GENETIC ANALYSIS OF A POPULATION OF *TRIBOLIUM*. VII. STABILITY: RESPONSE TO GENETIC AND DEMOGRAPHIC PERTURBATIONS

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A biological counterpart to mathematical stability analysis was demonstrated using the *Tribolium* model. The responses to deliberate demographic and genetic perturbations of *T. castaneum* populations, initially homozygous for the corn oil sensitive (*cos*) allele and near demographic equilibrium, were examined experimentally. An equilibrium was said to be ecologically stable if it was locally stable to perturbations in population numbers alone and genetically stable if it was locally stable to changes in allele frequencies alone. A qualitative stability analysis of the within population age-class interactions indentified several conditions for stability, such as, the product of the rate of egg cannibalism by larvae times the rate of pupal cannibalism by adults must be greater than the product of the "net" fecundity of adults times the overall larval viability. The populations which were subjected to the addition or removal of genetically similar adults or immature life stages were ecologically stable. The cultures altered by the introduction of the + allele were genetically unstable to this perturbation and converged to an average polymorphic *cos* equilibrium allele frequency of 0.42. The incorporation of the + allele into populations initially homozygous for the *cos* allele was examined in terms of natural selection in a density-regulated population. The experimental data supported the theoretical prediction that selection is expected to move an ecologically stable system toward an evolutionarily stable strategy resulting in the maximization of adult numbers.

La contre partie biologique de l'analyse de stabilité mathématique a pu être effectuée à l'aide d'un modèle basé sur le genre *Tribolium*. Pour cela, on a examiné expérimentalement la réaction de populations de *T. castaneum* homozygotes pour le gène de sensibilité à l'huile de maïs (*cos*) et proches du point d'équilibre démographique à des perturbations démographiques et génétiques volontaires. D'un point de vue écologique, un équilibre est stable lorsqu'il n'est pas modifié localement par des perturbations ne touchant que la population; pour être stable sur le plan génétique, il doit pouvoir résister localement aux modifications qui n'affectent que la fréquence des allèles. Une analyse de stabilité qualitative des interactions âge-classe existant au sein d'une même population a permis d'identifier plusieurs conditions de stabilité comme le produit arithmétique du taux de cannibalisme des œufs par les larves et du taux de cannibalisme des pupes par les adultes, qui doit être plus grand que le produit mathématique de la fécondité "nette" des adultes et de la viabilité globale des larves. Les populations auxquelles on avait ajouté ou enlevé des adultes génétiquement semblables ou des stades de vie immatures sont restées écologiquement stables. L'introduction de l'allèle + a toutefois rompu l'équilibre génétique des populations qui ont convergé vers une fréquence d'équilibre allélique de type *cos* polymorphe d'une moyenne de 0,42. Les effets de l'incorporation de l'allèle + aux populations originalement homozygotes pour l'allèle *cos* ont été étudiés en fonction de la sélection naturelle qui se fait dans un milieu où la densité de population est régularisée. Les résultats expérimentaux appuient la théorie selon laquelle le processus de sélection tend à changer un système écologiquement stable en système stable sur le plan de l'evolution mais dans lequel le nombre d'adultes augmente jusqu'à un chiffre optimum.

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Introduction

Much of the recent theory in population biology has focused simultaneously upon genetic and demographic equilibria. Anderson and King (1970) and Charlesworth and Giesel (1972) have shown that changes in gene frequency and age structure may be intimately related; Charlesworth (1972) gives the conditions for the co-

occurrence of these equilibria. For density-dependent population growth, the work of Roughgarden (1971), Charlesworth (1971, 1972), Anderson (1971), Ginzburg (1977), and Hastings (1978) suggests that natural selection will move an ecologically stable system toward an evolutionarily stable strategy resulting in a maximization of population size. In this paper we experimentally investigated genetic and demographic stability using the *Tribolium* model.

A mathematical analysis of the stability of a dynamic system involves imposing a perturbation in the neighborhood of an equilibrium and then characterizing the subsequent response of the system. Although population biologists have used this method with theoretical models (e.g. Lewontin, 1969; Crow and Kimura, 1970; Coale, 1972; May, 1973), a similar approach has not been widely adopted by the experimentalist. Our design of experiment was to establish populations of the flour beetle *Tribolium castaneum* at or near both demographic and genetic (homozygous for the corn oil sensitive, *cos* allele) equilibrium and then to examine the local stability properties of the equilibria by deliberate perturbation. Specifically, ecological stability was examined by monitoring the response of populations to the addition or to the removal of individuals genetically similar (*cos/cos*) to members of the equilibrium population. The question of genetic stability was addressed by observing both the genetic and the age structure changes following the introduction of genetically different (+/+) individuals into the equilibrium populations. An equilibrium was said to be ecologically stable if it was locally stable to perturbations in population numbers alone and genetically stable if it was locally stable to changes in allele frequency.

Our study encompasses three interconnected areas of inquiry: (i) a stability analysis of the age class (eggs, larvae, pupae, adults) interactions within this species, (ii) a characterization of the responses of the equilibrium populations to perturbations in population number and allele frequency at the *cos* locus, and (iii) an examination of natural selection in a density-regulated population.

Materials and Methods

Twenty-two cultures of *Tribolium castaneum*, homozygous for the corn oil sensitive allele (*cos/cos*), were initiated with the equilibrium age distribution experimentally observed during a pilot study: 64 adults, 16 pupae, 20 large larvae, and 70 small larvae. Each population was contained in a one-half pint milk bottle with 20 grams of corn oil medium (percentage composition: 90% wheat flour, 5% dried brewers yeast, and 5% liquid corn oil) and kept in an unlighted incubator at $33 \pm 1°C$ and $56 \pm 11\%$ relative humidity. All age classes were censused and placed in fresh medium at biweekly intervals for 80 weeks. For a review of the biology of the *cos* mutant, readers are referred to Costantino *et al.* (1967, 1968), Costantino *et al.* (1970), Costantino and Rowe (1972), Scully and Costantino (1975), and Moffa and Costantino (1977).

At the end of ten weeks three replicates were randomly assigned to each of six treatments: three involving demographic perturbations and three involving genetic perturbations. The demographic manipulations consisted of adding 100 adults, removing all adults, and removing all juveniles. For the three remaining treatments the genetic structure was perturbed by the introduction of one female adult, or three female adult or three male and three female adults with the +/+ genotype. In the latter treatments the frequency of the + allele was estimated every four weeks. Four undisturbed replicates remained as a control.

Genetic data were obtained by sampling 25% of the pupae, to a maximum of 25 and a minimum of 10, and mating these with *cos/cos* individuals. Twenty eggs were collected from each cross and placed on corn oil media. Based on the statistical analysis of 60 crosses involving individuals of known genotype (20 per each genotype), the unknown pupae were classified using the following linear discriminant functions:

$$cos/cos : 0.4877 \text{ A} + 1.7665 \text{ P} + 1.9305 \text{ L} - 2.2812$$
$$+/cos : 1.4087 \text{ A} + 2.2518 \text{ P} + 2.6637 \text{ L} - 7.3883$$
$$+/+ : 1.3805 \text{ A} + 2.3049 \text{ P} + 2.2817 \text{ L} - 12.091$$

where A, P, and L are the number of adults, pupae, and larvae respectively, recovered after 27 days. The unknown individual was assigned the genotype with the largest discriminant function value. The percentages of correct classifications for the 60 individuals of known genotype were 85% for *cos/cos*, 75% for *+/cos* and 95% for ±/±.

Results

Stability Analysis of the Age Class Interactions

Laboratory cultures of *Tribolium* are composed of several age classes, among which there are directed interactions. These interactions can be described by a system of differential equations:

$$dL_i/dt = g_i (L_1, L_2, ..., L_n; B_1, B_2, ...). \tag{1}$$

where the rate of change in life stage L_i (eggs, larvae, pupae, adults) is some function g_i of these life stages and of a set of parameters B. In *Tribolium*, these parameters may reflect such things as culture medium, temperature, humidity and/or biological entities such as the number of pupae produced per parent, rates of cannibalism or the death rate among adults. The behavior of the system in the vicinity of an equilibrium, $dL_i/dt = 0$, can be determined by the properties of a matrix with elements $Y_{ij} = \partial g_i/\partial L_j$ evaluated at the equilibrium point. Qualitatively, these elements represent the interactions among the various life stages at equilibrium, but, if the functions, g_i, are non-linear, deriving exact expressions for the Y_{ij}'s can be quite tedious.

It has been shown (Quirk and Ruppert, 1965; May, 1973) that much information about the stability of a system can be obtained from a knowledge of only the signs of the non-zero elements of the matrix $\{Y_{ij}\}$. Levins (1974, 1975) presented a graphical interpretation of this analysis which we shall employ.

The diagram for the *Tribolium* system appears in Fig. 1 and is based on a great deal of experimental work summarized in the *Biology of Tribolium* (Sokoloff, 1972, 1974). Each interaction is labeled using the following notation: ± Y_{ij} = the positive or negative effect of age-class j on age-class i. The wide arrows indicate the positive age-class interactions, that is, the life cycle. The narrow arrows indicate the direction of the negative interactions, which, with the exceptions of −Yaa and −Yla, represent cannibalism. The adult self-loop, −Yaa, is based on the fact that adult mortality increases with adult density, although this is probably not due to cannibalism (Park *et al.*, 1965; Moffa and Costantino, 1977). The adult-larval interaction, −Yla, is an inhibition of larval metamorphosis which occurs in *T. brevicornis* but not in *T. castaneum* (Jillson and Costantino, 1980). We will comment on this interaction later. The "net" effect of adults on eggs, Yea, is positive, but is the result of both egg laying and egg cannibalism (Rich, 1956). The cannibalism of pupae by large larvae may also occur to some small extent, but at equilibrium Ypl is positive.

The "feedback" at level k is defined as

$$F_k = \sum_{m=1}^{k} (-1)^{m+1} * L (m,k) \tag{2}$$

where L(m,k) is the sum of the products of all possible paths (Y_{ij}'s) that make up m disjunct loops involving k life stages. Two loops are said to be disjunct if they do not involve one or more of the same life stages. For a system to be stable

(Levins, 1975), the feedback at each level must be negative. For *T. castaneum* the "feedbacks" at each level are:

$$F1 = -Yaa \tag{3}$$
$$F2 = -(Ypa * Yap + Yel * Yle) \tag{4}$$
$$F3 = -(Yaa * Yel * Yle) \tag{5}$$
$$F4 = (Yea * Yle * Ypl * Yap) - (Yle * Yel * Yap * Ypa). \tag{6}$$

The feedbacks, Fk, are equivalent (but of opposite sign) to the coefficients of the characteristic polynomial for the matrix $\{Y_{ij}\}$. The roots of this polynomial are the eigenvalues of the matrix; they must have negative real parts for the system to
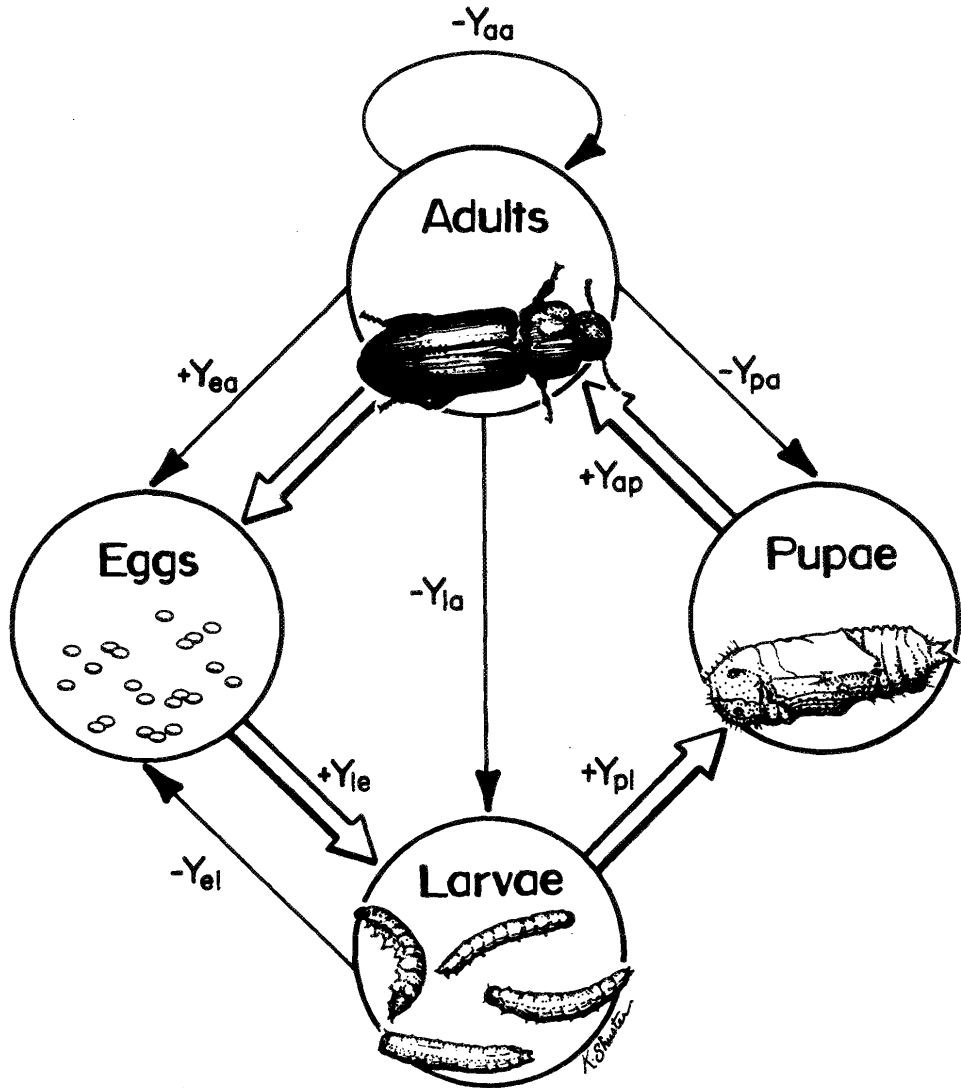

Fig. 1.   Loop analysis diagram for the *Tribolium* system. The wide arrows indicate positive interactions and the narrow arrows indicate the direction of negative interactions. See text for a complete explanation.

be stable. Thus, based on the Routh-Hurwitz criteria (e.g. May, 1973, p. 196), the following inequalities must also hold

$$Yaa * Ypa * Yap > 0 \tag{7}$$

and

$$Y^a_{aa} * Yea * Yle * Yap * Ypl > 0. \tag{8}$$

We can now identify several conditions for stability. First, the feedbacks are negative for every level except F4, which is ambiguous. For stability, Yel * Ypa > Yea * Ypl, or expressing this inequality in words: the product of the rate of egg cannibalsim by larvae times the rate of pupal cannibalism by adults must be greater than the product of the ''net'' fecundity of adults times the overall larval viability. Second, at level F1 the self-dampening effect of adults is critical, otherwise the system is not stable. Third, cannibalism of adults on pupae and larvae on eggs is an important interaction for population stability; in particular, the cannibalistic effects provide that levels F2 and F3 are negative and the possibility that level F4 can be negative.

We now consider a change in one of the parameters of the system, and examine this effect on the equilibrium densities of each life stage. If a biological parameter, B_v, changes under natural selection, then $\partial g / \partial B_v > 0$ for one or more of the life stages of that species. We are interested in the case where B_v is a parameter describing larval viability and the rate of larval development. (We shall present evidence for an increase in B_v in a later section.) An increase in B_v would enter the equation for larvae as positive input, that is $\partial g_1 / \partial B_v > 0$, and would increase Y_{pl}, thus also entering the equation for pupae as positive input, $\partial g_p / \partial B_v > 0$. We will assume all other developmental and cannibalistic rates remain unchanged ($\partial g_a / \partial B_v = \partial g_e / \partial B_v = 0$)

To determine the effect of natural selection on the densities of each life stage, we must find expressions for $\partial L_i / \partial B_v$ in the vicinity of the equilibrium. Differentiating the functions to the right of equation (1) with respect to B_v and setting these derivatives equal to zero, we use the implicit function theorem to obtain the following system of equations:

$$\begin{pmatrix} -Y_{aa} & Y_{ap} & 0 & 0 \\ -Y_{pa} & 0 & Y_{pl} & 0 \\ 0 & 0 & 0 & Y_{le} \\ Y_{ea} & 0 & -Y_{el} & 0 \end{pmatrix} \begin{pmatrix} \partial L_a/\partial B_v \\ \partial L_p/\partial B_v \\ \partial L_l/\partial B_v \\ \partial L_e/\partial B_v \end{pmatrix} = \begin{pmatrix} 0 \\ -\partial g_p/\partial B_v \\ -\partial g_l/\partial B_v \\ 0 \end{pmatrix} \tag{9}$$

The solutions to this system are:

$$\partial L_a/\partial B_v = (\partial g_p/\partial B_v) * Yap * Yel * Yle / (-F4) \tag{10}$$
$$\partial L_p/\partial B_v = (\partial g_p/\partial B_v) * Yaa * Yel * Yle / (-F4) \tag{11}$$
$$\partial L_l/\partial B_v = (\partial g_p/\partial B_v) * Yap * Yle * Yea / (-F4) \tag{12}$$
$$\partial L_e/\partial B_v = (\partial g_l/\partial B_v) * Yap * (Ypl * Yea - Yel * Ypa) / (-F4) \tag{13}$$

for adults, pupae, larvae, and eggs, respectively. (See Levins, 1975, pp. 36-38 and Trench, 1978, pp. 437-451 for an expository presentation.) If the system is stable, the fourth level feedback is negative, and solutions (10), (11), and (12) are positive. F4 < 0 implies $Y_{el} * Y_{pa} > Y_{pl} * Y_{ea}$, hence solution (13) is negative. Thus, one would predict that selection for increased larval viability and developmental rate would increase the equilibrium numbers of adults, pupae, and larvae but would decrease the equilibrium number of eggs.

TABLE I

Number of adults for the control, genetically perturbed and demographically perturbed cultures

Week	Control	Genetic perturbations			Demographic perturbations		
		One +/+ female added	Three +/+ females added	Three +/+ pairs added	Adults removed	Immatures removed	100 adults added
0	64.0 ± 0.0*	64.0 ± 0.0	64.0 ± 0.0	64.0 ± 0.0	64.0 ± 0.0	64.0 ± 0.0	64.0 ± 0.0
2	82.2 ± 2.8	77.7 ± 3.0	82.0 ± 0.6	77.7 ± 3.3	77.7 ± 1.5	81.7 ± 3.0	75.7 ± 0.9
4	80.2 ± 1.3	76.3 ± 1.7	81.0 ± 1.5	75.3 ± 1.7	76.3 ± 0.3	80.7 ± 2.4	74.7 ± 1.9
6	80.2 ± 2.7	78.3 ± 1.5	77.7 ± 0.9	75.0 ± 2.1	74.0 ± 2.6	81.3 ± 4.7	78.3 ± 2.9
8	67.7 ± 4.5	72.7 ± 4.4	74.3 ± 2.7	72.7 ± 4.3	66.7 ± 3.5	68.0 ± 2.5	74.3 ± 5.9
10	70.7 ± 8.5	52.0 ± 5.1	66.3 ± 3.8	74.3 ± 6.7	59.7 ± 3.8	88.3 ± 4.1	51.3 ± 8.3
	--------		Perturbation	Perturbation		Perturbation	
10	70.7 ± 8.5	53.0 ± 5.1	69.3 ± 3.8	80.3 ± 6.7	0.0 ± 0.0	88.3 ± 4.1	151.3 ± 8.3
12	87.0 ± 7.6	82.7 ± 9.4	84.7 ±12.4	86.7 ± 6.7	106.3 ±33.1	63.7 ± 4.1	130.3 ± 6.0
14	84.5 ± 7.6	85.3 ± 4.8	108.0 ± 5.9	94.0 ± 3.1	109.7 ±29.6	61.3 ± 4.8	110.3 ± 4.6
16	108.7 ± 4.3	115.7 ±10.8	152.7 ±13.9	127.7 ±13.2	111.3 ±29.2	147.0 ±16.1	104.3 ± 2.2
18	106.0 ± 6.5	116.0 ±13.9	145.7 ±17.8	132.0 ± 9.1	112.3 ±25.0	130.3 ±11.9	94.3 ± 5.9
20	114.7 ± 7.2	119.3 ± 8.7	139.3 ± 9.5	128.7 ± 5.7	110.3 ±18.3	118.0 ± 5.0	116.7 ± 5.8
22	105.5 ± 5.4	121.7 ± 4.7	126.3 ±12.1	119.0 ± 4.0	140.7 ± 6.9	106.7 ± 5.5	106.3 ± 3.8
24	111.5 ± 4.7	137.3 ± 3.4	146.0 ± 7.1	125.7 ± 1.5	129.0 ± 5.7	128.3 ± 7.3	110.7 ±11.0
26	101.0 ± 6.1	125.7 ± 5.4	147.3 ± 5.5	112.3 ± 2.6	137.0 ± 7.5	118.0 ±11.3	100.0 ± 5.7
28	120.7 ± 9.5	144.3 ± 4.9	162.3 ±11.6	144.3 ± 5.6	120.7 ± 7.8	136.7 ± 7.1	106.3 ± 9.2
30	118.0 ± 4.1	135.0 ± 6.1	147.0 ±16.3	138.0 ± 8.7	118.0 ± 4.0	116.7 ± 4.3	106.7 ± 6.4
32	116.5 ± 7.4	144.7 ± 3.4	152.3 ± 9.2	157.3 ±23.2	109.3 ± 4.3	138.0 ± 3.1	108.3 ± 5.4
34	112.2 ± 4.0	141.3 ± 2.8	153.3 ± 8.7	160.0 ±13.5	132.7 ± 4.6	121.7 ± 5.6	118.0 ± 6.8
36	113.7 ± 9.6	146.3 ± 6.4	164.0 ±16.5	162.7 ± 3.8	118.0 ± 8.2	135.0 ± 6.4	135.0 ± 7.1
38	117.2 ± 9.1	135.0 ± 4.0	172.7 ±28.3	150.7 ± 2.3	113.3 ± 8.8	117.0 ± 6.5	142.0 ±11.7
40	115.0 ± 5.7	140.0 ± 7.2	169.3 ±21.4	155.7 ±13.3	101.0 ± 5.0	127.0 ± 9.0	125.3 ± 9.7
42	103.8 ± 2.7	112.3 ± 6.2	152.0 ±20.2	121.7 ± 7.3	111.0 ± 1.5	117.0 ± 6.5	126.0 ± 3.8
44	105.3 ± 7.7	140.3 ±19.0	148.0 ±10.8	144.7 ± 2.7	106.6 ± 8.8	124.6 ± 7.9	111.0 ± 3.8
46	112.5 ± 4.0	147.0 ± 3.0	155.7 ± 6.1	143.0 ± 7.0	122.6 ± 6.6	111.3 ± 0.3	123.3 ± 6.4
48	114.8 ± 9.5	144.0 ± 6.2	161.7 ± 1.2	154.3 ± 1.7	113.3 ±11.8	114.7 ± 6.4	125.0 ± 8.7
50	117.0 ± 4.8	134.7 ± 8.1	171.0 ± 6.7	147.0 ± 4.6	118.6 ± 7.1	120.6 ± 7.7	141.3 ± 6.7
52	117.3 ± 4.6	150.7 ± 7.8	177.7 ± 7.3	164.3 ±18.5	135.0 ± 5.7	134.0 ± 6.4	135.0 ± 6.0

TABLE I (CONT.)

Number of adults for the control, genetically perturbed and demographically perturbed cultures

Week	Control	Genetic perturbations			Demographic perturbations		
		One +/+ female added	Three +/+ females added	Three +/+ pairs added	Adults removed	Immatures removed	100 adults added
54	131.8 ± 5.5	152.7 ± 14.8	184.0 ± 11.9	153.0 ± 15.4	135.0 ± 6.1	124.0 ± 7.1	136.6 ± 6.4
56	123.5 ± 5.0	155.0 ± 8.1	173.0 ± 13.6	165.7 ± 7.7	146.0 ± 8.3	136.6 ± 8.7	126.3 ± 10.4
58	135.3 ± 5.5	148.0 ± 6.7	170.0 ± 2.0	160.0 ± 1.7	140.3 ± 5.5	142.6 ± 6.8	155.3 ± 9.8
60	127.0 ± 8.6	167.0 ± 5.1	167.3 ± 8.7	161.7 ± 2.8	153.6 ± 3.9	144.0 ± 3.8	144.0 ± 8.1
62	125.5 ± 8.8	169.6 ± 2.6	166.0 ± 6.9	154.6 ± 1.2	143.6 ± 2.3	141.6 ± 10.8	149.3 ± 14.1
64	128.2 ± 8.9	172.0 ± 2.5	170.6 ± 10.4	181.6 ± 20.9	143.3 ± 1.5	155.6 ± 6.7	164.6 ± 27.0
66	154.2 ± 12.1	174.6 ± 11.1	178.0 ± 1.5	197.3 ± 13.6	157.0 ± 9.5	170.6 ± 18.8	162.3 ± 18.2
68	154.7 ± 15.6	171.7 ± 10.3	170.0 ± 4.2	177.7 ± 12.5	157.0 ± 1.0	167.0 ± 13.7	165.7 ± 22.5
70	150.2 ± 10.2	167.0 ± 8.7	169.3 ± 9.2	164.7 ± 7.5	145.3 ± 2.9	166.3 ± 4.4	161.0 ± 17.4
72	146.0 ± 7.8	176.7 ± 9.0	186.0 ± 3.0	147.5 ± 8.5	151.7 ± 9.9	148.7 ± 2.4	148.0 ± 14.4
74	148.3 ± 10.2	183.3 ± 15.1	188.5 ± 13.5	146.0 ± 4.0	155.0 ± 11.7	151.3 ± 3.2	146.3 ± 2.7
76	148.8 ± 13.0	190.3 ± 9.0	180.5 ± 14.5	151.5 ± 10.5	174.3 ± 12.4	170.3 ± 15.6	153.0 ± 15.6
78	154.5 ± 13.6	201.6 ± 8.3	181.0 ± 29.0	151.5 ± 2.5	164.0 ± 14.8	178.3 ± 14.8	171.0 ± 14.6
80	149.0 ± 12.8	195.0 ± 13.0	187.5 ± 16.5	169.0 ± 16.0	161.7 ± 3.5	170.6 ± 12.1	158.3 ± 10.7

*Mean ± standard error

Demographic Perturbations

In the first demographic treatment, all adults were removed at week 10. This resulted in the elimination of adult cannibalism on pupae (the term −Ypa in Fig. 1), so that pupae then moved smoothly to adulthood and in merely two weeks the adult cohort was completely replaced (Table I) and equilibrium was restored.

In the second treatment, all immature life stages (eggs, larvae, pupae) were removed. The adult numbers showed no change at week 10, of course, but with no recruits available these average numbers decreased for the next four weeks (Table I). During this interval, however, a new cohort of eggs had been produced and with no larval cannibalism (the term −Yel in Fig. 1) large numbers underwent pupation. At week 16 adult numbers more than doubled. This is a classical "overshoot" response and now with the self-dampening effect of adults and the cannibalism by adults of pupae enhanced (the terms −Yaa and −Ypa in Fig. 1), adult numbers declined for the next six weeks.

The third age structure alteration of adding adults may be viewed as an artificially induced overshoot. The response was similar in this regard to the removal of all immature life stages.

Genetic Perturbations

The interpretation of the effect of introducing the + allele into populations homozygous for the *cos* allele is more complex than the age structure alterations. In addition to demographic equilibrium, we must now consider genetic equilibrium at the *cos* locus. The allele and genotypic frequencies for the genetically disturbed cultures appear in Table II. The data for the *cos* allele at week 10 represents its actual frequency among the adults at the time the + allele was introduced. The data for subsequent sample periods are estimates of the allelic and genotypic frequencies among the pupae. The binomial confidence intervals for these estimates are based on the sample sizes alone, and do not reflect possible errors in classification of the sampled genotypes.

Examination of the estimated allele frequencies show that the homozygous corn oil sensitive populations were genetically *unstable* to the introduction of the wild-type gene. From week 10 to 22, the *cos* allele frequency decreased in each of the treatments and then leveled off for the remainder of the experiment at an overall mean value of 0.42. The populations also seemed to converge to a common genotypic array. At equilibrium, that is from weeks 22 to 34, the pupae were composed of approximately 35% wild-type homozygotes, 43% heterozygotes, and 22% sensitive homozygotes. Due to small sample sizes, we could not detect any significant differences among the treatments.

The mean adult numbers (Table I) indicate that the age-class altered cultures had adult population sizes similar to the control cultures, but that selection for the + allele resulted in adult densities larger than the latter two groups. In order to statistically verify this, we performed a simple univariate analysis of variance to compare the mean levels of adult numbers among the treatments from weeks 22 to 80 (Table III). (Two cultures, which became diseased prior to week 70, were excluded from this analysis.) Orthogonal contrasts of the treatment means indicated that statistically significant differences ($p < 0.05$) existed between the control and the polymorphic cultures and also between the demographic and polymorphic cultures, but no statistical difference was found between the control and demographic cultures. We conclude that these populations were ecologically stable, but not genetically stable.

Natural Selection in a Density-Regulated Population

In this section we formulate a simple model for adult numbers in *Tribolium*, and use the results of Charlesworth (1971) and Hastings (1978) to examine the effect of natural selection on population size.

Based on Lloyd's (1968) equation, our discrete-time model can be expressed as

$$A_{t+1} = A_t [1 + \bar{X}^{(t)} (1 - C)^{A_t} - D] \qquad (14)$$

where A_t is the number of adults at time t, $\bar{X}^{(t)}$ is the average rate at which pupae are produced per parent at time t, C is the probability that a single adult will cannibalize a single pupae during one time interval, and D is the death rate for adults. The term $(1 - C)A_t$ represents the proportion of pupae surviving cannibalism. If we only consider genetic changes at the *cos* locus, we can write

$$\bar{X}^{(t)} = p_t^2 X_1 + 2p_t q_t X_2 + q_t^2 X_3$$

where p_t is the frequency of the sensitive allele at time t ($q_t = 1 - p_t$), and X_1, X_2 and X_3 are the expected number of pupae, with genotypes *cos/cos*, $+/cos$ and $+/+$ respectively, that are produced per parent. The *cos* allele frequency among adults at time t+1 is

$$p_{t+1} = \frac{p_t [1 + (p_t X_1 + q_t X_2) (1 - C)^{A_t} - D]}{[1 + \bar{X}^{(t)} (1 - C)^{A_t} - D]}. \qquad (15)$$

Two points about equations (14) and (15) are important. First, since the X_i are constants, this is *not* a model of density-dependent selection, but it is, rather, a model of selection in a density-regulated population. Second, examination of equation (15) indicates that the generations need not be discrete and do, in fact, overlap. This is easily seen by multiplying the numerator and denominator of the right-hand side of (15) by $2A_t$ to obtain numbers of alleles.

To apply the results of Charlesworth (1971) we let

$$W_i^{(t)} = 1 + f_i (A_t) \qquad (16)$$

where $f_i(A_t) = X_i (1 - C)^{A_t} - D$ for $i = 1, 2, 3$. We note that substitution of (16) into (14) and (15) yields Charlesworth's (1971) equations (1) and (2). Furthermore, since the $f_i(A_t)$ are monotonic decreasing functions of A_t, we can incorporate Charlesworth's results into our analysis.

A stable, non-trivial equilibrium will exist if $D < \bar{X}^* < D \exp(2/D)$ and $X_2 > X_1$, $X_3 > 0$. For the *cos* locus, Moffa and Costantino (1977) have shown the latter to be true. Also, this equilibrium will be a *global* maximum of adult numbers on the open interval $0 < p < 1$ (assuming no other genetic changes). Thus, in the words of Charlesworth (1971), "Selection tends to change gene frequencies in such a way that A* is always increased. If the initial state of the population is such that population size is approximately stationary, this fact suggests that the trajectory of population size with change in p will approximate a monotonically increasing curve". This is what we observed in these *Tribolium* data.

Throughout the course of the experiment, increases in adult numbers were also realized by both the control and demographically altered cultures (Table I). Experimental evidence was obtained which suggests that these changes were due to increases in larval viability. Specifically, random samples of eggs were collected at week 38 from some of the control and polymorphic cultures and from the *cos/cos* base population and placed into creamers on corn oil medium. The mean ($\pm$ standard deviation) survival to the pupal stage was $62 \pm 3.6\%$ for the controls and $72 \pm 3.3\%$ for the polymorphic cultures as compared to $23 \pm 3.2\%$ for the original or base population, which was maintained on medium containing no corn oil. At

TABLE II

Estimated *cos* allele frequencies and the genotypic arrays along with their 95% binomial confidence intervals

Week	1 +/+ ♀ added		3 +/+ ♀'s added		3 +/+ pairs added		Overall mean	
	cos allele frequency	Genotypic array	*cos* allele frequency	Genotypic array	*cos* allele frequency	Genotypic array	*cos* Allele frequency	Genotypic array
		cos/cos / +/cos / +/+		*cos/cos* / +/cos / +/+		*cos/cos* / +/cos / +/+		*cos/cos* / +/cos / +/+
10	0.98	0.98 / 0.00 / 0.02	0.96	0.96 / 0.00 / 0.04	0.92	0.92 / 0.00 / 0.08	0.95	0.95 / 0.00 / 0.05
14	0.66 (0.54,0.76)	0.32 (0.17,0.49) / 0.68 (0.51,0.83) / 0.00 (------)	0.74 (0.64,0.83)	0.49 (0.32,0.65) / 0.51 (0.35,0.68) / 0.00 (------)	0.57 (0.46,0.70)	0.35 (0.21,0.52) / 0.42 (0.27,0.58) / 0.23 (0.11,0.38)	0.65 (0.58,0.72)	0.39 (0.29,0.48) / 0.53 (0.43,0.62) / 0.08 (0.04,0.15)
22	0.52 (0.42,0.65)	0.31 (0.15,0.51) / 0.41 (0.23,0.62) / 0.28 (0.12,0.48)	0.40 (0.29,0.58)	0.19 (0.07,0.36) / 0.43 (0.27,0.61) / 0.38 (0.22,0.56)	0.36 (0.27,0.47)	0.16 (0.07,0.30) / 0.43 (0.28,0.58) / 0.41 (0.26,0.56)	0.41 (0.34,0.52)	0.21 (0.12,0.30) / 0.42 (0.32,0.51) / 0.37 (0.27,0.46)
26	0.44 (0.29,0.59)	0.17 (0.04,0.38) / 0.54 (0.32,0.75) / 0.29 (0.12,0.52)	0.44 (0.32,0.57)	0.20 (0.08,0.37) / 0.50 (0.32,0.68) / 0.30 (0.16,0.49)	0.47 (0.35,0.58)	0.30 (0.16,0.45) / 0.35 (0.22,0.51) / 0.35 (0.22,0.51)	0.45 (0.38,0.56)	0.24 (0.13,0.34) / 0.44 (0.31,0.54) / 0.32 (0.22,0.41)
30	0.50 (0.39,0.61)	0.27 (0.15,0.44) / 0.47 (0.31,0.63) / 0.26 (0.13,0.42)	0.50 (0.33,0.67)	0.33 (0.18,0.50) / 0.35 (0.20,0.52) / 0.32 (0.18,0.50)	0.33 (0.25,0.43)	0.09 (0.03,0.20) / 0.47 (0.33,0.59) / 0.44 (0.31,0.58)	0.42 (0.37,0.47)	0.21 (0.14,0.30) / 0.44 (0.31,0.53) / 0.36 (0.27,0.46)
34	0.50 (0.37,0.63)	0.23 (0.10,0.41) / 0.54 (0.36,0.72) / 0.23 (0.10,0.41)	0.40 (0.25,0.55)	0.13 (0.02,0.33) / 0.54 (0.32,0.75) / 0.33 (0.15,0.56)	0.38 (0.29,0.48)	0.22 (0.12,0.34) / 0.33 (0.22,0.47) / 0.45 (0.32,0.59)	0.42 (0.35,0.49)	0.20 (0.13,0.29) / 0.44 (0.34,0.54) / 0.36 (0.27,0.46)

TABLE III

Analysis of variance of the mean levels of adult numbers for weeks 22 to 80.

Source of variation	Degrees of freedom	Mean square
TREATMENTS	6	14,982*
Control vs. Demographic	1	8,618
Control vs. Genetic	1	71,430*
Demographic vs. Genetic	1	49,180*
Residual	13	1,965

*Significant at the 0.05 level.

week 80 a similar measurement was made using large sample sizes from all the cultures. The mean survival to pupation was $72 \pm 1.4\%$ for the controls, $71 \pm 1.0\%$ for the demographically altered cultures, $80 \pm 1.0\%$ for the polymorphic cultures and $19 \pm 1.4\%$ for the base *cos/cos* population. We suggest that these observed increases in larval viability are the result of selection for gene loci which decrease the sensitivity to corn oil. In cultures in which the + allele was introduced, the rapidly altered genotypic array at the *cos* locus (Table II) was the major genetic basis for this group's increased adult density. In the other cultures, the slower rate of increase in adult numbers may have been due to selection at modifier loci. The details of the genetic basis of these changes are unknown, but our data suggest that these changes are leading to a maximization of fitness, i.e. $\bar{X}$ increasing. Therefore, we can consider the general results of Hastings (1978).

Consider the differential equation model

$$dA/dt = A(t) G(a, A(t)) \tag{17}$$

where a is a set of parameters under evolutionary control. For Lloyd's (1968) model, $a = (X, C, D)$ and $G(a, A(t)) = X(1 - C)^{A(t)} - D$. The set of parameters a are an evolutionarily stable strategy (ESS) if they lead to an equilibrium population that is stable to small perturbations in the parameters of a (Hastings, 1978).

With our data set, it is reasonable to let the parameters C and D remain constant and allow X to increase slowly under selection. Also, we assume that X is constrained to a value $X \leqslant X_{max}$. Thus X is the only parameter under evolutionary control, and the genetic system leads to a maximization of fitness.

At equilibrium, $G(a^*, A^*) = 0$ and for all biologically meaningful equilibria $(A^* > 0)$ $X > D$ and $(\partial G / \partial A) \mid_{a^*, A^*} < 0$. The equilibria are, therefore, stable to perturbations in population number alone and are said to be ecologically stable. Since these equilibria are stable, the maxima of population size and ESS's are identical (Hastings, 1978). Thus, if $X < X_{max}$, then selection is expected to move the ecologically stable system toward an ESS resulting in a maximization of adult numbers. Again, this agrees with our experimental observations (Table I).

Discussion

Based on the experimental data, two general points concerning population stability analysis can be made: First, an analysis of ecological stability of equilibrium populations without a consideration of genetic stability would have yielded paradoxical results. In one treatment, after adding 100 adults, there was a return to equilibrium; however, in another treatment, the introduction of just one adult female led to an irreversible trend away from this equilibrium. The paradox is resolved by noting that the equilibrium was *ecologically stable* but was not *genetically* (or *evolutionarily*) *stable*. Also, an ecological viewpoint that evolutionary effects are unimportant over an ecological time scale would be misleading. Both ecological and

genetical stability are required for an equilibrium to be truly stable and, in this case, the genetic and demographic processes appear to be tightly coupled. Although this example was experimentally contrived, a similar situation could occur under more natural circumstances, either by migration or mutation.

Secondly, population stability is not an all-or-nothing characteristic. The degree of stability can be measured by the rate of return to equilibrium. For a linearized system, this is determined mathematically by the real part of the maximum eigenvalue of the community matrix. Thus, for perturbation of a single variable, the rate to return to equilibrium is a function of the structure of the system and is not dependent upon the size of the perturbation. But for the empiricist, this may not be the case, even for variables with constant equilibrium values. In the present study, when adults were added, the rate of return to equilibrium was much slower than when adults were removed. This suggests that experimentalists should exercise caution and consider both the direction and the magnitude of perturbations when using this approach to evaluate population stability.

As demonstrated with *T. castaneum*, qualitative stability analysis can provide information about complex systems which is not intuitively obvious. It may also prove useful in evaluating and comparing different density-regulating behavioral strategies in related species. For example, in another *Tribolium* species, *T. brevicornis*, an inhibition interaction has been identified between the adults and larvae (Jillson and Costantino 1980). This adult inhibition of larval metamorphosis introduces the term $-Yla$ (Fig. 1), which adds an additional negative term ($-Yap * Ypl * Yla$) to level F3, but does not alter the ambiguity at level F4. However, the biological effect of the adult-larval interaction is to delay pupation and thereby reduce the magnitude of transition from larva to pupa, and increase the level of larval cannibalism on eggs. The reduction of Ypl, and increase in Yel enhances the likelihood that the inequality $Yel * Ypa > Yea * Ypl$ is satisfied. Furthermore, it may allow the cannibalistic effect of adults on pupae, Ypa, to be reduced and still keep F4 negative. However, the term -Yla also introduces two additional ambiguities, based on the Routh-Hurwitz criteria, which were not found for *T. castaneum*. An experimental stability analysis of *T. brevicornis* is underway.

The model described by equations (14) through (17) is unquestionably a biological simplification. Yet, by focusing upon the adult age class, we have been able to incorporate the results of Charlesworth (1971) and Hastings (1978). For genetic changes at the *cos* locus, Charlesworth's results predicted a global maximum of adult numbers at equilibrium. For frequencies of the sensitive allele above the observed p^* of 0.42, our results support this prediction. Unfortunately, none of our cultures were started with an initial *cos* allele frequency less than 0.42; with our results we are neither able to confirm nor deny that a global maximum exists at p^*.

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