

$$[5] \quad q_j(\alpha,\beta) = F(s_j) - F(s_{j-1})$$

where $F(s)$ is the gamma cumulative distribution function (area under $f(x)$ between 0 and s). If the observations are spaced far enough apart in time, then the probability of obtaining the entire data set $y_1, y_2, \ldots, y_m$ is approximately given by the multinomial likelihood function

$$[6] \quad L(\alpha,\beta) = n!\prod_{j=1}^{m} \frac{q_j(\alpha,\beta)^{y_j}}{y_j!}$$

Here n represents the total number of observations: $n = \Sigma\, y_j$. The maximum likelihood estimates of α and β are the values that maximize the probability [6]. These values can be found by performing a nonlinear least squares regression (Jennrich and Moore 1975). The regression uses the values $y_1, y_2, \ldots, y_m$ as observations on the dependent variable, and $nq_1(\alpha,\beta), nq_2(\alpha,\beta), \ldots, nq_m(\alpha,\beta)$, the expected values of the y_j's, as the model to be fit. The nonlinear regression must be "iteratively reweighted"; that is, weights of $[nq_j(\alpha,\beta)]^{-1}$ must be computed with updated values of α and β at every iteration. After convergence, the resulting values of α and β are not least squares estimates; the (Gauss–Newton) least squares algorithm has been "tricked" into maximizing the likelihood function [6].

TABLE 2. Parameter estimates for the stationary distributions for the cultures segregating at the *cos* locus

Initial allele frequency	$\hat{\alpha}$	$\hat{\beta}$	$\hat{v}_1$	$\hat{v}_2$[a]	$\hat{v}_{12}$[a]
0.1	9.03374	0.16136	1.51814	2.79853	4.13939
0.2	3.82764	0.06591	0.67745	1.27291	0.81800
0.3	5.26045	0.10102	0.89092	1.80502	1.54108
0.4	7.83768	0.13026	1.38067	2.36396	3.18192
0.5	6.93378	0.12260	0.90757	1.67680	1.47282
0.6	5.90461	0.14693	0.76979	1.98636	1.47068
0.7	5.54226	0.12377	0.71929	1.67640	1.15675
0.8	5.70670	0.11154	0.86106	1.76567	1.46077
0.9	7.03089	0.16506	1.04143	2.52653	2.54385

[a]Each entry $\times 10^{-2}$.

The maximum likelihood estimates have an asymptotic multivariate normal distribution as $n \to \infty$ (see, for example, Bishop et al. 1975, p. 509). The inverse of the variance–covariance matrix is obtained by differentiating the log likelihood and taking the expected values of the second derivatives. The variance–covariance matrix is

$$[7] \quad \Sigma(\alpha,\beta) = \begin{bmatrix} \dfrac{\theta_1}{\theta_1\theta_3 - \theta_2^2} & \dfrac{-\theta_2}{\theta_1\theta_3 - \theta_2^2} \\ \dfrac{-\theta_2}{\theta_1\theta_3 - \theta_2^2} & \dfrac{\theta_3}{\theta_1\theta_3 - \theta_2^2} \end{bmatrix}$$

where

$$\theta_1 = n\sum_{j=1}^{m} q_j^{-1}\left(\frac{\partial q_j}{\partial \beta}\right)^2$$

$$[8] \quad \theta_2 = n\sum_{j=1}^{m} q_j^{-1}\left(\frac{\partial q_j}{\partial \alpha}\right)\left(\frac{\partial q_j}{\partial \beta}\right)$$

$$\theta_3 = n\sum_{j=1}^{m} q_j^{-1}\left(\frac{\partial q_j}{\partial \alpha}\right)^2$$

with $q_j = q_j(\alpha,\beta)$ as defined in [5]. The elements in $\Sigma(\alpha,\beta)$ are estimated by substituting the maximum likelihood estimates of α and β; the partial derivatives in [8] can be computed numerically. The square roots of the diagonal elements in $\Sigma(\alpha,\beta)$ provide standard errors for α and β.

Testing the goodness of fit of the gamma distribution was accomplished using the following Pearson χ^2 test. The maximum likelihood estimates $\hat{\alpha}$ and $\hat{\beta}$ are used to calculate the Pearson χ^2 statistic given by

$$[9] \quad \chi^2 = \sum_{j=1}^{m} \frac{[y_j - nq_j(\hat{\alpha},\hat{\beta})]^2}{nq_j(\hat{\alpha},\hat{\beta})}$$

If the iteratively reweighted least squares algorithm is used

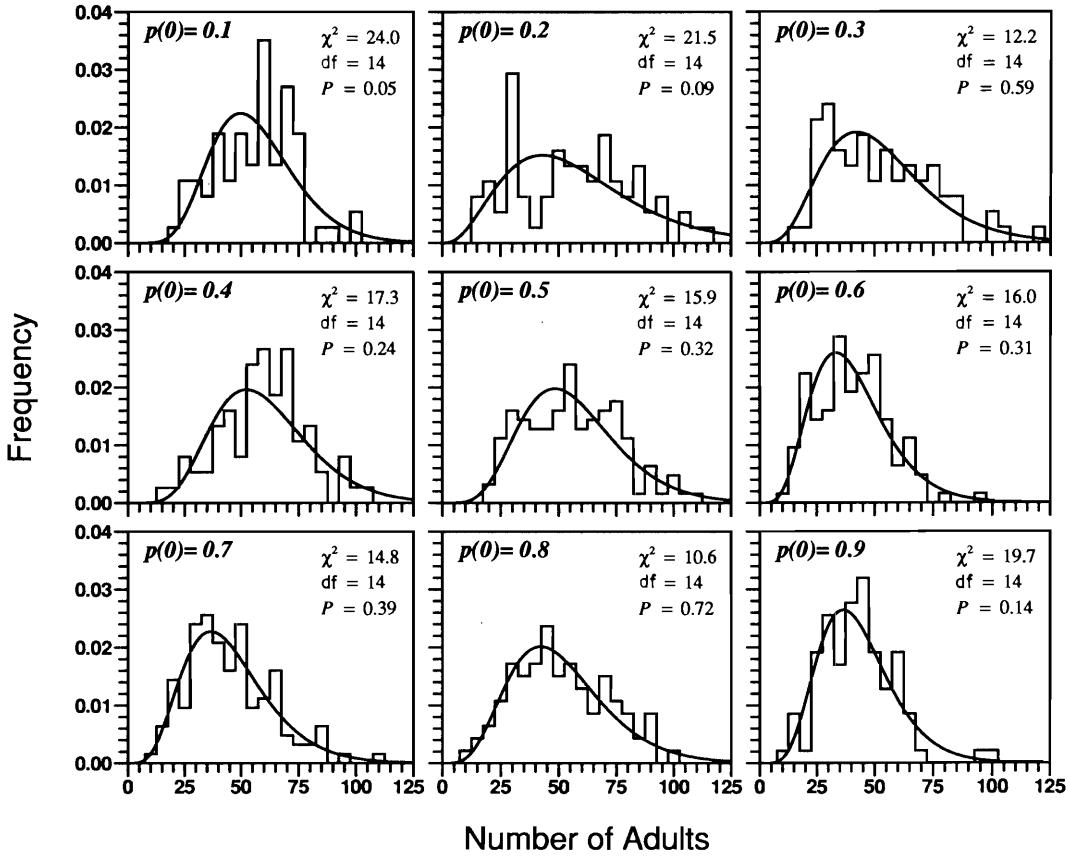

FIG. 1. The observed frequency distributions (histograms) and fitted gamma density functions [4] for the nine experimental treatments that were initially segregating at the *cos* locus. The test statistics (χ^2), degrees of freedom (df), and probability levels (P) are given for the goodness of fit tests.

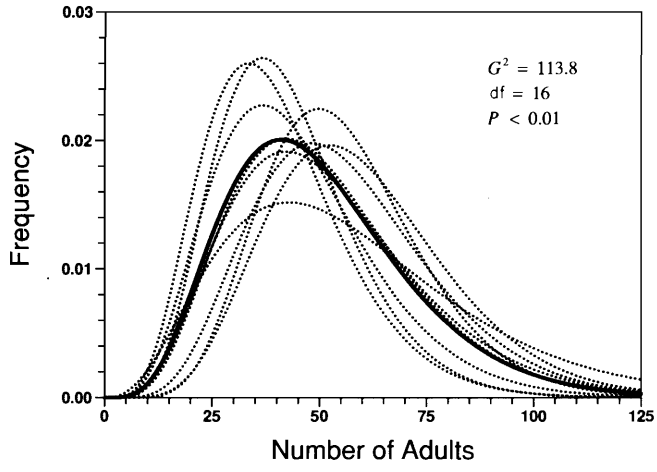

FIG. 2. A comparison of the nine gamma distributions fitted independently to each of the polymorphic groups (dotted curves) versus the single best fit gamma distribution for all the populations combined (but not pooled). The test statistic (G^2), degrees of freedom (df), and probability level (P) are given for a test of the null hypothesis that all these distributions are the same.

to obtain parameter estimates, χ^2 is the final value (at convergence) of the weighted residual sum of squares. Under the null hypothesis that the gamma model fits, χ^2 has a large-sample χ^2 distribution with $m - 3$ degrees of freedom.

The multivariate normal distribution of the maximum likelihood estimates was used for comparing parameters from two gamma distributions. Let $\hat{\alpha}_1$ and $\hat{\beta}_1$ represent the parameter estimates from the first gamma distribution and let $\hat{\alpha}_2$ and $\hat{\beta}_2$ be the estimates from the second distribution. The null hypothesis is H_0: $(\alpha_1, \beta_1)' = (\alpha_2,\beta_2)'$, i.e., both distributions have identical parameters, while the alternative hypothesis is H_1: $(\alpha_1,\beta_1)' \neq (\alpha_2,\beta_2)'$. Since $(\hat{\alpha}_1,\hat{\beta}_1)'$ and $(\hat{\alpha}_2,\hat{\beta}_2)'$ both converge in distribution to multivariate normals, the difference

$$[10] \quad \hat{\delta} = \begin{pmatrix} \hat{\alpha}_1 - \hat{\alpha}_2 \\ \hat{\beta}_1 - \hat{\beta}_2 \end{pmatrix}$$

will also converge to a multivariate normal with mean vector $(\alpha_1,\beta_1)'$ and variance-covariance matrix $\Sigma(\alpha_1,\beta_1) + \Sigma(\alpha_2,\beta_2)$. The statistic

$$[11] \quad D^2 = \hat{\delta}'[\Sigma(\hat{\alpha}_1,\hat{\beta}_1) + \Sigma(\hat{\alpha}_2,\hat{\beta}_2)]^{-1}\hat{\delta}$$

can be used to test H_0, where $\hat{\delta}$, $\Sigma(\hat{\alpha}_1,\hat{\beta}_1)$, and $\Sigma(\hat{\alpha}_2,\hat{\beta}_2)$ are computed from [7] using the maximum likelihood estimates. Under the null hypothesis, D^2 has a (large sample) χ^2 distribution with two degrees of freedom.

Observed stationary distributions

Cultures segregating at the cos *locus*

The observed numbers of adults, in the region of the steady state, are presented in Table 1 for the nine experimental treatments that were initially segregating at the *cos* locus. We computed the maximum likelihood (ML) estimates for the parameters α and β in the gamma model [4] using these grouped data and the multinomial likelihood function [6]. Goodness of fit was tested using the χ^2 statistic [9] at

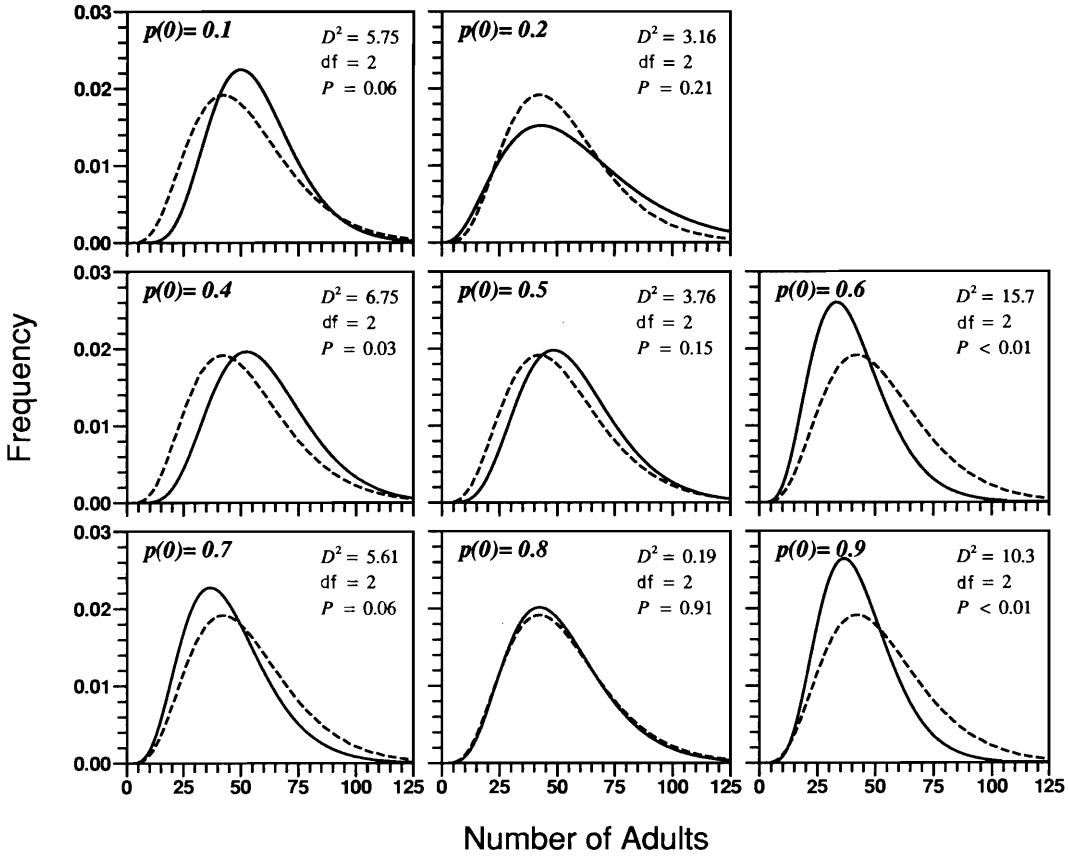

FIG. 3. Pairwise comparisons of the fitted gamma distributions for the nonequilibrium polymorphic populations (continuous curves) versus the reference population with $p(0) = 0.3$ (broken curve). The test statistics (D^2), degrees of freedom (df), and probability levels (P) are given for a test of the null hypothesis that the two distributions are the same.

the 0.01 significance level. In every case, the null hypothesis that the gamma model fits these data was accepted. The observed distributions, fitted gamma density functions, and test results are presented in Fig. 1. The ML estimates, $\hat{\alpha}$ and $\hat{\beta}$, their estimated variances, $\hat{v}_1$ and $\hat{v}_2$, and estimated covariance, $\hat{v}_{12}$, are given in Table 2.

Did the nine test cultures converge to a common gamma (see Fig. 2)? We used a likelihood ratio test of H_0: one gamma versus H_1: more than one gamma for the nine data sets ($p(0) = 0.1, 0.2, \ldots, 0.9$). For the null hypothesis, one gamma was fitted simultaneously to the nine data sets (not pooled). We computed

$$[12] \quad G_0^2 = 2\sum_{i=1}^{9}\sum_{j=1}^{17} y_{ij} \log\left(\frac{y_{ij}}{n_i q_j(\hat{\alpha},\hat{\beta})}\right)$$

where y_{ij} is the frequency count in the jth abundance interval for the ith population and

$$[13] \quad q_j(\hat{\alpha},\hat{\beta}) = \int_{s_j-1}^{s_j} \left(\frac{\hat{\beta}^{\hat{\alpha}}}{\Gamma(\hat{\alpha})}\right) x^{\hat{\alpha}-1} \exp(-\hat{\beta}x)\,dx$$

for $0 = S_0 < S_1 < \ldots < S_9 = \infty$. The maximum likelihood estimates $\hat{\alpha} = 5.43383$ and $\hat{\beta} = 0.10798$ minimize [12] (solid curve in Fig. 2). Under the alternative hypothesis of one or more of the α's, β's not equal, nine gammas were fitted separately to the nine data sets (dotted curves in Fig. 2). We computed

$$[14] \quad G_1^2 = 2\sum_{i=1}^{9}\sum_{j=1}^{17} y_{ij} \log\left(\frac{y_{ij}}{n_i q_j(\hat{\alpha}_i,\hat{\beta}_i)}\right)$$

where y_{ij} is the frequency count and $\hat{\alpha}_i,\hat{\beta}_i$ are the maximum likelihood estimates of the ith population. The likelihood ratio test statistic of H_0 vs. H_1 is

$$[15] \quad G^2 = G_0^2 - G_1^2 = 275.1707 - 161.3843 = 113.7864$$

with 16 degrees of freedom (18 parameters estimated under H_1 minus 2 parameters estimated under H_0). The null hypothesis of one common gamma is rejected.

In the deterministic genetic theory of natural selection if $p(0) = p^*$, then the population is in genetic equilibrium; if $p(0) \neq p^*$, then eventually p is expected to reach p^*. In these data, to a rough approximation, $p^* \approx 0.3$, so the experimental cultures with that allele frequency were near genetic equilibrium at the beginning of the experiment and all nonequilibrium cultures should, eventually, be similar to these cultures. With respect to the dynamics of adult numbers the $p(0) = 0.3$ treatment acts as a "reference population" (Desharnais and Costantino 1982*b*; Desharnais 1986). Did any of the nonequilibrium cultures converge to the stationary distribution of adult numbers attained by the $p(0) = 0.3$ cultures? To answer this question we used the D^2 test statistic [11] for the pairwise comparison of the gamma parameters for the $p(0) = 0.3$ treatment with the other eight treatments (Fig. 3). We accepted the null hypothesis at the 0.05 level of significance that the gammas for

TABLE 3. Observed number of adults (y_j) and class interval boundaries (s_j) for the wild-type and sensitive populations

Left (s_{j-1})	Right (s_j)	Observed (y_j) Wild[a]	Sensitive[b]
0	7.5	0	2
7.5	12.5	0	7
12.5	17.5	0	9
17.5	22.5	0	16
22.5	27.5	0	11
27.5	32.5	3	13
32.5	37.5	3	14
37.5	42.5	4	15
42.5	47.5	5	11
47.5	52.5	7	9
52.5	57.5	6	5
57.5	62.5	8	4
62.5	67.5	6	4
67.5	72.5	4	1
72.5	77.5	5	0
77.5	82.5	4	1
82.5	87.5	5	1
87.5	92.5	2	0
92.5	97.5	3	2
97.5	102.5	2	0
102.5	107.5	0	0
107.5	112.5	0	0
112.5	117.5	1	0
117.5	122.5	2	0
122.5	127.5	0	0
127.5	132.5	0	0
132.5	∞	4	0
Total		74	125

[a]Three replicate cultures; one observation was lost as a result of a data recording error.
[b]Five replicate cultures.

$p(0) = 0.1, 0.2, 0.5, 0.7$, and 0.8 are equal to the $p(0) = 0.3$ cultures. On the other hand, the null hypotheses for the $p(0) = 0.4$, 0.6, and 0.9 cultures were rejected.

While the idea of a shared or common gamma for all nine cultures was not accepted using the G^2 test statistic [15], the individual pairwise comparisons suggest that some convergence to a common stochastic equilibrium did occur (Fig. 3). Overall the polymorphic populations seem more alike than different.

Wild-type and sensitive populations

The observed stationary distributions for the wild-type ($p(0) = 0.0$) and sensitive ($p(0) = 1.0$) populations are given in Table 3. The observed histograms and fitted gamma density functions are sketched in Fig. 4. The parameters of the fitted gamma distributions are listed in Table 4. As with the test cultures, we accepted the null hypothesis that the gamma model fits these data at the 0.01 significance level.

These strains have statistically different parameter values according to the D^2 test [11] (Fig. 4). Nevertheless, the distributions do overlap. Deterministic ecological–genetic theory predicts that the wild-type populations ($p(0) = 0.0$) will attain a larger equilibrium population size than the sensitive populations ($p(0) = 1.0$). The gamma distribution provides a way to refine this prediction. Consider two populations, N_1 and N_2, both subject to independent random fluctuations. Let $f_1(x)$ and $f_2(x)$ represent the stationary probability density functions for N_1 and N_2. The probability that N_1 exceeds N_2 is given by

$$[16] \quad \text{Prob}(N_1 > N_2) = \int_0^\infty f_1(x)\,\text{Prob}(N_2 < x)dx = \int_0^\infty f_1(x)F_2(x)dx$$

where $F_2(x)$ is the cumulative distribution function for N_2. Substituting the gamma density function [4] in [16] gives

$$[17] \quad \text{Prob}(N_1 > N_2) = \left(\frac{\beta_1^{\alpha_1}\beta_2^{\alpha_2}}{\Gamma(\alpha_1)\Gamma(\alpha_2)}\right)\int_0^\infty x^{\alpha_1-1}\exp(-\beta_1 x) \times \left(\int_0^x z^{\alpha_2-1}\exp(-\beta_2 z)dz\right)dx$$

The integrals in [17] can be computed numerically. Using the ML estimates (Table 4) for the wild-type populations as α_1 and β_1 and for the sensitive populations as α_2 and β_2, the probability that adult numbers in a wild-type culture exceed the adult numbers in a sensitive culture was computed as 0.8666. Although it is clear that the wild-type populations have higher densities of adults (Fig. 4), it is interesting to note that 13.34% of the time one would observe *cos/cos* cultures with larger numbers of adults.

The fitted gamma density functions for each of the populations initially segregating at the *cos* locus were compared with the fitted gammas for the sensitive (*cos/cos*) and wild-type (+/+) populations. In Fig. 5 we show the pairwise comparisons of the gamma parameters for the $p(0) = 1.0$ (*cos/cos*) cultures with the other nine treatments ($p(0) = 0.1, 0.2, 0.3, \ldots, 0.9$). Using the D^2 test statistic [11], we concluded that each polymorphic treatment differs from the sensitive population. In Fig. 6, we present similar pairwise comparisons with the $p(0) = 0.0$ (+/+) distribution. In this case, we rejected the null hypothesis at the 0.05 level of probability for every treatment except for $p(0) = 0.4$. In general, the polymorphic cultures have gamma distributions that differ from the wild-type and sensitive populations.

Maximization of population size

Does the maximization theorem apply just to the underlying deterministic equilibrium? Do the data support the hypothesis of maximization of point equilibrium abundances? The equilibrium from the deterministic model ([1] or [2]), $N(\infty)$, can be written as a function of the gamma distribution parameters:

$$[18] \quad N(\infty) = \frac{\alpha + 1}{\beta}$$

The maximum likelihood estimate, given by $\hat{N}(\infty) = (\hat{\alpha} + 1)/\hat{\beta}$, has an approximate large-sample normal distribution with mean $N(\infty)$ and variance

$$[19] \quad V = \frac{\nu_1^2}{\beta^2} + \frac{(\alpha + 1)^2\nu_2^2}{\beta^4} - \frac{2(\alpha + 1)\nu_{12}}{\beta^3}$$

TABLE 4. Parameter estimates for the stationary distributions for the wild-type and sensitive populations

Population	$\hat{\alpha}$	$\hat{\beta}$	$\hat{\nu}_1$	$\hat{\nu}_2{}^a$	$\hat{\nu}_{12}{}^a$
Wild type	7.0016	0.10209	1.26556	1.90457	2.34070
Senstive	3.8884	0.11015	0.50263	1.53039	0.72561

[a]Each entry $\times 10^{-2}$.

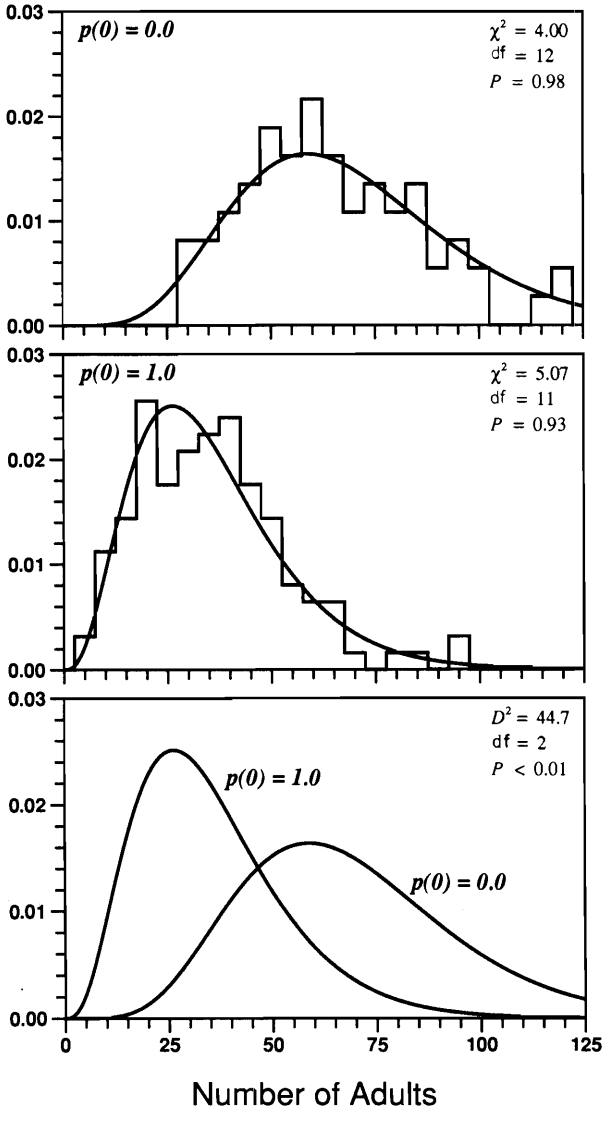

FIG. 4. The observed frequency distributions (histograms) and fitted gamma density functions [4] for the two experimental treatments that were homozygous at the *cos* locus. The test statistics (χ^2), degrees of freedom (df), and probability levels (P) are given for the goodness of fit tests. The two fitted gamma distributions are compared in the lower panel. The test statistic (D^2), degrees of freedom (df), and probability level (P) are given for a test of the null hypothesis that the two distributions are the same.

where ${\nu_1}^2$, ${\nu_2}^2$, and ν_{12} are the elements of the large-sample variance–covariance matrix [7] for $\hat{\alpha}$ and $\hat{\beta}$ (see Appendix 1). A test of H_0: $N_1(\infty) = N_2(\infty)$ versus H_1: $N_1(\infty) \neq N_2(\infty)$ can be conducted using

$$[20] \quad W = \frac{\hat{N}_1(\infty) - \hat{N}_2(\infty)}{\sqrt{\hat{V}_1 + \hat{V}_2}},$$

where $\hat{N}_i(\infty)$ and $\hat{V}_i$ are $N(\infty)$ in [18] and V in [19] evaluated using the maximum likelihood estimates $\hat{\alpha}_i$ and $\hat{\beta}_i$. Under the null hypothesis W converges to the normal (0,1) distribution.

In pairwise fashion, we compared the underlying point equilibria for the cultures initially segregating at the *cos* locus with the +/+ and *cos/cos* cultures and also with the "reference population" of $p(0) = 0.3$ (Table 5). In comparisons with the +/+ treatment we rejected H_0 in every case except for $p(0) = 0.2$ at the 0.05 level of significance. In comparisons with the *cos/cos* treatment we rejected H_0 for all initial allele frequencies except $p(0) = 0.6$ and 0.9. In comparisons with the reference population we rejected H_0 for $p(0) = 0.6$, 0.7, and 0.9; we accepted H_0 for $p(0) = 0.1$, 0.2, 0.4, 0.5, and 0.8.

Finally, to emphasize the stochastic nature of these equilibria, we used [17] to make pairwise comparisons of the random variables for population size. For each treatment segregating at the *cos* locus, we computed the probability that the population size will exceed that of the homozygous and reference populations (Table 6). Values near 0.5 indicate no tendency for a higher or lower population size. In comparison with the wild-type homozygotes, the probabilities for the polymorphic populations are consistently below 0.5; they range from 0.17 for $p(0) = 0.6$ to 0.41 for $p(0) = 0.4$. In comparison with the sensitive homozygotes, the probabilities for the polymorphic populations are consistently above 0.5; they range from 0.59 for $p(0) = 0.6$ to 0.82 for $p(0) = 0.4$. The reference population is intermediate; the probabilities range from 0.34 for $p(0) = 0.6$ to 0.61 for $p(0) = 0.4$. It is worth noting that none of these probabilities falls outside the range 0.15–0.85. In general, there is a significant overlap among all the stationary distributions.

Discussion

According to the single-locus genetic theory of natural selection with density-dependent population growth, the (deterministic) equilibrium population size of the polymorphic populations should be larger than either homozygote equilibrium. Furthermore, since the genetic equilibrium is globally stable, all populations initially segregating at the genetic locus should converge to the same equilibrium population size. Neither of these predictions was supported by the data. Overall, *Tribolium* populations segregating at the *cos* locus were intermediate in population size to the two homozygous groups. This result is true whether one uses the estimates of the deterministic equilibria (Table 5) or the probability comparisons for population size (Table 6). Among the polymorphic populations, there was a significant amount of heterogeneity for the estimated gamma density functions (Fig. 1), although on the whole, the polymorphic populations are more alike than different when compared with the two homozygous distributions (Fig. 3 vs.

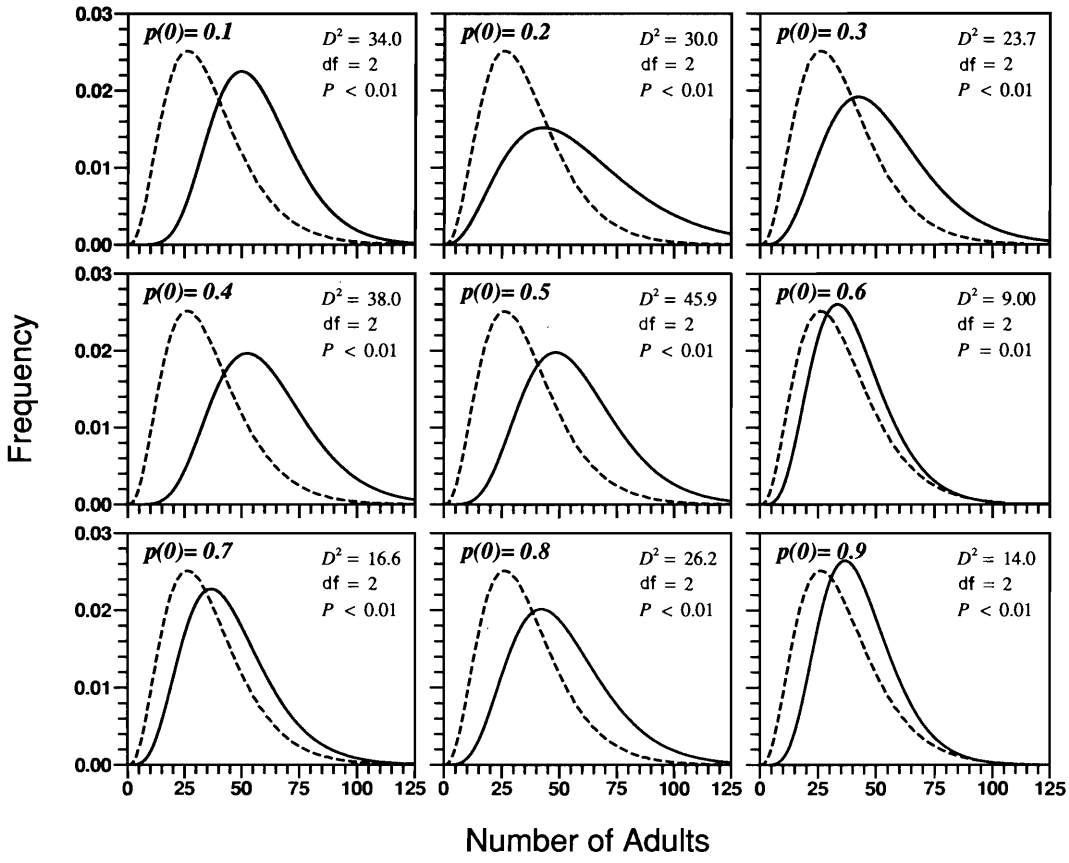

FIG. 5. Pairwise comparisons of the fitted gamma distributions for the polymorphic populations (continuous curves) versus the homozygous *cos/cos* population (broken curve). The test statistics (D^2), degrees of freedom (df), and probability levels (P) are given for a test of the null hypothesis that the two distributions are the same.

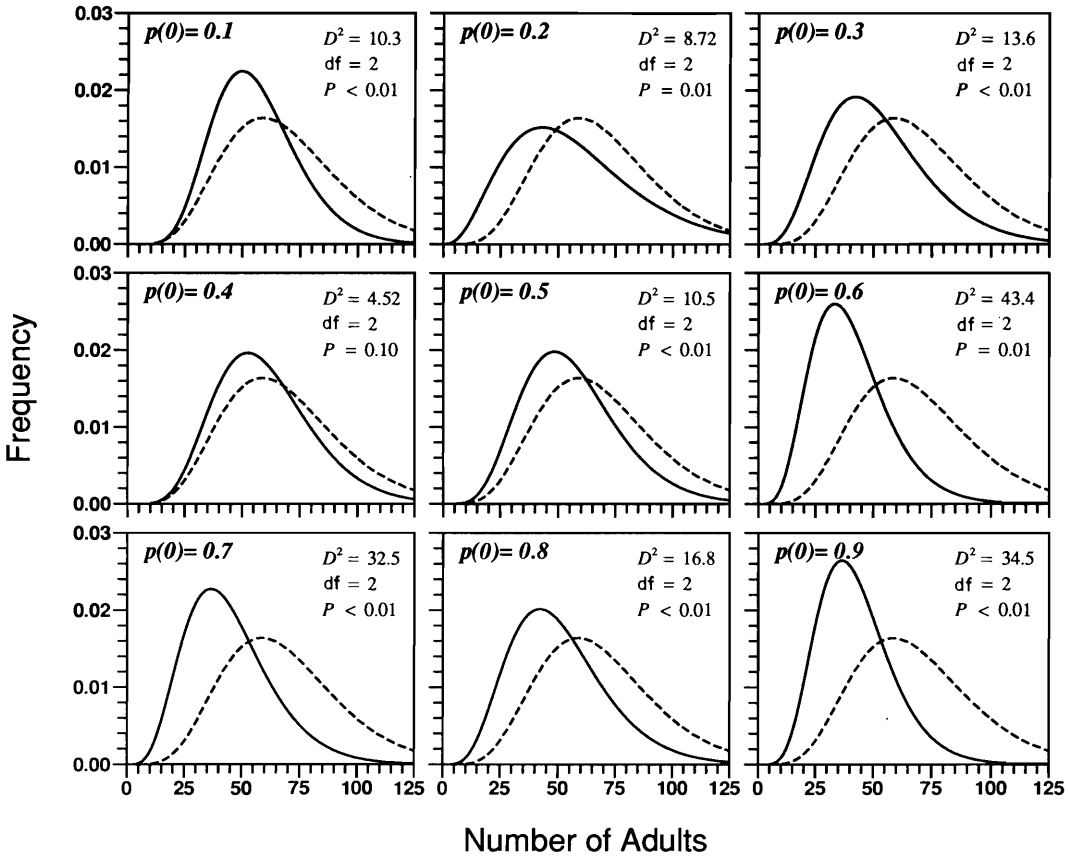

FIG. 6. Pairwise comparisons of the fitted gamma distributions for the polymorphic populations (continuous curves) versus the homozygous +/+ population (broken curves). The test statistics (D^2), degrees of freedom (df), and probability levels (P) are given for a test of the null hypothesis that the two distributions are the same.

TABLE 5. Pairwise comparisons of deterministic point equilibria

		Test statistics, W, for pairwise comparisons		
$p(0)$	$\hat{N}(\infty) \pm \sqrt{\hat{V}}$	$p(0)$ vs. wild type	$p(0)$ vs. sensitive	$p(0)$ vs. reference
0.1	62.182 ± 2.666	3.412**	−4.963**	−0.047
0.2	73.246 ± 5.469	0.762	−4.833**	−1.717
0.3	61.972 ± 3.636	3.065**	−4.038**	—
0.4	67.846 ± 3.080	2.110*	−6.010**	−1.233
0.5	64.713 ± 2.513	2.931**	−5.851**	−0.620
0.6	46.993 ± 1.945	7.161**	−0.846	3.632**
0.7	52.858 ± 2.281	5.618**	−2.561*	2.123*
0.8	60.128 ± 2.999	3.693**	−4.100**	0.391
0.9	48.654 ± 2.101	6.673**	−1.340	3.171**

NOTE: $\hat{N}(\infty) \pm \sqrt{\hat{V}}$ equals 78.378 ± 3.928 for the wild-type populations and 44.380 ± 2.400 for the sensitive populations.
*$P < 0.05$; **$P < 0.01$.

TABLE 6. Probabilities of observing a greater population size than the wild-type, sensitive, and reference populations

$p(0)$	Prob(N > wild type)	Prob(N > sensitive)	Prob(N > reference)
0.1	0.3544	0.8013	0.5710
0.2	0.3743	0.7465	0.5483
0.3	0.3094	0.7268	0.5000
0.4	0.4062	0.8243	0.6141
0.5	0.3616	0.7880	0.5663
0.6	0.1696	0.5947	0.3411
0.7	0.2235	0.6522	0.4065
0.8	0.2979	0.7246	0.4919
0.9	0.1917	0.6384	0.3777

Figs. 5 and 6). Although there is little doubt that natural selection has affected the dynamics of population size, the maximization principle was not supported.

What could account for these differences? Clearly, the theory is an oversimplification of any real biological system. The model ignores age structure, age-class interactions such as larvae eating eggs, and many of the other details that influence population dynamics in *Tribolium* (Hastings and Costantino 1987; Desharnais and Liu 1987; Costantino and Desharnais 1990). It is possible that the theory, while sufficiently general and robust to support the prediction of a gamma stationary density function, lacks sufficient detail to account for the influence of natural selection. On the other hand, we must point out that our stochastic model is for population size alone; it does not explicity include any genetic dynamics. The maximization prediction is based on a model that includes genetic and population size dynamics (Roughgarden 1971; Charlesworth 1971), but this model is deterministic. It may be the case that a stochastic model that combines both genetic and population size dynamics may yield new predictions that are in agreement with the experimental observations.

The need for a combined stochastic theory of natural selection and population growth can be illustrated with a simple example. Consider two populations, N_1 and N_2, which are subject to independent stochastic fluctuations. Assume that the first population is polymorphic and has gamma parameters $\alpha_1 = 3$ and $\beta_1 = 0.01$, and that the second population is homozygous and has gamma parameters $\alpha_2 = 34$ and $\beta_1 = 0.1$. The deterministic equilibria are $N_1(\infty) = (\alpha_1 + 1)/\beta_1 = 400$ and $N_2(\infty) = (\alpha_2 + 1)/\beta_2 = 350$, and according to the theory, $N_1(\infty) > N_2(\infty)$. However, from [17] we compute Prob $(N_1 > N_2) = 0.3526$. Even though the homozygous population has the smaller deterministic equilibrium, N_2 will exceed N_1 about 65% of the time! Although this is a contrived example, it points out the need to reformulate the genetic maximization principle in the context of a stochastic model.

Finally, one should not overlook a major success of the stochastic model: the prediction of a gamma stationary probability distribution for population size. For each of the 11 experimental groups, the gamma distribution provided an adequate description of the variation in adult numbers. The present success of the gamma supports several previous studies involving *Tribolium* in which the gamma was also a useful paradigm (Costantino and Desharnais 1981; Desharnais and Costantino 1985; Dennis and Costantino 1988). Through the gamma stationary distribution, we can use variability of population size as yet another source of information about the population.

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Appendix 1

The underlying point equilibrium $N(\infty)$ can be written as a differentiable function of the gamma distribution parameters α and β:

$$[A1] \quad N(\infty) = h(\alpha,\beta) = \frac{(\alpha + 1)}{\beta}$$

The maximum likelihood estimate of $N(\infty)$ becomes a function of the estimates of α and β:

$$[A2] \quad \hat{N}(\infty) = h(\hat{\alpha},\hat{\beta}) = \frac{(\hat{\alpha} + 1)}{\hat{\beta}}$$

The distribution of $(\hat{\alpha},\hat{\beta})'$ converges to a multivariate normal distribution with mean vector $(\alpha,\beta)'$ and variance-covariance matrix $\Sigma(\alpha,\beta)$ given by [7]. These facts allow use of the the "delta method," or the "method of statistical differentials," to obtain the large sample distribution of $\hat{N}(\infty)$ (Rao 1973, p. 388). Let ν_1^2 and ν_2^2 be the elements on the main diagonal of $\Sigma(\alpha,\beta)$ (variance of $\hat{\alpha}$ and of $\hat{\beta}$, respectively), and let ν_{12} be the off-diagonal element (covariance of $\hat{\alpha}$ and $\hat{\beta}$). The delta method arises through a Taylor series approximation of a function $h(\hat{\alpha},\hat{\beta})$ near α and β. The main result is that the distribution of $h(\hat{\alpha},\hat{\beta})$ converges to a normal distribution with mean $h(\alpha,\beta)$ and variance

$$[A3] \quad V(\alpha,\beta) = \left(\frac{\partial h}{\partial \alpha}, \frac{\partial h}{\partial \beta}\right) \Sigma(\alpha,\beta) \left(\frac{\partial h}{\partial \alpha}, \frac{\partial h}{\partial \beta}\right)'$$

By calculating the indicated partial derivatives from [A1] and performing the indicated matrix multiplications, the text expression [19] for the large sample variance of $\hat{N}(\infty)$ is obtained.

Also if $\hat{N}_1(\infty)$ converges to a normal $(N_1(\infty), V(\alpha_1,\beta_1))$ distribution, and $\hat{N}_2(\infty)$ converges to a normal $(N_2(\infty), V(\alpha_2, \beta_2))$ distribution, then

$$[A4] \quad W = \frac{\hat{N}_1(\infty) - \hat{N}_2(\infty)}{\sqrt{V(\hat{\alpha}_1,\hat{\beta}_1) + V(\hat{\alpha}_2,\hat{\beta}_2)}}$$

converges to a normal $(N_1(\infty) - N_2(\infty), 1)$ distribution. Under the null hypothesis that $N_1(\infty) = N_2(\infty)$, W has a large-sample normal (0,1) distribution.

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