NATURAL SELECTION WITH NUCLEAR AND CYTOPLASMIC TRANSMISSION. I. A DETERMINISTIC MODEL

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ABSTRACT

A deterministic model allowing variation at a nuclear genetic locus in a population segregating two cytoplasmic types is formulated. Additive, multiplicative and symmetric viability matrices are analyzed for existence and stability of equilibria. The protectedness of polymorphisms in both nuclear genes and cytoplasmic types is also investigated in the general model. In no case is a complete polymorphism protected with this deterministic model. Results are discussed in light of the extensive variation in mtDNA that has recently been reported.

A complete understanding of the evolution of cytoplasmic variation requires not only its description and quantification but also information about the phenotypic expression of the variation. The notion that cytoplasmic variation may be relevant to adaptive evolution is supported by evidence from the plant kingdom, in which cytoplasmic male sterility (EDWARDSON 1970) and leaf variegation (KIRK and TILNEY-BASSETT 1967) are well documented. Even in the absence of obvious morphological effects, the nature of mtDNA sequence variation suggests that cytoplasmic variation can confer differences in phenotypic fitness.

Stable transmission of traits through the cytoplasm is important to their evolutionary dynamics. In many cases, the mode of transmission can be ascribed to particular cytoplasmic organelles. Petite mutants of yeast are known to be due to defective mitochondria, lacking cytochromes $a + a_3$ and b (EPHRUSSI 1953). Poky mutants of Neurospora are also clearly mitochondrial mutants (MITCHELL and MITCHELL 1952; LAMBOWITZ, CHUA and LUCK 1976). A number of drugs, including chloramphenicol (CAP) and erythromycin, specifically inhibit protein synthesis in mitochondria by affecting mitochondrial ribosomes but do not inhibit cytoplasmic protein synthesis. Yeast mutants resistant to these drugs often show non-Mendelian inheritance (LINNANE *et al.* 1968), and proof that these are mtDNA mutants was most convincingly demonstrated by mapping the genes that confer resistance to different drugs on the mitochondrial genome (MOLLOY, LINNANE and LUKINS 1975). Drug-resistant mitochondrial mutants have also been isolated in Aspergillus (ROWLANDS

and TURNER 1975), Podospora (BELCOUR and BEGEL 1977) and Paramecium (BEALE, KNOWLES and TAIT 1972). In human HeLa cells, CAP resistance was shown to be determined by the cytoplasm by fusing enucleated CAP-resistant cells with nuclei of CAP-sensitive cells and observing that these "cybrids" were CAP resistant (SPOLSKY and EISENSTADT 1972). Subsequently, WALLACE (1981) proved that these were mitochondrial mutants, with sequences differing in the large rRNA gene (WALLACE *et al.* 1982).

The polymorphic nature of mitochondria had been shown in a number of organisms by agarose gel electrophoresis of restriction endonuclease-digested mtDNA. These include Drosophila (SHAH and LANGLEY 1979; POWELL 1983), sheep and goats (UPHOLT and DAWID 1977), Peromyscus (AVISE, LANSMAN and SHADE 1979), Mus (FERRIS *et al.* 1983a,b; LANSMAN *et al.* 1981), pocket gopher (AVISE *et al.* 1979), rat (BROWN and SIMPSON 1982), humans (BROWN 1980; AQUADRO and GREENBERG 1983; BLANC *et al.* 1983; GREENBERG, NEWBOLD and SUGINO 1983; DENARO *et al.* 1981; CANN, BROWN and WILSON 1982; CANN and WILSON 1983) and other primates (FERRIS, WILSON and BROWN 1981; BROWN *et al.* 1982). Although these data do not suggest any adaptive role for mitochondrial variation, they have been useful in elucidating mtDNA transmission and in constructing evolutionary phylogenies. Introgression of mtDNA has been inferred in both Drosophila (POWELL 1983) and mice (FERRIS *et al.* 1983), demonstrating that mitochondria can have unexpected evolutionary dynamics. The data suggest strict maternal inheritance of mitochondria in higher eukaryotes, even after many generations of substitution backcrossing (LANSMAN, AVISE and HUETTEL 1983).

Mitochondrial DNA sequence analysis in man, mouse and rat (MIYATA *et al.* 1982; BROWN, GEORGE and WILSON 1979; and AVISE *et al.* 1979) demonstrates that silent substitutions (those not changing amino acid sequence in translated genes) occur at six to ten times the rate of silent substitutions in nuclear genes. On the other hand, the rates of substitutions causing amino acid sequence changes are similar in mitochondrial and nuclear genes (BROWN *et al.* 1979). HAUSWIRTH and LAIPIS (1982) directly observed divergence among 15 Holstein cows within a single maternal lineage spanning 13 generations. The bulk of the variation in Drosophila mtDNA sequence occurs in the A-T rich region and is apparently not transcribed (WALSTENHOLME, FAURAN and GODDARD 1980). Sequence variation in the human D-loop region indicates a number of significant biases (AQUADRO and GREENBERG 1983). The crucial point is that mtDNA sequence variation is not completely random, and the nature of the nonrandomness may suggest evolutionary constraints. Despite this, the degree to which phenotypic variation in a natural population of higher eukaryotes is mediated by variation in mitochondria is not known.

It is clear at the biochemical level that nuclear and mitochondrial genes must retain tight integration in their expression. The mitochondrial proteins ATPase, cytochrome oxidase and cytochrome *b* have subunits encoded by both nuclear and mitochondrial genes (BEALE and KNOWLES 1978). Nuclear-cytoplasmic interactions are also manifest at the phenotypic level, and systems of cytoplasmic male sterility are particularly well studied.

In this paper a population genetic model allowing nuclear-cytoplasmic interaction in viability is studied with the intention of understanding the nature of cytoplasmic variation.

THEORY

Consider an infinite randomly mating population segregating at one nuclear genetic locus with two alleles, A and a. Let there be two cytoplasmic types, m and n, and let them be strictly maternally inherited. The frequencies of the six cytogenotypes can be written:

	Cytoplasm	
Genotype	m	n
AA	x_{11}	x_{12}
Aa	x_{21}	x_{22}
aa	x_{31}	x_{32}

so that x_{ij} is the frequency of the ith genotype in the jth cytoplasm, and $\Sigma_i \Sigma_j x_{ij} = 1$. Define:

$$\begin{aligned} p_m &= x_{11} + \tfrac{1}{2}x_{21} & q_m &= x_{31} + \tfrac{1}{2}x_{21} \\ p_n &= x_{12} + \tfrac{1}{2}x_{22} & q_n &= x_{32} + \tfrac{1}{2}x_{22} \\ p &= p_m + p_n & q &= q_m + q_n = 1 - p \end{aligned} \tag{1}$$

$$m = x_{11} + x_{21} + x_{31}$$

$$n = x_{12} + x_{22} + x_{32}$$

where p is the frequency of A, q is the frequency of a and m and n are the frequencies of the respective cytoplasmic types. Consider the case with no selection. Adults mate randomly with respect to cytogenotype, so the probability of each mating type is the product of the frequencies of the cytogenotypes involved. The expected fraction of progeny occurring in the six cytogenotypic classes is easily gotten from Mendelian segregation and maternal transmission of cytoplasm. From a table of mating types and resultant progeny, we get the recurrence relations:

$$x'_{11} = x_{11}^2 + \tfrac{1}{2}x_{11}x_{21} + x_{11}x_{12} + \tfrac{1}{2}x_{11}x_{22} + \tfrac{1}{2}x_{21}x_{11} + \tfrac{1}{4}x_{21}^2 + \tfrac{1}{2}x_{21}x_{12} + \tfrac{1}{4}x_{21}x_{22}$$

$$x'_{21} = \tfrac{1}{2}x_{11}x_{21} + x_{11}x_{31} + \tfrac{1}{2}x_{21}x_{11} + \tfrac{1}{2}x_{21}^2 + \tfrac{1}{2}x_{21}x_{31} + x_{31}x_{11} + \tfrac{1}{2}x_{31}x_{21} + \tfrac{1}{2}x_{11}x_{22} + x_{11}x_{32} + \tfrac{1}{2}x_{21}x_{12} + \tfrac{1}{2}x_{21}x_{22} + \tfrac{1}{2}x_{21}x_{32} + x_{31}x_{12} + \tfrac{1}{2}x_{31}x_{22}$$

$$x'_{31} = \tfrac{1}{4}x_{21}^2 + \tfrac{1}{4}x_{21}x_{22} + \tfrac{1}{2}x_{21}x_{31} + \tfrac{1}{2}x_{21}x_{32} + \tfrac{1}{2}x_{31}x_{21} + \tfrac{1}{2}x_{31}x_{22} + x_{31}x_{32} + x_{31}^2$$

$$
\begin{aligned}
x'_{12} &= x_{11}x_{12} + \tfrac{1}{2}x_{11}x_{21} + x_{12}^2 + \tfrac{1}{2}x_{12}x_{22} + \tfrac{1}{2}x_{22}x_{11} + \tfrac{1}{4}x_{22}x_{21} \\
&\quad + \tfrac{1}{2}x_{22}x_{12} + \tfrac{1}{4}x_{22}^2 \\
x'_{22} &= \tfrac{1}{2}x_{12}x_{21} + x_{12}x_{31} + \tfrac{1}{2}x_{12}x_{22} + x_{12}x_{32} + \tfrac{1}{2}x_{22}x_{11} + \tfrac{1}{2}x_{22}x_{21} \\
&\quad + \tfrac{1}{2}x_{22}x_{31} + \tfrac{1}{2}x_{22}x_{12} + \tfrac{1}{2}x_{22}^2 + \tfrac{1}{2}x_{22}x_{32} + x_{32}x_{11} + \tfrac{1}{2}x_{32}x_{12} \\
&\quad + x_{32}x_{21} + \tfrac{1}{2}x_{32}x_{22} \\
x'_{32} &= \tfrac{1}{4}x_{22}x_{21} + \tfrac{1}{2}x_{22}x_{31} + \tfrac{1}{2}x_{22}x_{32} + \tfrac{1}{2}x_{32}x_{21} + x_{32}x_{31} + \tfrac{1}{2}x_{32}x_{22} \\
&\quad + x_{32}^2 + \tfrac{1}{4}x_{22}^2
\end{aligned}
\tag{2}
$$

After algebraic rearrangement and collecting terms, this simplifies to:

$$
\begin{aligned}
x'_{11} &= pp_m \\
x'_{21} &= 2pq - p_mq_n - p_nq_m - 2p_nq_n \\
x'_{31} &= qq_m \\
x'_{12} &= pp_n \\
x'_{22} &= 2pq - p_mq_n - p_nq_m - 2p_mq_m \\
x'_{32} &= qq_n
\end{aligned}
\tag{3}
$$

First observe what happens to the allelic frequency:

$$p' = x'_{11} + x'_{12} + \tfrac{1}{2}x'_{21} + \tfrac{1}{2}x'_{22} = p \tag{4}$$

So the frequencies of the alleles of the nuclear locus do not change in the absence of selection. Now consider what happens to cytoplasmic frequencies:

$$m' = x'_{11} + x'_{21} + x'_{31} = m \tag{5}$$

So cytoplasmic frequencies do not change either.

The recurrence system given in equation 2 is suitable for extensions of the model in which cytogenotypes are acted upon by selection is such a way that cannot be reduced in dimension. In other cases, a more compact formulation is desirable. By analogy, in two-locus theory the recurrences can be expressed in terms of ten genotypic frequencies or four gametic frequencies or two allelic frequencies and a coefficient of linkage disequilibrium.

Define the egg frequencies as follows:

	Cytoplasm	
Allele	m	n
A	e_1	e_2
a	e_3	e_4

where $e_1 + e_2 + e_3 + e_4 = 1$. Barring sex-specific viability differences or segregation distortion, sperm frequencies will be the same as respective egg frequencies. The recurrence equations can be written:

$$\begin{aligned} e_1' &= e_1^2 + e_1e_2 + e_1e_3 + \tfrac{1}{2}e_3e_2 + \tfrac{1}{2}e_1e_4 \\ e_2' &= e_2^2 + e_2e_1 + \tfrac{1}{2}e_2e_3 + e_2e_4 + \tfrac{1}{2}e_4e_1 \\ e_3' &= e_3^2 + e_1e_3 + \tfrac{1}{2}e_2e_3 + e_3e_4 + \tfrac{1}{2}e_1e_4 \\ e_4' &= e_4^2 + \tfrac{1}{2}e_1e_4 + e_2e_4 + e_3e_4 + \tfrac{1}{2}e_2e_3 \end{aligned} \tag{6}$$

Defining an association parameter $A = e_1e_4 - e_2e_3$ this can be written,

$$\begin{aligned} e_1' &= e_1 - \tfrac{1}{2}A \\ e_2' &= e_2 + \tfrac{1}{2}A \\ e_3' &= e_3 + \tfrac{1}{2}A \\ e_4' &= e_4 - \tfrac{1}{2}A. \end{aligned} \tag{7}$$

Now the parallel to two-locus theory is readily apparent, where the association parameter A is identical in formula to the linkage disequilibrium parameter D, and there is free recombination ($r = 0.5$ in the two-locus model). From this parallel it is clear that allelic and cytoplasmic frequencies do not change, and egg frequencies change such that the magnitude of the association parameter, A, halves each generation.

At equilibrium we have $\hat{A} = 0$ and,

$$\hat{e}_1 = pm \qquad \hat{e}_2 = pn \qquad \hat{e}_3 = qm \qquad \hat{e}_4 = qn. \tag{8}$$

The equilibrium cytogenotypic frequencies are the products of respective allelic and cytoplasmic frequencies, analogous to ROBBINS' (1918) proportions:

$$\begin{aligned} \hat{x}_{11} &= p^2m \qquad & \hat{x}_{12} &= p^2n \\ \hat{x}_{21} &= 2pqm \qquad & \hat{x}_{22} &= 2pqn \\ \hat{x}_{31} &= q^2m \qquad & \hat{x}_{32} &= q^2n. \end{aligned} \tag{9}$$

The general viability model allows each of the six cytogenotypes to have a different viabilities:

	Cytoplasmic type	
Genotype	*m*	*n*
AA	v_1	v_4
Aa	v_2	v_5
aa	v_3	v_6

This can be put into the format of the usual viability matrix **W** with elements $w_{ij}(i, j = 1, 2, 3, 4)$ as follows:

	Sperm (pollen)			
Egg	*Am*	*An*	*am*	*an*
Am	v_1	v_1	v_2	v_2
An	v_4	v_4	v_5	v_5
am	v_2	v_2	v_3	v_3
an	v_5	v_5	v_6	v_6

The marginal fitness of the egg type i is:

$$w_i = \sum_j e_j w_{ij} \tag{10}$$

and the marginal fitness of the jth type sperm is:

$$w_j = \sum_i e_i w_{ij} \tag{11}$$

Notice that the model departs from two-locus theory in that **W** is not symmetric, and the marginal fitnesses of the male and female gametes are different. Define the mean fitness $\bar{w}$ as the sum of the products of gametic frequencies weighted by the corresponding fitness:

$$\bar{w} = \sum_i \sum_j e_i e_j w_{ij} \tag{12}$$

The derivation of the recurrence equations in egg frequency will be given in some detail, because the resulting lack of influence of sperm/pollen frequency is somewhat nonintuitive.

$$\begin{aligned}\bar{w}e_1' &= w_{11}e_1^2 + w_{12}e_1e_2 + \tfrac{1}{2}w_{13}e_1e_3 + \tfrac{1}{2}w_{14}e_1e_4 + \tfrac{1}{2}w_{31}e_3e_1 + \tfrac{1}{2}w_{32}e_3e_2 \\ &= e_1w_1 - \tfrac{1}{2}(w_{14}e_1e_4 - w_{32}e_3e_2) \\ &= e_1w_1 - \tfrac{1}{2}w_{14}A.\end{aligned}$$

Similarly, (13)

$$\begin{aligned}\bar{w}e_2' &= e_2w_2 + \tfrac{1}{2}w_{41}A \\ \bar{w}e_3' &= e_3w_3 + \tfrac{1}{2}w_{14}A \\ \bar{w}e_4' &= e_4w_4 - \tfrac{1}{2}w_{41}A.\end{aligned}$$

This can be somewhat more succinctly written as:

$$\begin{aligned}\bar{w}e_i' &= e_iw_i + \epsilon_iA \\ \epsilon_i &= -\tfrac{1}{2}w_{14},\ \tfrac{1}{2}w_{41},\ \tfrac{1}{2}w_{14},\ -\tfrac{1}{2}w_{41} \quad \text{for} \quad i = 1, 2, 3, 4.\end{aligned} \tag{14}$$

When $w_{14} = w_{41}$, the heterozygote viabilities are the same in the two cytoplasms, and the recurrence equations are identical with the two-locus model with $r = \frac{1}{2}$ (with restrictions on the viability matrix).

A convenient way to express these equations is to use the allelic frequencies:

$$p = e_1 + e_2 \quad \text{and} \quad q = e_3 + e_4$$

$$\begin{aligned}
\bar{w}e_1' &= e_1(pv_1 + qv_2) - \tfrac{1}{2}v_2A \\
\bar{w}e_2' &= e_2(pv_4 + qv_5) + \tfrac{1}{2}v_5A \\
\bar{w}e_3' &= e_3(pv_2 + qv_3) + \tfrac{1}{2}v_2A \\
\bar{w}e_4' &= e_4(pv_5 + qv_6) - \tfrac{1}{2}v_5A
\end{aligned} \tag{15}$$

Additive viability model

Let the viabilities be:

Genotype	m	n
AA	$\alpha_1 + \beta_1$	$\alpha_1 + \beta_2$
Aa	$\alpha_2 + \beta_1$	$\alpha_2 + \beta_2$
aa	$\alpha_3 + \beta_1$	$\alpha_3 + \beta_2$

so that genotypic and cytoplasmic fitness effects are additive. Without loss of generality, let $\beta_1 = 0$. From (15) we obtain:

$$\begin{aligned}
\bar{w}e_1' &= e_1(p\alpha_1 + q\alpha_2) - \tfrac{1}{2}\alpha_2A \\
\bar{w}e_2' &= e_2(p\alpha_1 + q\alpha_2 + \beta_2) + \tfrac{1}{2}(\alpha_2 + \beta_2)A \\
\bar{w}e_3' &= e_3(p\alpha_2 + q\alpha_3) + \tfrac{1}{2}\alpha_2A \\
\bar{w}e_4' &= e_4(p\alpha_2 + q\alpha_3 + \beta_2) - \tfrac{1}{2}(\alpha_2 + \beta_2)A
\end{aligned} \tag{16}$$

It is immediately clear that there is no internal equilibrium with $\hat{A} = 0$, since this would require $w_1 = w_2 = w_3 = w_4$. In particular $w_1 = p\alpha_1 + q\alpha_2$ and $w_2 = p\alpha_1 + q\alpha_2 + \beta_2$, so if $w_1 = w_2$ then β_2 must be zero, implying neutrality of cytoplasmic types.

Observe that the mean fitness is independent of A:

$$\begin{aligned}
\bar{w} &= e_1(p\alpha_1 + q\alpha_2) + e_2(p\alpha_1 + q\alpha_2 + \beta_2) + e_3(p\alpha_2 + q\alpha_3) \\
&\quad + e_4(p\alpha_2 + q\alpha_3 + \beta_2) \\
&= p(p\alpha_1 + q\alpha_2) + q(p\alpha_2 + q\alpha_3) + n\beta_2 \\
&= p^2(\alpha_1 - 2\alpha_2 + \alpha_3) + 2p(\alpha_2 - \alpha_3) + \alpha_3 + n\beta_2
\end{aligned} \tag{17}$$

To determine the change in mean fitness, substitute (14) into (12) to obtain:

$$\bar{w}' = \bar{w}^{-2} \sum_i \sum_j w_{ij}(e_iw_i + \epsilon_iA)(e_jw_j + \epsilon_jA) \tag{18}$$

Note that w_j here refers to the marginal egg fitness, so $w_i = w_j$ if $i = j$. Expanding this expression we get,

$$\bar{w}' = \bar{w}^{-2} \sum_i \sum_j w_{ij}e_iw_ie_jw_j + 2\bar{w}^{-2} \sum_i \sum_j w_{ij}e_iw_i\epsilon_jA + \bar{w}^{-2} \sum_i \sum_j w_{ij}\epsilon_i\epsilon_jA^2. \tag{19}$$

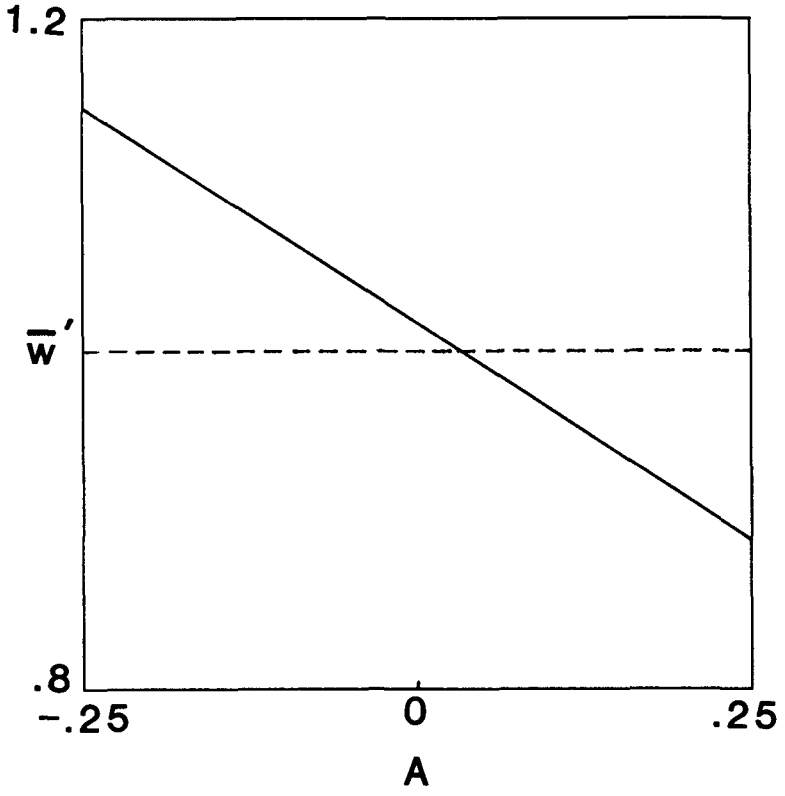

FIGURE 1.—The relationship between $\bar{w}'$ and A is plotted with a solid line using the additive viability matrix when $\alpha_1 = 1$, $\alpha_2 = 0.5$, $\alpha_3 = 0.2$, $\beta_1 = 0$ and $\beta_2 = 0.5$ and $p = m = 0.5$. The dashed line represents the four-allele case and is referred to as $\bar{w}'_{\text{classical}}$ in the text. $\bar{w}$ in this case is 0.8 for all values of A.

Observe that the first term is the same as the mean fitness in the subsequent generation in the classical four-allele viability model. Denoting this term by $\bar{w}'_{\text{classical}}$, a considerable amount of algebra yields:

$$\bar{w}' = \bar{w}'_{\text{classical}} + A\beta_2 w^{-2}\{p^2(\alpha_1^2 - 2\alpha_1\alpha_2 + 2\alpha_2\alpha_3 - \alpha_3^2) + p(2\alpha_3^2 - 3\alpha_2\alpha_3 + \alpha_1\alpha_2) + \alpha_2\alpha_3 - \alpha_3^2 + \beta_2[pn(\alpha_1 - \alpha_2) + qn(\alpha_2 - \alpha_3) - A(\alpha_1 - 2\alpha_2 + \alpha_3)]\} + \tfrac{1}{4}A^2\beta_2^2\bar{w}^{-2}(\alpha_1 - 2\alpha_2 + \alpha_3). \quad (20)$$

When $A = 0$ the mean fitness will not decrease for at least one generation because $\bar{w}' = \bar{w}'_{\text{classical}}$, and as KINGMAN (1961) showed, $\bar{w}'_{\text{classical}}$ is nondecreasing. This is true for the general nuclear-cytoplasmic viability model and is analogous to the two-locus viability model where $D = 0$.

$\bar{w}'$ can be examined by considering the effect of varying A when allelic and cytoplasmic frequencies are held constant. Equation (20) is then a quadratic function relating A and $\bar{w}'$ (Figure 1).

Either of the two rightmost terms of (20) can be either positive or negative, so it is possible to have $\bar{w}' < \bar{w}'_{\text{classical}}$. In particular, the function depicted in Figure 1 is defined for $A_{\min} < A < A_{\max}$ where

$$A_{\min} = \bar{}\min(pm, qn), \quad \text{and}$$
$$A_{\max} = \min(pn, qm). \tag{21}$$

If $\bar{w}'|_{A = A_{\min}} \leq \bar{w}'_{\text{classical}}$ then $\bar{w}' \leq \bar{w}'_{\text{classical}}$ for $A_{\min} < A < 0$ and $\bar{w}' \geq \bar{w}'_{\text{classical}}$ for $0 < A < A_{\max}$, and conversely. $\bar{w}'$ is minimized at either $A_{\min}$, $A = 0$ or $A_{\max}$, depending on the viability matrix. If, for all p and m, $\bar{w}'$ is minimized at $A_{\min}$, then by showing

$$\bar{w}'|_{A = A_{\min}} \geq \bar{w} \tag{22}$$

we will demonstrate that $\bar{w}'$ is nondecreasing. First observe that the equation (20) graphed in Figure 1 is nearly linear, so that $\bar{w}' \approx \bar{w}'_{\text{classical}} - A \cdot S$. Collecting terms in A and solving the derivative,

$$\frac{\partial \bar{w}'}{\partial A} = A\left[-\frac{3}{2}\beta_2^2\bar{w}^{-2}(\alpha_1 - 2\alpha_2 + \alpha_3)\right] + \beta_2\bar{w}^{-2}\{p^2(\alpha_1^2 - 2\alpha_1\alpha_2 + 2\alpha_2\alpha_3 - \alpha_3^2) + p(2\alpha_3^2 - 3\alpha_2\alpha_3 + \alpha_1\alpha_2) + \alpha_2\alpha_3 - \alpha_3^2 + \beta[pn(\alpha_1 - \alpha_2) + n(\alpha_2 - \alpha_3)]\} \tag{23}$$

so S can be approximated by the second term. By substituting $A_{\min} = pm$ we obtain,

$$\bar{w}' - A_{\min}S > \bar{w}; \tag{24}$$

so provided that the linear approximation of (20) is adequate, this demonstrates that $\bar{w}$ is nondecreasing in the additive model. Numerical simulations have shown the approximation $\bar{w}' \approx \bar{w}'_{\text{classical}} - A \cdot S$ to be very good, and direct simulations (see following data) fail to find a case in which $\bar{w}$ decreases.

Accepting that $\bar{w}$ is a Lyapunov function, equilibrium properties of the recurrence system can be determined directly. Equilibrium allelic frequencies are obtained from

$$\frac{\partial \bar{w}}{\partial p} = 2p(\alpha_1 - 2\alpha_2 + \alpha_3) + 2(\alpha_2 - \alpha_3) = 0, \quad \text{so}$$
$$\hat{p} = \frac{\alpha_3 - \alpha_2}{\alpha_1 - 2\alpha_2 + \alpha_3} \tag{25}$$

A necessary and sufficient condition for a globally stable nuclear polymorphism is $\alpha_2 > \alpha_1, \alpha_3$. If $\alpha_1 < \alpha_2 < \alpha_3$, then allele a will fix, whereas if $\alpha_1 > \alpha_2 > \alpha_3$, allele A will fix. If $\alpha_2 < \alpha_1, \alpha_3$, then either allele a or allele A will fix depending on initial conditions. The fate of the cytoplasmic variation can also be obtained from $\bar{w}$:

$$\frac{\partial \bar{w}}{\partial n} = \beta_2 \tag{26}$$

If $\beta_2 > 0$, then cytoplasm n increases in frequency until all individuals possess this cytoplasm, whereas if $\beta_2 < 0$, the m cytoplasm increases to fixation. The additive model cannot maintain a cytoplasmic polymorphism.

The $\bar{w}$ surface has interesting geometric properties. Equation (26) implies that, even though $\bar{w}$ is a quadratic surface, it is composed of straight lines parallel to the m axis (Figure 2). This is also true in the general viability model. A consequence of this is that it is topologically impossible to have a closed loop in a contour map of the surface, and hence there is never an internal local maximum in $\bar{w}$.

Multiplicative viability model

Let the viabilities be

Genotype	m	n
AA	$\alpha_1\beta_1$	$\alpha_1\beta_2$
Aa	$\alpha_2\beta_1$	$\alpha_2\beta_2$
aa	$\alpha_3\beta_1$	$\alpha_3\beta_2$

The recurrence equations in this case are,

$$\begin{aligned}
\bar{w}e_1' &= e_1(p\alpha_1\beta_1 + q\alpha_2\beta_1) - \tfrac{1}{2}\alpha_2\beta_1 A \\
\bar{w}e_2' &= e_2(p\alpha_1\beta_2 + q\alpha_2\beta_2) + \tfrac{1}{2}\alpha_2\beta_2 A \\
\bar{w}e_3' &= e_3(p\alpha_2\beta_1 + q\alpha_3\beta_1) + \tfrac{1}{2}\alpha_2\beta_1 A \\
\bar{w}e_4' &= e_4(p\alpha_2\beta_2 + q\alpha_3\beta_2) - \tfrac{1}{2}\alpha_2\beta_2 A
\end{aligned} \tag{27}$$

where $\bar{w} = (e_1\beta_1 + e_2\beta_2)(p\alpha_1 + q\alpha_2) + (e_3\beta_1 + e_4\beta_2)(p\alpha_2 + q\alpha_3)$. This model contrasts sharply with the behavior of the two-locus multiplicative viability model. By analysis similar to the additive case, it can be shown that $\bar{w}$ is nondecreasing, unlike the two-locus model. A consequence of $\bar{w}$ being a Lyapunov function is that a cytoplasmic polymorphism cannot be maintained, and the nuclear polymorphism then behaves as in the classical one-locus model.

The multiplicative nuclear-cytoplasmic and two-locus models are similar in the domains of the association parameter and D. Observe that

$$\begin{aligned}
A' &= e_1'e_4' - e_2'e_3' \\
&= \frac{e_1e_4(p\alpha_1\beta_1 + q\alpha_2\beta_1)(p\alpha_2\beta_2 + q\alpha_3\beta_2)}{\bar{w}^2} \\
&\quad - \frac{e_2e_3(p\alpha_1\beta_2 + q\alpha_2\beta_2)(p\alpha_2\beta_1 + q\alpha_3\beta_1)}{\bar{w}^2}
\end{aligned} \tag{28}$$

If $A = 0$, then $A' = 0$. Furthermore, if $A < 0$ for some starting condition, then A will remain less than zero. As KARLIN (1975) states, R_+ and R_- are invariant domains of D in the two-locus viability model. Here, R_+ and R_- are invariant domains of A.

Symmetric viability model

One type of symmetric model can be specified by the following viability matrix:

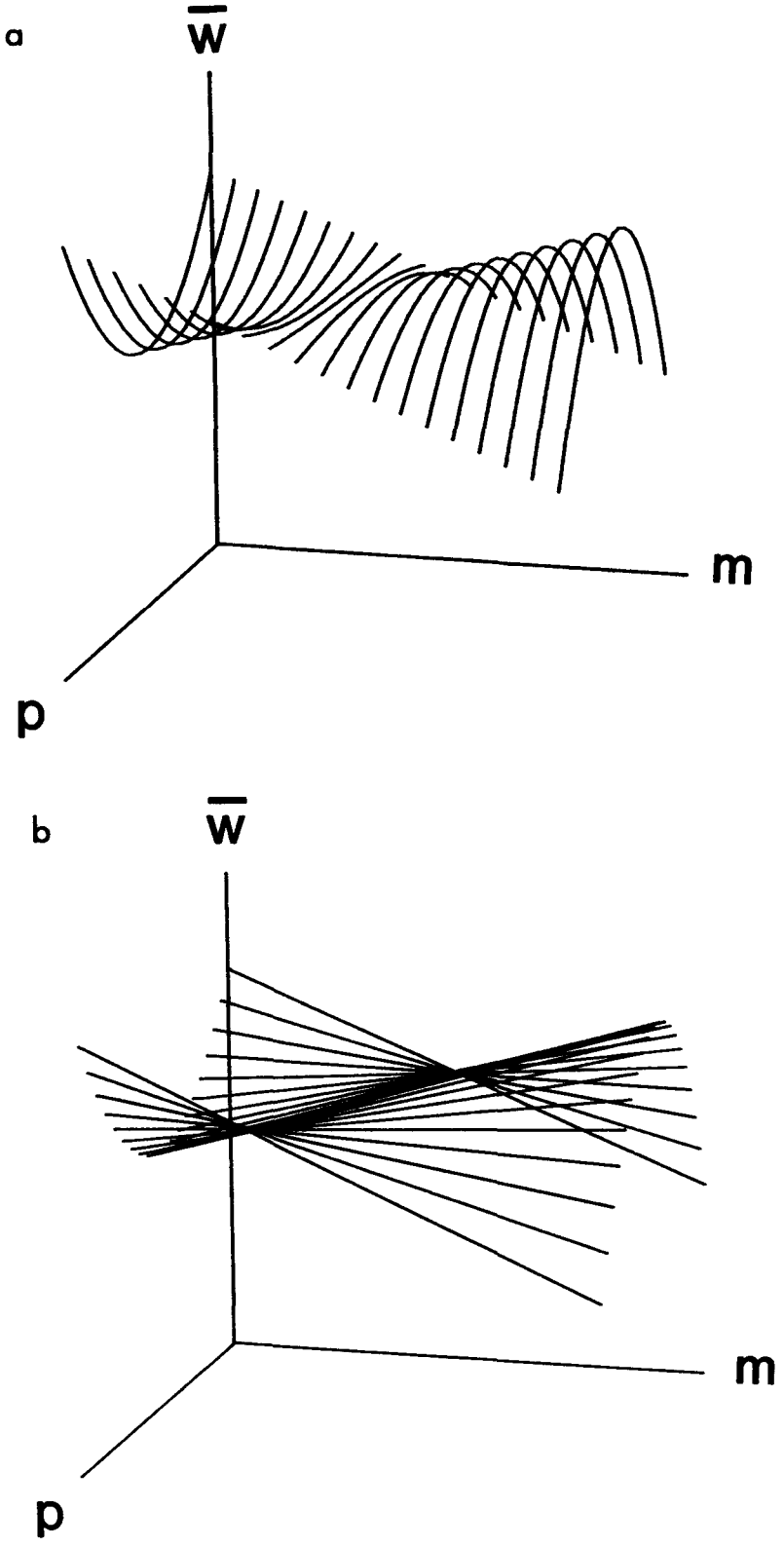

FIGURE 2.—Two perspective plots in the $\bar{w}$ surface for the viability matrix where v_i = 0.3, 1.3, 0.4, 0.9, 0.2 and 0.8 for i = 1 to 6. Axes are of unit length.

Genotype	m	n
AA	v_1	v_3
Aa	v_2	v_2
aa	v_3	v_1

(29)

Without loss of generality, let $v_2 = 1$ so only two parameters determine the model.

The recurrence equations become

$$\begin{aligned}
\bar{w}e_1' &= e_1(pv_1 + q) - \tfrac{1}{2}A \\
\bar{w}e_2' &= e_2(pv_3 + q) + \tfrac{1}{2}A \\
\bar{w}e_3' &= e_3(p + qv_3) + \tfrac{1}{2}A \\
\bar{w}e_4' &= e_4(p + qv_1) - \tfrac{1}{2}A
\end{aligned} \tag{30}$$

If $\hat{A} = 0$, then the marginal fitnesses are equal and we immediately obtain the condition $v_1 = v_3$. Therefore, if $v_1 \neq v_3$, then an interior equilibrium must have $\hat{A} \neq 0$.

The symmetry of the model suggests equilibria of the form $\hat{p} = \hat{m} = 0.5$; therefore, substituting into (30) we get,

$$\begin{aligned}
\bar{w}\hat{e}_1 &= \hat{e}_1(0.5v_1 + 0.5) - \tfrac{1}{2}A \\
\bar{w}\hat{e}_2 &= \hat{e}_2(0.5v_3 + 0.5) + \tfrac{1}{2}A \\
\bar{w}\hat{e}_3 &= \hat{e}_3(0.5 + 0.5v_3) + \tfrac{1}{2}A \\
\bar{w}\hat{e}_4 &= \hat{e}_4(0.5 + 0.5v_1) - \tfrac{1}{2}A.
\end{aligned} \tag{31}$$

Therefore, symmetric equilibria (if they exist) are of the form $\hat{e}_1 = \hat{e}_4$, $\hat{e}_2 = \hat{e}_3$. At these equilibria $\hat{A} = \hat{e}_1^2 - \hat{e}_2^2$, and the recurrence equations become,

$$\begin{aligned}
\bar{w}\hat{e}_1 &= 0.5(v_1 + 1)\hat{e}_1 - \tfrac{1}{2}(\hat{e}_1^2 - \hat{e}_2^2) \\
\bar{w}\hat{e}_2 &= 0.5(1 + v_3)\hat{e}_2 + \tfrac{1}{2}(\hat{e}_1^2 - \hat{e}_2^2)
\end{aligned} \tag{32}$$

Therefore, $\bar{w} = (v_1 + 1)\hat{e}_1 + (v_3 + 1)\hat{e}_2$. Substituting $\hat{e}_2 = \tfrac{1}{2} - \hat{e}_1$ we get $\bar{w} = \hat{e}_1(v_1 - v_3) + \tfrac{1}{2}(v_3 + 1)$. When this is substituted into (32), then $\hat{e}_1$ can be solved directly.

$$\hat{e}_1 = \frac{0.5(v_1 + 1)\hat{e}_1 - \tfrac{1}{2}[\hat{e}_1^2 - (\tfrac{1}{2} - \hat{e}_1)^2]}{\hat{e}_1(v_1 - v_3) + \tfrac{1}{2}(v_3 + 1)}. \tag{33}$$

Some algebra yields,

$$\hat{e}_1 = \frac{(v_1 - v_3 - 1) \pm \sqrt{(v_1 - v_3)^2 + 1}}{4(v_1 - v_3)}, \tag{34}$$

and $\hat{A} = \hat{e}_1 - \tfrac{1}{4}$.

The (+) root is valid for all non-negative values of v_1 and v_3 (provided $v_1 \neq v_3$), whereas the (−) root is never valid; therefore, a unique symmetric

equilibrium always exists. The marginal fitness pattern in one cytoplasm can be overdominant, underdominant or directional, and this result still holds. If $v_1 = v_3$, then the model is degenerate because there is no cytoplasmic effect on viability.

The stability of the symmetric equilibrium is analyzed by evaluating the eigenvalues of the linearized transformation evaluated at the equilibrium point. The linearized system is,

$$\begin{aligned} \bar{w}e_1' &= \tfrac{1}{2}e_1(v_1 + 1) \\ \bar{w}e_2' &= \tfrac{1}{2}e_2(v_3 + 1) \end{aligned} \tag{35}$$

Eigenvalues are obtained from

$$\begin{vmatrix} \tfrac{1}{2}(v_1 + 1) - \lambda\hat{\bar{w}} & 0 \\ 0 & \tfrac{1}{2}(v_3 + 1) - \lambda\hat{\bar{w}} \end{vmatrix} = 0 \tag{36}$$

where $\hat{\bar{w}} = \hat{e}_1(v_1 - v_3) + \tfrac{1}{2}(v_3 + 1) = \hat{e}_2(v_3 - v_1) + \tfrac{1}{2}(v_1 + 1)$. The roots are

$$\lambda = \frac{\tfrac{1}{2}(v_1 + 1)}{\hat{\bar{w}}}, \qquad \frac{\tfrac{1}{2}(v_3 + 1)}{\hat{\bar{w}}}. \tag{37}$$

If $\lambda < 1$, then

$$\tfrac{1}{2}(v_1 + 1) < \hat{e}_1(v_1 - v_3) + \tfrac{1}{2}(v_3 + 1) \Rightarrow \hat{e}_1 > \tfrac{1}{2} \tag{38}$$

and

$$\tfrac{1}{2}(v_3 + 1) < \hat{e}_3(v_3 - v_1) + \tfrac{1}{2}(v_1 + 1) \Rightarrow \hat{e}_2 > \tfrac{1}{2}.$$

Since neither $\hat{e}_1$ nor $\hat{e}_2$ can exceed $\tfrac{1}{2}$ in the symmetric case, the contradiction implies that $\lambda > 1$. Hence, the symmetric equilibrium is never stable. The same result is obtained when the system is treated as one dimensional. A numerical simulation of the symmetric case is presented in Figure 3.

Rather than pursue the uniqueness of the symmetric equilibrium, the possibility of maintaining a complete polymorphism (A, a, m and n all segregating) will be investigated in the general viability model.

General viability model

First consider the conditions for the stability of the corner equilibrium $\hat{e}_1 = 1$. [The terms "corner" and "edge" refer to the tetrahedral simplex representation of gametic frequencies as in the two-locus model (KARLIN and FELDMAN 1970)]. A small perturbation from this point can be represented as,

$$\begin{aligned} e_1 &= 1 - d \\ d &= e_2 + e_3 + e_4, \end{aligned}$$

where d is arbitrarily small. If $e_4 = 0$ initially, then $e_4' = (\tfrac{1}{2}w_{41}e_2e_3)/\bar{w}$ is obtained from (13). Equation (13d) can be written as,

$$\bar{w}(e_4' - e_4) = e_4(w_4 - \tfrac{1}{2}w_{41}e_1 - \bar{w}) + \tfrac{1}{2}w_{41}e_2e_3. \tag{39}$$

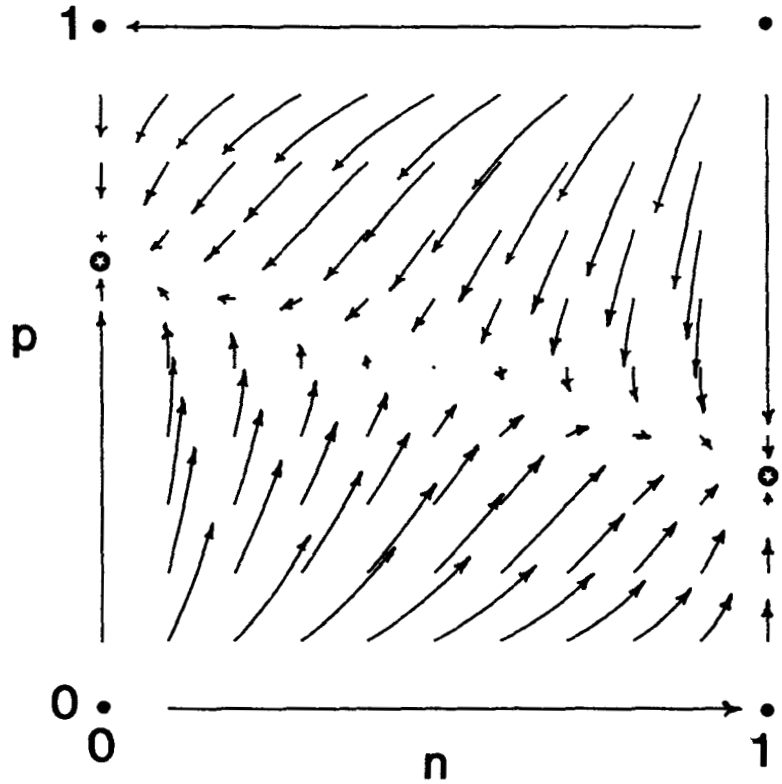

FIGURE 3.—Phase plot showing 10 generation iterations from each of 100 starting points, using the symmetric viability matrix where $v_1 = 1$, $v_2 = 1.2$ and $v_5 = 1.1$. Stars indicate stable edge equilibria, and the center is an unstable equilibrium.

A sufficient condition for e_4 to increase is, therefore,

$$w_4 - \tfrac{1}{2}w_{41}e_1 - \bar{w} > 0, \tag{40}$$

which is equivalent to $\tfrac{1}{2} < (w_4 - \bar{w})/w_{41}$. When e_4 is close to unity, we have

$$w_i = w_{i1} + 0(e_i),$$

and $\bar{w} = w_{11} + 0(e_i)$. Equation (40) is equivalent to $\tfrac{1}{2} < (w_{41} - w_{11})/w_{41}$. In the v_i notation, a sufficient condition for e_4 to increase (hence for the $e_1 = 1$ corner to be unstable) is $v_1 < \tfrac{1}{2}v_5$.

Assuming that e_4 is of the order e_2e_3, and ignoring quadratic terms in e_2, e_3 and d, we obtain,

$$\begin{aligned} e_2' &= e_2w_2/\bar{w} = e_2w_{21}/w_{11} = e_2v_4/v_1 \\ e_3' &= e_3w_3/\bar{w} = e_3w_{31}/w_{11} = e_3v_2/v_1. \end{aligned} \tag{41}$$

These equations indicate that the $e_1 = 1$ equilibrium is unstable if $v_1 < v_4$ or $v_1 < v_2$. This result is rather similar to the two-locus situation with free recombination, and the analysis follows that of BODMER and FELSENSTEIN (1967). In summary, any of the three conditions $v_1 < v_2$, $v_1 < v_4$ or $v_1 < \tfrac{1}{2}v_5$ is sufficient to guarantee instability of the equilibrium $\hat{e}_1 = 1$.

Next consider the conditions for the increase of a novel cytoplasm in a homoplasmic population with a balanced nuclear polymorphism. Let $e_1 = e_3 = 0$, $e_2 = \hat{p} = (v_5 - v_6)/(2v_5 - v_4 - v_6)$ and $e_4 = 1 - e_2$. The m cytoplasm is introduced into this population by letting $e_2 = p - d_2$, $e_4 = q - d_4$ and $e_1 + e_3 = d_2 + d_4$. Assuming e_1, e_3, d_2 and d_4 are small enough to ignore quadratic terms, we get

$$\begin{aligned} \bar{w} &= p^2v_4 + 2pqv_5 + q^2v_6 \\ w_1^* &= \sum_j e_j w_{ij} = pv_1 + qv_2 \\ w_3^* &= \sum_j e_j w_{3j} = pv_2 + qv_3. \end{aligned} \tag{42}$$

w_1^* and w_3^* represent the marginal fitnesses of the gametes Am and am introduced near the edge equilibrium. Substituting into the recurrence equations (13) and ignoring quadratic terms in e_1, e_3, d_2 and d_4 we obtain,

$$\begin{aligned} \bar{w}e_1' &= e_1w_1^* - \tfrac{1}{2}v_2e_1(q - d_4) + \tfrac{1}{2}v_2(p - d_2)e_3 = e_1(w_1^* - \tfrac{1}{2}vq) + \tfrac{1}{2}v_2pe_3 \\ we_3' &= e_3w_3^* + \tfrac{1}{2}v_2e_1(q - d_4) - \tfrac{1}{2}v_2(p - d_2)e_3 = e_3(w_3^* - \tfrac{1}{2}v_2p) + \tfrac{1}{2}v_2qe_1. \end{aligned} \tag{43}$$

We also get equations for d_2' and d_4' and note that they are independent of e_1 and e_3, whereas equation (43) is independent of d_2 and d_4. The eigenvalues of the full system can be obtained from each pair of equations separately, since the fourth order characteristic equation can be factored into two quadratic terms. The equations in d_2 and d_4 simply indicate that $v_5 > v_4$, v_6 is a condition for stability of the edge equilibrium. Equations (43) yield the characteristic equation,

$$\begin{vmatrix} w_1^* - \tfrac{1}{2}v_2q - \lambda\bar{w} & \tfrac{1}{2}v_2p \\ \tfrac{1}{2}v_2q & w_3^* - \tfrac{1}{2}v_2p - \lambda\bar{w} \end{vmatrix} = 0$$

which is equivalent to,

$$w^2\lambda^2 - \lambda\bar{w}[w_1^* + w_3^* - \tfrac{1}{2}v_2] + w_1^*w_3^* - \tfrac{1}{2}v_2(pw_1^* + qw_3^*) = 0 \tag{44}$$

Letting $w_1^* = w_3^* = w^*$, this factors into

$$(\bar{w}\lambda - w^*)[\bar{w}\lambda - (w^* - \tfrac{1}{2}v_2)] = 0 \tag{45}$$

so the roots are $\lambda = w^*/\bar{w}$ and $(w^* - \tfrac{1}{2}v_2)/\bar{w}$. Invasion of the noval cytoplasm can occur if $w^* > \bar{w}$, implying that the marginal fitnesses of the rare gametes are greater than the mean fitness of the population at the edge equilibrium.

The characteristic equation (44) can be written $\lambda^2 + X\lambda + Y = 0$, where

$$\begin{aligned} X &= \frac{w_1^* + w_3^* - \tfrac{1}{2}v_2}{\bar{w}} \\ Y &= \frac{w_1^*w_3^* - \tfrac{1}{2}v_2(pw_1^* + qw_3^*)}{\bar{w}^2} \end{aligned} \tag{46}$$

As BODMER and FELSENSTEIN (1967) indicate, the condition $\lambda > 1$ is equivalent to either $X > 2$, or, if $X < 2$, then $Y > X - 1$. These conditions reveal that when $w_1^* > \bar{w}$ and $w_3^* > \bar{w}$ invasion occurs, whereas when $w_1^* < \bar{w}$ and $w_3^* < \bar{w}$ invasion cannot occur. When $w_1^* < \bar{w} < w_3^*$ or $w_3^* < \bar{w} < w_1^*$, the edge equilibrium is either stable or unstable, depending on the earlier described conditions on X and Y.

Armed with the stability conditions for all possible equilibria that are not complete polymorphisms, we can now examine the conditions for the protection of nuclear and cytoplasmic polymorphisms. By explicitly examining all possible patterns of viability matrices, it is shown that the conditions for instability of an edge or corner guarantee the stability of another edge or corner. A detailed case analysis is presented in the APPENDIX. Degenerate viability matrices can be found that do not satisfy the strict inequalities for the corner and edge equilibria (*e.g.*, $v_i = 0, 2, 0, 1, 1, 1$ for $i = 1$ to 6). The analysis presented here does not allow prediction of stability in these cases, but numerical simulation shows that both cytoplasms can be maintained. However, these matrices are degenerate in the sense that any small change in viabilities results in instability of interior equilibria. It can be concluded that the general viability model cannot protect a complete polymorphism.

Numerical studies

A variety of numerical simulations further corroborated the analytical results. In the case of additive viabilities, 1000 matrices fitting this pattern were chosen at random, and ten random starting points were selected for each by choosing e_1, e_2, e_3 and e_4 from a uniform distribution on [0, 1]. $\bar{w}$ and $\bar{w}'$ were calculated and, as expected, $\bar{w}' > \bar{w}$ in all 10,000 cases. In a similar fashion, $\bar{w}$ failed to decrease when the viability pattern was multiplicative. A and A' were also calculated and, in the multiplicative case, the association parameters never changed sign (even when allowed to iterate to equilibrium). In the case of symmetric viabilities, the location and instability of the center equilibrium were verified numerically, and $\bar{w}$ and A did not have monotone trajectories. In both the symmetric and general cases, the mean fitness could decrease.

The conditions for invasion of a novel cytoplasm from an edge or corner were verified in the general viability model by comparing the eigenvalues with actual iterations with 5000 random matrices. Following this, the edge and corner stability was tested for 100,000 randomly chosen matrices (all six viabilities chosen from U[0, 1], and results appear in Table 1. Note that the two edges can be simultaneously stable and that in all cases at least one edge or corner is stable.

Proving that protection cannot be guaranteed does not preclude the possibility of a stable interior equilibrium, but this was addressed numerically. Ten thousand random matrices were generated as described earlier, and one random initial gametic frequency vector was generated for each. The recurrence equations were iterated until a gamete had a frequency less than 10^{-12}. By this criterion, in all 10,000 cases the model failed to maintain a complete polymorphism.

DISCUSSION

The general viability model allowing Mendelian transmission of nuclear genes

TABLE 1

Numerical results

One corner stable	20,516
One edge stable	15,874
One corner and opposite edge stable	16,074
Diagonally opposite corners stable	21,697
Two corners in the same cytoplasm stable	22,590
Two edges stable	1,241
One edge and two opposite corners stable	2,008
Total	100,000

Conditions for stability of edge and corner equilibria were tested in 100,000 randomly generated general viability matrices.

and strict maternal transmission of cytoplasmic factors cannot maintain a polymorphism in both nuclear genes and cytoplasms. The observation of extensive variation in both nuclear and mitochondrial DNA forces consideration of means other than viability selection that can maintain polymorphism. Genetic drift can occur in subcellular organelles both within and among individuals. The theory of neutral organelle genes is being developed (BIRKY, MARUYAMA and FUERST 1983; TAKAHATA and MARUYAMA 1981; CHAPMAN *et al.* 1982), and results indicate that the relative importance of genetic drift to nuclear and organelle genes depends on paternal contribution, the number of organelles per cell, mutation rates and sex ratio. It is not the case that genetic drift can always maintain more genetic diversity in organelles than in nuclear genes. The observation of gene sequence conservation despite the elevated mutation rate of mtDNA suggests selective constraints, but this does not address the issue of the importance of drift in maintaining mtDNA sequence diversity (not all sites are constrained).

Even in the absence of genetic drift, deterministic selection models may be able to maintain nuclear and cytoplasmic polymorphism. Components of selection including gametic viability, fecundity and sexual selection may be important. Observations of cytoplasmic male sterility in crop plants as well as natural polymorphisms for cytoplasmic sterility factors (MICHAELIS 1954) are well documented. Theoretical results of GREGORIUS and ROSS (1984) show that sexual asymmetry in fertility can protect a nuclear and cytoplasmic polymorphism. Multiple alleles and multiple loci may be relevant to the question, and mitochondrial recombination cannot be excluded. Allowing even a small component of paternal transmission of cytoplasmic factors would greatly change the results of these models. Introgression of mtDNA has already been documented in two independent cases (POWELL 1983 and FERRIS *et al.* (1983), representing an unexpected source of diversity. In summary, our knowledge of the degree and causes of mtDNA sequence diversity is far from complete, but the necessary theoretical and experimental tools are now at our disposal.

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APPENDIX

It is shown that it is not possible to have all corners and edges simultaneously unstable by using the general conditions for stability of the corner and edge equilibria. When taken together, these cases prove that the general viability model cannot protect a complete polymorphism.

Case 1a: Directional selection in both cytoplasms ($v_1 > v_2 > v_3$ and $v_4 > v_5 > v_6$). The two corner equilibria $\hat{e}_1 = 1$ and $\hat{e}_3 = 1$ cannot both be unstable, because if $v_1 > v_4$, then $\hat{e}_1 = 1$ is stable and, if $v_4 > v_1$, then $\hat{e}_3 = 1$ is stable.

Case 1b: Opposing directional selection in both cytoplasms ($v_1 > v_2 > v_3$ and $v_4 < v_5 < v_6$). Assume that $\hat{e}_1 = 1$ is unstable, so that either $v_1 < v_4$ or $v_1 < \frac{1}{2}v_5$. In either of these cases, the conditions for stability of $\hat{e}_4 = 1$ are met (*i.e.*, $v_6 > v_5$, $v_6 > v_3$ and $v_6 > \frac{1}{2}v_2$).

Case 2a: Directional selection in one cytoplasm and overdominance in the other. Let $\hat{e}_1 = 1$ be unstable because $v_1 < v_4$. The viability matrix can be written,

	Cytoplasm	
Genotype	*m*	*n*
AA	$1 + a + b$	$1 + a + b + c$
Aa	$1 + a$	$1 + a + b + c + d$
aa	1	$1 + a + b + c + d - e$

where $a, b, c, d, e > 0$, and all viabilities are non-negative. Let $\hat{e}_1 = 1$ be unstable because $v_1 < v_4$. The equilibrium on the *An-an* edge has $\hat{p} = e_2 = e/(d + e)$ and $\bar{w} = 1 + a + b + c + 2pqd - q^2e$. The marginal fitnesses of *Am* and *am* are $w_1^* = 1 + a + pb$ and $w_3^* = 1 + pq$. If $\bar{w} > w_1^*$ we have,

$$1 + a + b + c + 2\left(\frac{e}{d+e}\right)\left(\frac{d}{d+e}\right)d - \left(\frac{d}{d+e}\right)^2 e \overset{?}{>} 1 + a + p\left(\frac{e}{d+e}\right),$$

which is equivalent to

$$\frac{bd}{d+e} + c + \frac{d^2e}{(d+e)} > 0. \tag{47}$$

This is clearly valid since all terms are positive. Since $w_1^* > w_3^*$, this implies that $\bar{w} > w_3^*$ as well, and the stability of the *An-an* edge is guaranteed.

Case 2b: As in case 2a, let the pattern of viabilities be directional in one cytoplasm and overdominant in the other. Now assume that $\hat{e}_1 = 1$ is unstable because $v_1 < \frac{1}{2}v_5$. The viability matrix can be written

Genotype	Cytoplasm	
	m	n
AA	$1 + a + b$	$2(1 + a + b) + c - d$
Aa	$1 + a$	$2(1 + a + b) + c$
aa	1	$2(1 + a + b) + c - e$

where $a, b, c, d, e > 0$, and all viabilities are non-negative. $\hat{p}$, w_1^* and w_3^* are the same as they were in case 2a, but now $\bar{w} = 2(1 + a + b) + c - p^2d - q^2e$. To see whether $\bar{w} > w_1^*$, choose d and e to minimize $\bar{w}$ for given a, b and c. This yields

$$\bar{w}_{\min} = 2\hat{p}\hat{q}^2(1 + a + b) + c, \qquad \text{where} \quad \hat{p} = 0.5, \qquad \text{so}$$
$$\bar{w}_{\min} = 1 + a + b + c. \tag{48}$$

Hence, $\bar{w} \geq \bar{w}_{\min} > w_1^*$ and $\bar{w} > w_3^*$, and the edge equilibrium is stable.

Case 3: Directional selection in one cytoplasm and underdominance in the other ($v_1 > v_2 > v_3$ and $v_5 < v_4, v_6$). Let $\hat{e}_1 = 1$ be unstable due to $v_1 < v_4$. This guarantees stability of $\hat{e}_2 = 1$ because $v_4 > v_1$, $v_4 > v_5$ and $v_4 > \frac{1}{2}v_2$. If $\hat{e}_1 = 1$ is unstable because $v_1 < \frac{1}{2}v_5$, this too guarantees stability of $\hat{e}_2 = 1$.

Case 4: Overdominance in both cytoplasms. The viability matrix in this case can be written:

Genotype	Cytoplasm	
	m	n
AA	$1 - a$	$c - d$
Aa	1	c
aa	$1 - b$	$c - e$

where $a, b, c, d, e > 0$, $a, b < 1$ and $d, e < c$. Let w_{1m}^* and w_{3m}^* be the marginal fitnesses of gametes Am and am near the equilibrium on the An-an edge. Furthermore, let $\bar{w}_n$ be the mean fitness at this equilibrium. Define w_{2n}^* and w_{4n}^* as the marginal fitnesses of An and an, respectively, near the equilibrium on the Am-am edge. The mean fitness here is $\bar{w}_m$.

Substituting the viability matrix (49) into formulas (42) we obtain the following:

$$w_{1m}^* = 1 - \frac{ae}{e + d} \qquad w_{3m}^* = 1 - \frac{db}{e + d}$$
$$\bar{w}_m = 1 - \frac{ab}{a + b}$$
$$w_{2n}^* = c - \frac{db}{a + b} \qquad w_{4n}^* = c - \frac{ae}{a + b} \tag{50}$$
$$\bar{w}_n = c - \frac{ed}{e + d}$$

Let X_m and Y_m be the coefficients of the characteristic equation determining the stability of the equilibrium on the Am-am edge. They are obtained by substituting w_{2n}^*, w_{4n}^* and $\bar{w}_m$ into equations (46). Define X_n and Y_n as described previously, except they pertain to the An-an edge and are obtained by substituting w_{1m}^*, w_{3m}^* and $\bar{w}_n$ into equations (46). These are,

$$X_m = \frac{\frac{3}{2}c(a+b) - bd - ae}{a+b-ab} \qquad Y_m = \frac{\frac{1}{2}c^2(a+b)^2 - c(bd+ae)(a+b) + \frac{1}{2}a^2ce + \frac{1}{2}cb^2d + abde}{(a+b-ab)^2}$$

$$X_n = \frac{\frac{3}{2}(e+d) - ae - bd}{c(e+d) - ed} \qquad Y_n = \frac{\frac{1}{2}(e+d)^2 - (ae+bd)(e+d) + \frac{1}{2}ae^2 + \frac{1}{2}bd^2 + abde}{[c(e+d) - ed]^2} \tag{51}$$

From these it will be shown that the conditions for instability of the *Am-am* edge guarantee stability of the *An-an* edge and *vice versa*. In the symmetric case, where $a = b$ and $d = e$, the coefficients become

$$X_m = \frac{3c - 2d}{2 - a}, \qquad Y_m = \frac{2c^2 - 3cd + d^2}{(2-a)^2}$$

$$X_n = \frac{3 - 2a}{2c - d}, \qquad Y_n = \frac{2 - 3a + d^2}{(2c-d)^2}. \tag{52}$$

In this case, the following conditions can be directly shown to be true:

$$\text{If } X_m > 2, \quad \text{then } X_n < 2 \text{ and } Y_n > X_n - 1.$$
$$\text{If } X_m < 2 \text{ and } Y_m < X_m - 1, \quad \text{then } X_n < 2 \text{ and } Y_n > X_n - 1. \tag{53}$$

These conditions indicate that, whenever the *Am-am* edge is unstable, the *An-an* edge must be stable. Although direct algebraic proof that the coefficients (51) guarantee conditions (53) will not be given here, numerical and graphical methods verify that both edge equilibria cannot be simultaneously unstable. They can, however, be simultaneously stable.

Case 5: Underdominance in one cytoplasm, overdominance in the other. For case 5a, let $e_1 = 1$ be unstable because $v_4 > v_1$. Let the viability matrix be:

	Cytoplasm	
Genotype	*m*	*n*
AA	$1 + b$	$1 + b + c$
Aa	1	$1 + b + c + d$
aa	$1 + a$	$1 + b + c + d - e$

where $a, b, c, d, e > 0$, and all viabilities are non-negative. In this case $w_1^* = 1 + \frac{be}{d+e}$, $w_3^* = 1 + \frac{ad}{d+e}$ and $\bar{w}_n = 1 + b + c + \frac{d^2}{(e+d)}$. The restrictions on the viabilities guarantee that $\bar{w} > w_1^*$. The condition $\bar{w} > w_3^*$ is equivalent to

$$a < d + (b+c)(d+e)/d = a^*. \tag{54}$$

If $a < a^*$ then the *An-an* edge is stable, whereas if a is sufficiently larger than a^* the *An-an* edge is not stable, but now the $\hat{e}_3 = 1$ corner stability is guaranteed.

In case 5b let the $\hat{e}_1 = 1$ equilibrium be unstable because $v_1 < \frac{1}{2}v_2$. Here the viability matrix is:

	Cytoplasm	
Genotype	*m*	*n*
AA	$1 + b$	$2(1 + b) + c - d$
Aa	1	$2(1 + b) + c$
aa	$1 + a$	$2(1 + b) + c - e$

where a, b, c, d, $e > 0$ and all viabilities are non-negative. w_1^* and w_3^* are as in case 5a, and $\bar{w} = 2(1 + b) + c\,\frac{ed}{e + d}$. In this case d and e can be chosen to minimize $\bar{w}$, so that $\bar{w}_{\min} = 2\hat{p}\hat{q}[2(1 + b) + c] = 1 + b + c$ because $\hat{p} = \frac{1}{2}$. Clearly, $\bar{w}_{\min} > w_1^*$ and $\bar{w}_{\min} > w_3^*$ if $a < (b + c)(d + e)/d = a^*$. As in case 5a, if $a < a^*$ this guarantees stability of the *An-an* edge, whereas if a is sufficiently greater than a^*, stability of the $\hat{e}_3 = 1$ corner equilibrium is guaranteed. Since $\bar{w} \geq \bar{w}_{\min}$, this holds when the restrictions on d and e are relaxed.

Case 6: Underdominance in both cytoplasms. The restrictions on the viabilities are $v_2 < v_1$, v_3 and $v_5 < v_4$, v_6. If $\hat{e}_1 = 1$ is unstable either because $v_1 < v_4$ or $v_1 < \frac{1}{2}v_5$, then stability of $\hat{e}_2 = 1$ is guaranteed. $\hat{e}_4 = 1$ may be simultaneously stable, but if it is not, then $\hat{e}_3 = 1$ must be stable. This viability pattern guarantees stability of two corner equilibria.

This exhausts the possible patterns of viabilities. In all cases it has been shown that the instability of one edge or corner equilibrium guarantees the stability of another edge or corner. This completes the proof that the general nuclear-cytoplasmic viability model cannot protect a complete polymorphism.