Effects of Differential Selection in the Sexes on Cytonuclear Polymorphism and Disequilibria

Christina S. Babcock and Marjorie A. Asmussen

Department of Genetics, University of Georgia, Athens, Georgia 30602

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ABSTRACT

We develop a series of models that examine the effects of differential selection between the sexes on cytonuclear polymorphism and disequilibria. A detailed analysis is provided for populations under constant fertility or viability selection censused at life stages without frequency differences in the sexes. We show analytically that cytonuclear disequilibria can be generated de novo if the cytoplasmic and nuclear loci each affect female fitness and there is a nonmultiplicative fitness interaction between them. While computer simulations demonstrate that the majority of disequilibria produced by random selection are transient and small in magnitude, measurable permanent disequilibria can result from selective differences both within and between the two sexes. We derive analytic conditions for a protected cytonuclear polymorphism and use numerical simulations to quantify the likelihood of obtaining permanent nuclear, cytoplasmic, and cytonuclear variation under various patterns of selection. The numerical analysis identifies special selection regimes more likely to generate disequilibria and maintain cytonuclear polymorphism and reveals a direct correlation to the strength of selection. As a byproduct, our models also provide the first decomposition of the different parental contributions to cytonuclear dynamics and the analytic conditions under which selection can cause cytoplasmic frequency changes or a cytonuclear hitchhiking effect.

Joint nuclear-cytoplasmic assays have become important tools for studying evolutionary processes in natural populations. The results have shown that significant nonrandom associations (cytonuclear disequilibria) can develop between nuclear and cytoplasmic markers within a population. Moreover, cytonuclear theory has demonstrated that such disequilibria can reveal new information about natural populations such as the occurrence and operation of nonrandom mating, migration, and population subdivision (Asmussen et al. 1987, 1989; Arnold et al. 1988; Asmussen and Arnold 1991; Arnold 1993; Asmussen and Basten 1994). However, one critical evolutionary phenomenon that cytonuclear disequilibrium theory has not adequately addressed is natural selection.

There is great potential for selection to favor particular joint cytonuclear genotypes over others due to the functional interdependence of nuclear and cytoplasmic gene products and thus for nonrandom associations to develop between the two genomes (Clark 1984; Muller et al. 1984; Jari et al. 1989; Cellino and Arnold 1993; Hutter and Rand 1995). A growing body of experimental evidence from Drosophila has suggested that there are detectable selective interactions between the mitochondrial and nuclear genomes (Clark and Lycegaard 1988; MacRae and Anderson 1988; Fos et al. 1990; Hutter and Rand 1995; D. M. Rand, personal communication). For example, in a test of the neutrality of mtDNA, MacRae and Anderson (1988) monitored the mitochondrial haplotype frequencies of two subspecies of Drosophila pseudoobscura (from Apple Hill, CA and Bogota, Colombia) on a mixed nuclear genetic background. A dramatic increase of Bogota mtDNA over the Apple Hill haplotype was observed in one of their initial cages, but the frequency change resulted in a polymorphic equilibrium, rather than fixation of one haplotype as predicted under a haploid selection model. These results have been taken as evidence of either a direct cytonuclear fitness interaction or the hitchhiking of the mitochondrial haplotype on a selected nuclear allele. In order to properly interpret such findings, further models of selection on cytonuclear genotypes are needed to determine the selective conditions under which these dramatic frequency changes can occur, together with the nonrandom cytonuclear associations that contribute to them.

The existing theory on the effects of selection on joint cytonuclear genotypes has primarily concentrated on the conditions necessary to maintain nuclear-cytoplasmic polymorphisms (Watson and Caspary 1960; Caspary et al. 1966; Clark 1984, 1985; Gregorius and Ross 1984; Ross and Gregorius 1985). The extensive analysis of Clark (1984) demonstrated that a deterministic, constant viability selection model cannot maintain joint, cytonuclear polymorphism; this means that, by
definition, permanent cytonuclear disequilibrium is also precluded under this model. However, joint cytonuclear variation is often found in natural populations, and the theoretical analysis by Gregorius and Ross (1985) has demonstrated that, under very specific life history characteristics such as selfing, polymorphism can be maintained under differential fertility selection between sexes.

In the following study we extend our understanding of the consequences of joint cytonuclear selection by developing and analyzing general models of differential fertility or viability selection between the sexes. Consideration is also made for the life stage at which the population is censused. We first derive the analytic conditions under which cytoplasmic frequency change, genetic hitchhiking, de novo disequilibria, or a protected polymorphism can occur in such cytonuclear systems. To better determine what biological forces may account for observed patterns of joint cytonuclear frequencies and nonrandom associations, we then provide detailed numerical investigations of (i) the conditions under which cytonuclear disequilibria can be generated de novo; (ii) the magnitude, duration, and patterns of resulting disequilibrium trajectories; and (iii) the likelihood of nuclear, cytoplasmic and joint cytonuclear polymorphism under differential selection between the sexes. In addition, we explore whether the occurrence of permanent polymorphism and disequilibria can be correlated with either particular patterns or the strength of selection.

**Analytic Study**

We have derived six models that differ in selection type and census time. These include either fertility or viability selection with censusing at either the zygote, adult, or gamete life stage. The basic assumptions of each model include the following: sufficiently large population size so that the effects of drift can be ignored, discrete and nonoverlapping generations, Mendelian segregation of nuclear alleles, strict maternal inheritance of the cytoplasmic locus, no mutation or migration, and random mating with respect to cytonuclear genotype. Inspection of the basic recursion equations for the cytonuclear genotypic frequencies reveals a close correspondence among the various models of differential selection as was previously found for nuclear systems (Bodmer 1965). The relationships of the six models are summarized schematically in Figure 1. Fertility models censused at the zygote or adult life stages are equivalent and mathematically identical to the viability model censused at the zygote stage, as indicated by the double headed arrows. Similarly, the fertility and viability models censused at the gamete stage are mathematically identical, and their recursion equations are subsets of the viability selection model with adult census (illustrated by the single headed arrow in Figure 1). The adult viability model is not only distinct, but it is also the most complex due to the required analysis of twice the number of variables to allow for different frequencies and disequilibria in males and females at census time. Here we examine the consequences of cytonuclear selection under the first three models (indicated by italics in Figure 1), within the context of the model of differential fertilities with zygote census. One reason we present this model is because it is highly likely that fertility selection differs between sexes due to the different modes of gamete production in males and females (Kidwell et al. 1977). Moreover, there is some direct experimental evidence of fertility differences among cytonuclear genotypes (Edwardson 1970; Hiraiumi 1985; Avise 1991; Hutter and Rand 1995).

**Nomenclature:** Here we present the general nomenclature used throughout the model. We consider a population with two alleles, A and a, at a diploid nuclear locus and two alleles (cytotypes), C and c, at a haploid cytoplasmic locus. Frequencies of the six possible cytonuclear genotypes in zygotes are denoted as in Table 1, with column sums providing the marginal frequencies of the three nuclear genotypes (u, v, w) and the row sums providing the marginal frequencies of the two cytotypes (x, y). The marginal nuclear allele frequencies can be calculated as \( p = u + \frac{1}{2}v = \text{freq}(A) \) and \( q = w + \frac{1}{2}v = \text{freq}(a) \).

Further frequency variables are needed to describe the two gamete pools. For instance, in the female gamete pool there are four possible cytonuclear allelic combinations, A/C, A/c, a/C, a/c, with frequencies denoted by \( P_1^f \), \( P_2^f \), \( Q_1^f \), and \( Q_2^f \), respectively; the corresponding marginal allele frequencies of A, a, C, and c, are denoted by \( P_1 \), \( P_2 \), \( Q_1 \), \( Q_2 \), \( X_1 \), and \( Y_1 \) (Table 2). Only the two nuclear alleles, A and a, are present in the male gamete pool, the frequencies of which are denoted by \( P^m \) and \( Q^m \), respectively.

For zygotes, the departures of cytonuclear frequencies from expectations under random associations are quantified by four disequilibrium statistics (Asmussen et al. 1987). Three are genotypic disequilibria, which mea-
Cytonuclear Disequilibria

TABLE 1

Genotypic frequencies in zygotes

<table>
<thead>
<tr>
<th>Cytoplasm</th>
<th>Nuclear genotype</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>AA</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>$u_1 = vx + d_1$</td>
<td>$v_1 = wx + d_2$</td>
</tr>
<tr>
<td>c</td>
<td>$u_2 = wy - d_1$</td>
<td>$v_2 = vy - d_2$</td>
</tr>
<tr>
<td>Total</td>
<td>$u$</td>
<td>$v$</td>
</tr>
</tbody>
</table>

Sure nonrandom associations between the two cytotypes and each of the three nuclear genotypes,

$$d_i = u_i - ux \quad d_2 = v_i - ux \quad d_3 = w_i - ux$$

where, for instance, the genotypic disequilibrium for the nuclear homozygote AA is defined as

$$d = freq(AA/C) - freq(AA) freq(C)$$

where, for instance, the genotypic disequilibrium for the nuclear homozygote AA is defined as

$$d = freq(AA/C) - freq(AA) freq(C)$$

The fourth measure of statistical association is the allelic disequilibrium,

$$d_a = freq(A) freq(a) - freq(A) freq(a)$$

which measures the association between cytoplasmic and nuclear alleles. The genotypic and allelic disequilibria are interrelated by the two formulas,

$$d = d_a + Xd$$

where, for instance, the genotypic disequilibrium for the nuclear homozygote AA is defined as

$$d = freq(AA/C) - freq(AA) freq(C)$$

The recursion equations for the marginal nuclear genotypic frequencies are

$$u' = P/p^m \quad v' = P/Q^n + Q/p^m \quad w' = Q/Q^n$$

for $i = 1, 2$. The recursion equations for the marginal nuclear genotypic frequencies are

$$u' = P/p^m \quad v' = P/Q^n + Q/p^m \quad w' = Q/Q^n$$

These involve the female gametic frequencies following fertility selection,

$$p_f' = \phi_f(u_1 + \frac{\phi_f}{\phi_a}v_1) \quad Q_f' = \phi_f(w_1 + \frac{\phi_f}{\phi_a}v_1)$$

$$p_m' = \phi_m(u_2 + \frac{\phi_m}{\phi_a}v_2) \quad Q_m' = \phi_m(w_2 + \frac{\phi_m}{\phi_a}v_2)$$

and the nuclear allelic frequencies in female and male gametes

$$p_f = \phi_f(u_1 + \frac{\phi_f}{\phi_a}v_1 + \frac{\phi_f}{\phi_a}w_1 + \frac{\phi_f}{\phi_a}v_2 + \frac{\phi_f}{\phi_a}w_2)$$

$$p_m = \phi_m(u_2 + \frac{\phi_m}{\phi_a}v_2 + \frac{\phi_m}{\phi_a}w_2 + \frac{\phi_m}{\phi_a}v_2 + \frac{\phi_m}{\phi_a}w_2)$$

where

$$\bar{p}_f = \phi_f(u_1 + \frac{\phi_f}{\phi_a}v_1 + \frac{\phi_f}{\phi_a}w_1 + \frac{\phi_f}{\phi_a}v_2 + \frac{\phi_f}{\phi_a}w_2)$$

$$\bar{p}_m = \phi_m(u_2 + \frac{\phi_m}{\phi_a}v_2 + \frac{\phi_m}{\phi_a}w_2 + \frac{\phi_m}{\phi_a}v_2 + \frac{\phi_m}{\phi_a}w_2)$$

are the mean fertilities in females and males, respectively.

Cytoplasmic frequency: The recursion equation for the cytoplasmic frequency is

$$x' = x' = \frac{\phi_f(u_1 + \frac{\phi_f}{\phi_a}v_1)}{\phi_{a_f}}$$

where $X'$ is the cytoplasmic frequency in female gametes. By substituting the genotypic frequencies written in terms of disequilibria (Table 1) into (7), the change

TABLE 2

Female gametic frequencies

<table>
<thead>
<tr>
<th>Cytoplasm</th>
<th>Nuclear allele</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>$P_f' = P_f'x' + D_f' \quad Q_f' = Q_f'x' - D_f'$</td>
<td>$X'$</td>
</tr>
<tr>
<td>c</td>
<td>$P_m' = P_m'y' - D_f' \quad Q_m' = Q_m'y' + D_f'$</td>
<td>$Y'$</td>
</tr>
<tr>
<td>Total</td>
<td>$P_f' \quad Q_f'$</td>
<td>1.0</td>
</tr>
</tbody>
</table>

TABLE 3

Cytonuclear fitnesses

<table>
<thead>
<tr>
<th>Cytoplasm</th>
<th>Nuclear genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>$\phi_f$</td>
</tr>
<tr>
<td>c</td>
<td>$\phi_f$</td>
</tr>
</tbody>
</table>

Superscripts of $* = f$ or $m$ represent female or male values.
in cytoplasmic frequency after one generation of selection can be written
\[
\phi' \Delta \mathbf{x} = \phi'(x' - x) = [(\phi' - \phi''_1)u + (\phi'_2 - \phi'')v + (\phi' - \phi''_1)w]\mathbf{y} + (\phi' d_1 + \phi'_2 d_2 + \phi(d_3)\mathbf{y}
+ (\phi' d_1 + \phi'_2 d_1 + \phi(d_3)\mathbf{y})x
\] (8)
where \(y = 1 - x\). It is immediately apparent that there must be selective differences among females in order for there to be a change in cytoplasmic frequencies. Also, disequilibria between the nuclear and cytoplasmic genes cannot by itself generate cytoplasmic frequency changes. Furthermore, while under this model selection in males cannot generate frequency change at a maternally inherited cytoplasmic locus, male selection can nonetheless affect the amount of change generated by selection in females through its effect on marginal nuclear frequencies (4) and disequilibria (as seen below), which all depend upon \(P^m\) (5). Closer inspection of (8) reveals that there are exactly two situations that will result in a change in cytoplasmic frequency: either the cytoplasmic gene must affect the female fitnesses directly in at least one nuclear background \((\phi'_1 \neq \phi'_2\) or \(\phi'_2 \neq \phi'_2\) or \(\phi'_2 \neq \phi''_1\)), or there must be cytonuclear genotypic disequilibria with a nuclear gene that affects the female fitnesses in at least one cytoplasmic background (either \(\phi'_1 = \phi'_2 = \phi''_1 = \phi''_2 = \phi''_2 = \phi''_2 = \phi''_2\)). The second case can be met by genetic hitchhiking of the cytoplasmic locus on a selected nuclear locus in females, at least two of whose genotypes are nonrandomly associated with the cytoplasmic gene.

**Nuclear allele frequency:** After one generation of selection, the nuclear allele frequency
\[
p' = \frac{1}{2}(p' + P^m)
\] (9)
is the average of the nuclear gene frequencies in the male and female gamete pools (5) and is thus, in contrast to the cytoplasmic marker, affected equally and symmetrically by selection in males and females. The mathematical expression for the change in nuclear allele frequency \((\Delta p = p' - p)\) is much more complicated than that for the cytoplasmic frequency and, as a result, is not informative in the general case. In light of this, we consider one case of particular interest due to the potential for a reverse hitchhiking effect from a selected cytoplasmic locus. If the nuclear locus is selectively neutral in both sexes \((\phi'_1 = \phi'_2 = \phi''_1 = \phi''_2 = \phi''_2 = \phi''_2 = \phi''_2)\), the change in the nuclear allele frequency over one generation is
\[
\Delta p = \frac{d'_1(2(\phi' d_1 x - \phi''_1 x) + (\phi'_2 d_2 + \phi''_1) (y - x))}{2(\phi' d_1 + \phi''_1) (\phi'_2 d_2 + \phi''_1) (y - x)}.
\] (10)

There can be a hitchhiking effect upon a nuclear locus \((\Delta p \neq 0)\) that is selectively neutral in both sexes provided there is nonzero allelic disequilibrium between it and a cytoplasmic gene that is selected in at least one of the sexes \((\phi' = \phi''_1 \text{ or } \phi''_1 = \phi''_2, \text{ where } \phi''_1 \text{ and } \phi''_2 \text{ represent the fitnesses of the two cytotypes in all nuclear backgrounds}). Note that a hitchhiking effect is precluded if the selection on the cytoplasmic gene is reversed in the two sexes \((\phi'_1 = \phi''_1 \text{ and } \phi'_2 = \phi''_2)\) and the cytoplasmic frequency \((x)\) is \(\frac{1}{2}\) or, more generally, if \(\phi' = \phi''_1 \text{ or } \phi' = \phi''_2\). It is also interesting that hitchhiking of the nuclear locus differs from the previous case of cytoplasmic hitchhiking where it is necessary to have genotypic rather than allelic disequilibrium with the selected marker.

**Disequilibrium dynamics:** The disequilibrium dynamics are obtained by substituting the zygote frequency recursions, (3), (4), (7), and (9), into the disequilibrium definitions in (1) and (2)
\[
d'_1 = P^m D'\]
\[
d'_2 = (Q^m - P^m) D'\]
\[
d'_3 = -Q^m D'\]
\[
d'_4 = \frac{1}{2} D'.
\] (10)
These three results are immediately apparent from the form of these recursions. First, these equations parallel those for random mating populations with no selection (Asmussen et al. 1987) in that each genotypic disequilibrium is the product of a nuclear gene frequency term and the allelic disequilibrium in the previous generation. There will therefore be nonzero genotypic associations if and only if there are nonzero allelic associations. Note that this means that after the first generation of selection, the distinction between the disequilibrium conditions for the two forms of hitchhiking is eliminated. More importantly, under this model selection will result in the same disequilibrium sign patterns found in the neutral case. However, allowing for differences between the sexes reveals several new insights. The disequilibrium dynamics of both this model of differential selection and the selectively neutral model presented in Asmussen et al. (1987) in fact depend on the nuclear gene frequency in the male gamete pool and the allelic disequilibrium, \(D'\), in the female gamete pool. The sign of the heterozygote disequilibrium \((d'_3)\) is consequently determined by the male nuclear allele frequency \((P^m)\) and its size relative to \(\frac{1}{2}\).

The second conclusion from (10) is that differential selection can generate cytonuclear disequilibrium de novo, with no disequilibrium in the original zygotes \((d = d_1 = d_2 = d_3 = 0\) initially). In particular, nonrandom cytonuclear associations will be produced in this model if and only if selection generates nonzero allelic disequilibrium in the female gamete pool \((D' \neq 0)\). To determine when this occurs, it is helpful to express \(D' = P' - P'X'\) in terms of the marginal frequencies and genotypic disequilibria in the original population of zygotes.
(\(\bar{y}'\))^2D' = [(\(\phi'_1\phi'_2 - \phi'_2\phi'_1\))uw + \(\frac{1}{2}{\phi'_1}\phi'_1 - \phi'_2\phi'_2\) uv]xy + \[(\phi'_1w + \frac{1}{2}\phi'_2u)(\phi'_1d_1 + \frac{1}{2}\phi'_2d_2)\]x + \[(\phi'_1w + \frac{1}{2}\phi'_2u)(\phi'_1d_3 + \frac{1}{2}\phi'_2d_4)\]y + \[(\phi'_1u + \frac{1}{2}\phi'_2v)(\phi'_1d_1 + \frac{1}{2}\phi'_2d_2)\]y + \[(\phi'_1u + \frac{1}{2}\phi'_2v)(\phi'_1d_3 + \frac{1}{2}\phi'_2d_4)\]y + \(\frac{1}{2}(\phi'_1\phi'_2 - \phi'_2\phi'_1)u_1d_2 + \frac{1}{2}(\phi'_1\phi'_2 - \phi'_2\phi'_1)d_1d_3\) (11)

where \(\bar{y}'\) is the mean fertility in females, given in (6). This reduces to

\[(\bar{y}')^2D' = [(\phi'_1\phi'_2 - \phi'_2\phi'_1)uw + \frac{1}{2}(\phi'_1\phi'_1 - \phi'_2\phi'_2)uv]xy\] (12)

when there is no initial disequilibrium. Inspection of (12) shows that disequilibria can be generated de novo only if there are selective differences in females involving a cytonuclear interaction in such a way that at least two of the following conditions hold:

\[\phi'_1\phi'_2 \neq \phi'_2\phi'_1, \quad \phi'_1\phi'_3 \neq \phi'_3\phi'_1, \quad \text{or} \quad \phi'_2\phi'_4 \neq \phi'_4\phi'_2.\] (13)

These inequalities require that the cytoplasmic and nuclear loci each affect female fitness. In other words, as for nuclear systems (Thomson 1977; Asmussen and Clegg 1981), disequilibria cannot be generated de novo by hitchhiking selection since \(D' = 0\) if female fitnesses are determined solely by nuclear genotype \(\phi'_1 = \phi'_4, \phi'_2 = \phi'_5, \text{and} \phi'_3 = \phi'_6\) or solely by cytoplasmic type \(\phi'_1 = \phi'_2 = \phi'_3\) and \(\phi'_4 = \phi'_5 = \phi'_6\). In addition, multiplicative fitnesses are not sufficient to generate disequilibria de novo, and there must be a nonmultiplicative fitness component between the two genes [for example, \(\phi(AA/C) \neq \phi(AA)\phi(C)\) where \(\phi\) denotes fitness]. A suitable reparameterization of the cytonuclear fitnesses demonstrates that these two requirements are in fact the complete conditions for the generation of nonrandom cytonuclear associations de novo. Details of this argument are given in Appendix A.

The final conclusion from (10) and (12) is that selective differences in males cannot generate cytonuclear disequilibria de novo in this model. However, just as male fitnesses have an indirect effect on cytoplasmic frequency dynamics, they may affect the amount of disequilibrium generated by selection in females through their influence on the nuclear genotypic frequencies and the nuclear allelic frequencies in the male gamete pool. The magnitude and nature of these effects are explored by computer simulations described below.

Equilibrium structure: Due to the analytic complexities, our equilibrium analysis focuses on the two types of boundary equilibria and the conditions for protected polymorphism. In each case, an equilibrium is locally stable if all five eigenvalues of its local stability matrix have magnitude less than one. The first and simplest boundary equilibria are the four corners corresponding to fixation in both markers: \(u_1 = 1, \tilde{w}_2 = 1, \tilde{w}_1 = 1,\) and \(\tilde{w}_1 = 1.\) For \(u_1 = 1\) (fixation for \(A\) and \(C\)), there are three nonzero eigenvalues

\[\lambda_1 = \frac{\phi'_2}{\phi'_1}, \quad \lambda_2 = \frac{\phi''_2}{\phi'_1 + \phi'_2}, \quad \lambda_3 = \frac{\phi'_3}{2\phi'_1}.\]

The resulting conditions for local stability of this and the other corner equilibria are given in Table 4. There are three components that must be satisfied for each to be locally stable. These involve differences between cytotypes within the same nuclear background (cytoplasmic component), between nuclear genotypes within the same cytoplasmic background (nuclear component), and between cytonuclear genotypes that differ at both markers (cytonuclear component). The conditions for local stability are similar to those derived by Clark (1984) for the constant viability selection model (without sex differences) except our model shows that the cytoplasmic and cytonuclear components in fact depend only on female fitnesses, while the nuclear component depends on the fitnesses of both sexes. In other words, differential selection between sexes, or more specifically selection in males, only affects the stability of the nuclear subsystem. For example, to meet the nuclear condition for the stability of \(u_1 = 1\) (fixed for alleles \(A\) and \(C\)), the heterozygous genotype \(Aa/C\) must have a lower fitness than the homozygous genotype \(AA/C\) in at least one sex \((\phi'_2 < \phi'_1 \text{ or } \phi''_2 < \phi''_1)\), and the average ratio of these two fitnesses across the sexes must be less than one. This will always hold if \(\phi'_2 < \phi'_1 \text{ and } \phi''_2 < \phi''_1\), which is the nuclear criterion without sex differences (Clark 1984).

The other type of boundary equilibria are edges corresponding to fixation in one genome and polymorphism in the other. The dynamics on the edges \((\tilde{p} = 1, 0 < \tilde{x} < 1 \text{ and } \tilde{p} = 0, 0 < \tilde{x} < 1)\) depend on female fitnesses alone and reduce to the classical model of haploid selection. Consequently, even with differential selection between the sexes, there are no equilibria fixed for the nuclear gene and polymorphic for the cytoplasmic gene. There can, however, be equilibrium polymorphic for the nuclear gene and fixed for the cytoplasmic gene, \((\tilde{k} = 1, 0 < \tilde{p} < 1 \text{ or } \tilde{k} = 0, 0 < \tilde{p} < 1)\). These two edges both have up to three equilibria, which are obtained as the roots of a cubic equation. These are specified in Appendix B in terms of the frequencies in adult females \((\tilde{p}')\) and males \((\tilde{p}'')\) to allow for a direct comparison with the classical model of differential selection on a nuclear locus (Kidwell et al. 1977), which assumed an adult census. The four nonzero eigenvalues at such an equilibrium satisfy a quartic polynomial, which, paralleling the analysis of the corner equilibria, decomposes into two quadratic equations: one corresponding to the stability of the nuclear subsys-
TABLE 4
Conditions for local stability of corner equilibria

<table>
<thead>
<tr>
<th>Component</th>
<th>$\hat{u}_1 = 1$</th>
<th>$\hat{u}_2 = 1$</th>
<th>$\hat{v}_1 = 1$</th>
<th>$\hat{v}_2 = 1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasmic</td>
<td>$\phi'_1 &gt; \phi'_2$</td>
<td>$\phi'_1 &gt; \phi'_2$</td>
<td>$\phi'_1 &gt; \phi'_2$</td>
<td>$\phi'_1 &gt; \phi'_2$</td>
</tr>
<tr>
<td>Nuclear</td>
<td>$\frac{1}{2} \left( \phi''_n + \phi''_n \right) &lt; 1$</td>
<td>$\frac{1}{2} \left( \phi''_n + \phi''_n \right) &lt; 1$</td>
<td>$\frac{1}{2} \left( \phi''_n + \phi''_n \right) &lt; 1$</td>
<td>$\frac{1}{2} \left( \phi''_n + \phi''_n \right) &lt; 1$</td>
</tr>
<tr>
<td>Cytonuclear</td>
<td>$\phi'_1 &gt; \frac{1}{4} \phi''_n$</td>
<td>$\phi'_1 &gt; \frac{1}{4} \phi''_n$</td>
<td>$\phi'_1 &gt; \frac{1}{4} \phi''_n$</td>
<td>$\phi'_1 &gt; \frac{1}{4} \phi''_n$</td>
</tr>
</tbody>
</table>

system and the other to the cytonuclear component. For the edge ($x = 1, 0 < y < 1$), the characteristic equation of the nuclear subsystem is $\lambda^2 - A\lambda + B = 0$, where

$$A = \hat{p}^n \left( \frac{\partial p'}{\partial u} \right) + \hat{p}^n \left( \frac{\partial p''}{\partial u} \right) + (1 - 2\hat{p}^n) \left( \frac{\partial p'}{\partial v} \right) + (1 - 2\hat{p}^n) \left( \frac{\partial p''}{\partial v} \right)$$

$$B = (\hat{p}^n - \hat{p}^n) \left[ \left( \frac{\partial p'}{\partial u} \right) \left( \frac{\partial p''}{\partial u} \right) - \left( \frac{\partial p'}{\partial v} \right) \left( \frac{\partial p''}{\partial v} \right) \right]$$

with $\frac{\partial}{\partial \phi}$ as in (15). This is similar to the characteristic equation of Clark's constant viability selection model without sex differences, but with the nuclear allele frequencies now partitioned into the contribution of each sex and the fitnesses now shown to be those of females only. Since this quadratic has real roots, the eigenvalues both have magnitude less than one if and only if $X < 2$ and $X < 1 + Y$ (Bodmer and Felsenstein 1967). In addition to completing the stability criterion for an edge equilibrium, the latter shows that a new cytotype ($c$) can successfully invade a population at a stable polymorphic nuclear equilibrium if $X > 2$ or $X > 1 + Y$. These conditions are difficult to resolve. However, one way in which $X > 2$ and successful invasion occurs is if $\phi'_1, \phi''_n > 2\hat{p}^n$, as would hold if in females the fitnesses of the new cytotype in the two homozygous backgrounds (AA/c and aa/c) are over twice the fitnesses of each genotype with the original cytotype (AA/C, Aa/C, and AA/C); this is obviously a very restrictive condition and suggests that even with differential selection between the sexes it may be difficult to maintain either a cytoplasmic or a joint cytonuclear polymorphism.

Unfortunately, due to the complexity of the model, it is not possible to gain much further analytic insight into the biological conditions under which the edge stability conditions will be met. However, we are able to use the above analysis to evaluate fitness sets numerically and determine if they will maintain joint cytonuclear variation through a protected polymorphism in which there are no stable boundary equilibria (results presented below).

NUMERICAL STUDY

We know from our analytic investigations that selective differences among females can produce cytonuclear disequilibria de novo. One of the goals of this study is to determine if measurable disequilibria detected in natural populations could be attributed to selection. Consequently, it is particularly important to analyze the magnitude of disequilibria and to provide a qualitative description of the disequilibrium trajectories. A second major goal is to quantify the effects of differential selection on the maintenance of nuclear, cytoplasmic, and cytonuclear polymorphism, including the extent to which the latter requires a protected poly-
morphism. We have investigated these issues through extensive computer simulations based on the four basic selection regimes:

1. **Differential selection between sexes**: All 12 fitnesses are independently generated by a random number generator with a uniform distribution on [0,1]. It is important to note that our subsequent use of the phrase “differential selection between sexes” always implies there is selection both within and between sexes.

2. **Equal selection between sexes**: All six female fitnesses are randomly generated from [0,1] and each male fitness set equal to the corresponding female parameter.

3. **Female selection only**: Female fitnesses are generated randomly from [0,1] while all male fitnesses are assigned a value of 1.0.

4. **Male selection only**: Male fitnesses are generated randomly from [0,1] while all female fitnesses are assigned a value of 1.0.

In the first simulation, we investigated the magnitude of disequilibria generated de novo after a single generation of selection. Three replicate runs, each with 50,000 random fitness sets, were analyzed for each of the four basic selection regimes. For each fitness set, marginal frequencies \((x, u, v)\) were chosen at random from [0,1] and used to calculate initial cytonuclear genotypic frequencies under the assumption of no initial disequilibrium. The first generation disequilibrium measures were calculated from (10) and classified according to magnitude in intervals of 0.01. Because the disequilibria generated are constrained by the marginal frequencies, we also calculated the maximum and minimum possible disequilibria and used these to normalize the de novo values as described by ASMUSSEN and BASTEN (1996).

A second simulation examined the duration of disequilibria generated by selection, the nature of their trajectories, and the likelihood of permanent genetic variation. For each run of the simulation, 100,000 random fitness sets were analyzed. Initial genotypic frequencies were calculated either from marginal frequencies as above (no initial disequilibria) or by directly drawing initial zygotic genotypic frequencies at random (arbitrary initial disequilibria). Because similar results were obtained in both cases, we present only those obtained with no initial disequilibria. In these runs, the full cytonuclear genotypic recursion equations (3) were iterated until either the population reached equilibrium (sum of absolute changes in genotypic frequencies at most \(10^{-7}\) in a single generation) or 10,000 generations, whichever came first. If the population did not reach equilibrium within 10,000 generations, the trajectory was excluded from the analysis below and stored in order to be checked for limit cycles. On average, the population did not reach equilibrium 218 times per run of 100,000 fitness sets. Many of these cases were investigated individually. While the standard progression from regular 2, 4, \ldots, 2^n-point cycles to chaos associated with real eigenvalues less than \(-1\) (MAY 1974; ASMUSSEN 1979; ALTENBERG 1991) was not evident, several cases of apparently long, nonintegral period cycles were found, reminiscent of those generated by complex eigenvalues with magnitude greater than 1 (HASTINGS 1981; ASMUSSEN 1986). The dynamics and robustness of these intriguing rare cases will be pursued elsewhere.

Disequilibria were considered measurable if they were greater than 0.01 in magnitude; this value is a typical minimum at which cytonuclear disequilibria can be detected at a 0.05 significance level given reasonable sample sizes and marginal frequencies (ASMUSSEN and BASTEN 1994). We have defined trajectories containing measurable disequilibria as permanent if they have measurable associations at equilibrium and as transient otherwise. Dynamical behavior was investigated by recording the following statistics for transient disequilibria: the number of generations that each disequilibrium was measurable, the final generation that each disequilibrium was measurable, and the number of times and generation in which each disequilibrium changed sign. For permanent disequilibria, the following statistics were recorded: the final magnitude at equilibrium, and the maximum value along the trajectory. The frequency of maintaining genetic variation was also determined for each of the four basic selection regimes. We considered polymorphism to be maintained at the nuclear or cytoplasmic locus if the equilibrium frequency of each allele was between 0.0001 and 0.9999 (ASMUSSEN and BASTEN 1990). Similarly, joint cytonuclear polymorphism was said to be maintained if at equilibrium the frequencies of each cytotype and nuclear allele were between 0.0001 and 0.9999.

**Initial generation of disequilibria**: To determine whether or not selection could account for disequilibria detected in natural populations, we first calculated the average magnitude of disequilibria generated from a single generation of selection. As expected from our analytic results, disequilibria were never generated in this model by selection in males only. For each of the three selection schemes capable of generating disequilibrium de novo (differential selection between sexes, male and female selection equal, and selection among females only), the average magnitude of each disequilibrium was 0.012 for the allelic \((d)\) and homozygote \((d_1, d_2)\) disequilibria, and 0.008–0.009 for the heterozygote \((d_3)\) disequilibrium. As expected, the averages for the symmetrical measures, \(d_1\) and \(d_2\), were always identical in value. Because not every set of random fitnesses and initial conditions generated measurable disequilibria, it should be stressed that all cases were included in calculating the average magnitude of disequilibrium produced, even those lacking measurable associations. Perhaps the most important result is that, although their magnitude is usually small, on average, the disequilibria initially produced by selection are nonetheless large enough that they are likely to be detected in
experimental and natural populations. Further practical insight is provided by the distribution of the corresponding normalized disequilibrium values (Asmussen and Basten 1994) shown in Figure 2. It is a highly skewed distribution, and most disequilibria are only a fraction of their maximum possible magnitude. However, differential selection can occasionally generate disequilibria that are at or near the maximum possible for the population.

The similar results for selection in females only and differential selection between sexes suggest that selection in males does not significantly influence the magnitude of disequilibria produced. In light of our analytic results, we investigated this issue further by closely examining one set of initial conditions for the influence of male fitnesses on the heterozygote disequilibrium, \( d_2 \). In this case, selection among males almost doubled the average magnitude of disequilibrium and resulted in measurable disequilibria much more frequently. Whereas, on average, male selection may not affect the disequilibrium generated, in any particular case it can have a noticeable effect.

**Duration of disequilibria:** Because our initial, single-generation analysis demonstrated that selection can produce measurable cytonuclear associations, we then studied the duration of the disequilibria produced. We have classified disequilibrium trajectories as follows: (i) *none*: disequilibria were never measurable (>0.01 in magnitude) in any generation of their trajectory; (ii) *transient*: disequilibria were measurable at some point along their trajectory, but were no longer so at equilibrium; or (iii) *permanent*: disequilibria were measurable at equilibrium. We have determined the frequency of producing these three types of trajectories under each of the basic selection regimes. With female selection alone or equal selection between sexes, measurable disequilibria are produced by 45–58% of the fitness sets, and *always* have a transient trajectory. Differential selection between sexes produces measurable disequilibria at about the same rate (51–60%), but can produce measurable permanent disequilibria as well as transient associations (Table 5). Permanent disequilibria are nonetheless rare, accounting for about 2.5% of measurable trajectories under random differential selection.

Knowing that such a large fraction of the disequilibria produced by selection are transient, the duration of these disequilibria becomes important. For instance, if they are measurable for many generations, then disequilibria are likely to be detected in populations undergoing selection. If, on the other hand, transient disequilibria decay to zero very rapidly, then detecting disequilibria in natural populations may be indicative of a recent selection event. An analysis of the duration of transient disequilibria for the three basic selection regimes which generate disequilibria *de novo* is presented in Table 6. When male selection is equal to female selection, or there is selection in females only, any generated disequilibrium is, on average, lost by generation 8 to 10. In the case of random differential selection between sexes, the transient disequilibria persist slightly longer and, on average, nonrandom associations are not all lost until generation 13. However, the standard deviation of this statistic is substantially higher than for the other selection regimes, implying that the last generation in which there are measurable disequilibria is much more variable under differential selection. Practically speaking, these disequilibria may therefore remain measurable much longer than the average suggests.

**Disequilibrium trajectories:** To better understand the nature of the disequilibrium trajectories, we analyzed the number of times each disequilibrium changed sign while still measurable. If a disequilibrium changes sign repeatedly or slowly, it may be undetectable for an intermediate portion of its trajectory, in which case the total number of measurable generations should be less than the final generation it is measurable. The results show that, on average, the number of measurable generations (Table 6) is indeed less than the final generation of measurable disequilibria for the three basic selection regimes with female selection, and, the ratio of these two quantities suggests that disequilibria are, on average, measurable for a large portion of the time before they are lost. In addition, no transient disequilibrium experienced more than two sign changes prior to

![Figure 2.—Frequency distribution of normalized disequilibria generated *de novo* following one generation of random differential fertility selection with zygote census.](image-url)
loss and very few trajectories (0.2–3.6%) changed sign twice (Table 6). Approximately 6% of transient disequilibria had a single sign change, except the heterozygote disequilibrium, $d_1$, which changed sign roughly 20% of the time. These results, together with the short duration of transient disequilibria, suggest that the majority of transient disequilibria have the trajectory illustrated in Figure 3, peaking quickly and then rapidly decaying to zero, rather than more complex patterns.

The nature of permanent disequilibrium trajectories is much more variable than that of transient associations. We have illustrated the patterns of permanent disequilibrium trajectories for four different sets of selection parameters in Figure 4. For example, in Figure 4A the disequilibria increase quickly in magnitude, have a period of about 10 generations in which they decrease, and then increase again to their final, permanent values; while, in Figure 4C the disequilibria remain near zero for close to 40 generations and then monotonically increase to their nonzero equilibrium values.

While, in general, the permanent disequilibria generated by differential selection are small, they are, on average, measurable and in rare cases reach a final magnitude of over 0.10. The average maximum magnitude obtained along the trajectories of permanent disequilibria is 0.0334, 0.0380, 0.0393, and 0.0383 for $d$, $d_1$, $d_2$, and $d_3$, respectively. The corresponding average final magnitudes are 0.0254, 0.0284, 0.0233, and 0.0277; the distribution of these final values is presented in Figure 5. (All averages are based solely on those selection patterns that differ both within and between sexes, the only basic selection regime allowing for permanent disequilibria.) As for the initial disequilibria, the maximum and final magnitudes of the heterozygote disequilibrium ($d_1$) are less than those of the two homozygote disequilibria ($d$ and $d_2$). Interestingly, the average final magnitude of each disequilibrium is less than (68–75%) its average maximum magnitude at equilibrium, which corroborates the suggestion that a monotonic increase to equilibrium is not the norm for permanent disequilibrium trajectories, as is suspected from the variable patterns illustrated in Figure 4.

**Permanent cytonuclear variation:** Since polymorphism maintained at one life stage must be maintained at all others (and fertility and viability selection are related by mathematically equivalent models), the following results apply to all six models of constant fertility or viability selection with any census time (Figure 1). We first investigated the equilibrium structure of the model by using our analytic local stability conditions to quantify the frequency of protected cytonuclear polymorphism for sets of 100,000 random fitnesses. Our results reveal that when there is selection in females, cytonuclear polymorphism can be protected (i.e., there are no stable boundary equilibria) by constant selection, only if there is differential selection both within and between sexes. In this case, a cytonuclear polymorphism is protected 2.0% of the time.

We next iterated the cytonuclear recursions with sets of random fitnesses and initial frequencies to determine the overall frequency of converging to a nuclear, cytoplasmic, or joint, cytonuclear polymorphism. The results for each of the four basic selection regimes are shown in the last three columns of Table 7. The frequency of maintaining cytonuclear polymorphism is highest (33.4%) with male selection only, but this is a degenerate case because, without selection in females, the cytotypes simply remain at their initial frequencies. Joint cytonuclear polymorphism is thus preserved in such instances whenever selection maintains nuclear variation; with selection only in males, this happens...
nuclear variation are maintained for males, cytoplasmic polymorphism can be maintained, these two measures, together with the low overall incidence of permanent cytoplasmic polymorphisms, shows fitnesses and initial conditions. The coincidence of internal equilibrium have no stable boundary equilibria. This means that the protected polymorphism conditions are fairly accurate predictors of when joint cytonuclear variation will be maintained. However, a full 23% of the time a stable interior equilibrium will occur in the absence of a protected cytonuclear polymorphism. In such cases, whether joint cytonuclear variation is ultimately lost or preserved will depend on the potential for maintaining cytonuclear polymorphism under random cytonuclear fitnesses and initial conditions. The coincidence of these two measures, together with the low overall incidence of permanent cytoplasmic polymorphisms, shows that the maintenance of cytoplasmic variation is clearly still a major limiting factor under cytonuclear selection (Clark 1984), even with sex differences.

These results demonstrate that there is a definite potential, albeit low, for cytonuclear polymorphism to be maintained when there is differential fertility or viability selection between the sexes. Combining this value (2.6%) with our earlier results provides further insight into two other aspects of the equilibrium structure under such selection regimes. First, a comparison with the frequency of a protected cytonuclear polymorphism (2.0%) shows that most (77%) of the cases with a stable internal equilibrium have no stable boundary equilibria. This means that the protected polymorphism conditions are fairly accurate predictors of when joint cytonuclear variation will be maintained. However, a full 23% of the time a stable interior equilibrium will occur in the absence of a protected cytonuclear polymorphism. In such cases, whether joint cytonuclear variation is ultimately lost or preserved will depend on the initial frequencies in the population as well as on the fitnesses. Second, a comparison with Table 5 shows that, although the potential for maintaining cytonuclear polymorphism is not large, the majority (58%) of the cases with permanent cytonuclear polymorphism must also have permanent cytonuclear disequilibria.

Our original discovery that constant cytonuclear selection alters the potential for permanent cytoplasmic variation, naturally raises the question of whether cytonuclear selection also affects the potential maintenance of nuclear variation. We addressed this issue by comparing the frequency of converging to a nuclear polymorphism under random cytonuclear fitnesses and initial cytonuclear frequencies (column 2 of Table 7) vs. ran-
While cytonuclear selection affords the opportunity for permanent cytoplasmic variation, it appears to some extent to which differential selection within a nuclear system, we also computed how often there actually is a stable internal nuclear equilibrium when male and female nuclear fitnesses are drawn at random from a uniform distribution on [0,1]. We did this by solving for all admissible polymorphic nuclear equilibrium (APPENDIX B) and evaluating their local stability criteria (14) viewed as equilibrium within the two-dimensional nuclear system itself. We found that under differential selection, 46.5% of random nuclear fitness sets have at least one stable internal nuclear equilibrium. This means that despite the potential for a simultaneously stable fixation equilibrium, over 95% of the time that there is a stable polymorphic equilibrium, nuclear genetic variation will be maintained in the population by constant differential selection at a nuclear locus.

**Special cases of selection:** Permanent disequilibria can be generated only when there is differential selection between sexes and permanent cytonuclear polymorphism is maintained in the population; however, this occurs relatively infrequently. To determine whether there are particular patterns of selection that have an increased likelihood of maintaining genetic variation or generating disequilibria, we examined the nine special cases of selection listed below. In each instance, the fitnesses represent cytonuclear genotypes as defined in Table 3.

**TABLE 7**

<table>
<thead>
<tr>
<th>Selection type</th>
<th>Nuclear system</th>
<th>Cytonuclear system*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nuclear</td>
</tr>
<tr>
<td>Random differential selection</td>
<td>0.4432</td>
<td>0.4056</td>
</tr>
<tr>
<td>Male = female selection</td>
<td>0.3321</td>
<td>0.2938</td>
</tr>
<tr>
<td>Female selection only</td>
<td>0.3338</td>
<td>0.3052</td>
</tr>
<tr>
<td>Male selection only</td>
<td>0.3350</td>
<td>0.3337</td>
</tr>
</tbody>
</table>

*Based on iterations of cytonuclear genotypic frequency recursions under sets of random cytonuclear fitnesses and initial cytonuclear genotypic frequencies.

*Based on iterations of nuclear genotypic frequency recursions under random sets of nuclear fitnesses and initial nuclear allele frequencies.

*Based on polymorphic criterion that allele frequency be in (0.0001, 0.9999).
1. **Hybrid inviability selection:** Complementary homozygous genotypes are most fit in both sexes. Female fitnesses, $\phi^w_l$ and $\phi^w_o$, and male fitnesses, $\phi^o_l$ and $\phi^o_o$, are assigned values of 1.0 while the other eight fitnesses are generated randomly from [0,1].

2. **Reverse hybrid inviability:** Different complementary homozygous genotypes are most fit in the two sexes. Female fitnesses, $\phi^w_l$ and $\phi^w_o$, and male fitnesses, $\phi^o_l$ and $\phi^o_o$, are assigned values of 1.0 while the other eight fitnesses are assigned randomly from [0,1].

3. **Reverse cytonuclear selection:** Male genotypes have fitnesses of the complementary cytonuclear genotype in females. Female fitnesses are chosen at random from [0,1] and male fitnesses are assigned such that $\phi^w_l = \phi^o_o$, $\phi^w_o = \phi^o_l$, $\phi^o_l = \phi^o_o$, $\phi^o_l = \phi^w_o$. and $\phi^o_o = \phi^w_l$.

4. **Reverse cytoplasmic selection:** Within each nuclear background, opposing cytotypes are favored in the two sexes. The female fitnesses are generated at random from [0,1] and the male fitnesses are assigned such that $\phi^w_l = \phi^o_l$, $\phi^w_o = \phi^o_o$, $\phi^o_l = \phi^w_o$, and $\phi^o_o = \phi^w_l$.

5. **Reverse directional selection:** Complementary cytonuclear homozygotes are favored in females and males. Female fitness $\phi^l_l$ and male fitness $\phi^l_o$ are assigned values of 1.0, while the other 10 fitnesses are generated at random from [0,1].

6. **Reverse directional selection with overdominance:** Selection favors complementary cytonuclear homozygotes in the two sexes, with overdominant selection in the other cytotype of each sex ($\phi^l_l = 1$, $\phi^l_l < \phi^l_o > \phi^l_l$ and $\phi^l_l < \phi^l_o > \phi^l_l$, $\phi^l_l = 1$).

7. **Hybrid superiority/overdominance:** All genotypes heterozygous at the nuclear locus are assigned a fitness of 1.0 in both sexes, while the other eight fitnesses are chosen at random from [0,1] ($\phi^l_l = \phi^l_l = 1$ and $\phi^l_l = \phi^l_l = 1$).

8. **Reverse overdominance:** Opposite heterozygotes in females and males are assigned values of 1.0 and the remaining 10 fitnesses are chosen at random from [0,1] ($\phi^l_l = \phi^l_l = 1$).

9. **Random reverse overdominance/underdominance:** All genotypes are generated at random from [0,1] such that opposite cytotypes in females and males are overdominant, and the complementary cytotypes are underdominant within each sex ($\phi^l_l < \phi^l_o > \phi^l_l$, $\phi^l_l > \phi^l_o < \phi^l_l$, $\phi^l_o < \phi^l_o > \phi^l_o$, $\phi^l_o < \phi^l_o < \phi^l_o$).

The results for the basic selection regime of random differential selection and the nine special cases described above are presented in Table 8. Although the special cases have widely varying potentials for the maintenance of nuclear polymorphism, they are much less variable in their cytonuclear effects. Just over half of these regimes have an increased frequency of permanent cytonuclear variation and disequilibria over random differential selection. One of these is **reverse cytoplasmic selection**, in which selection on each cytotype in females is equal to selection on the opposite cytotype in males. This form of selection is plausible if, for example, there is a direct interaction between the cytotype and a sex-determining locus. In this case, the frequency of permanent cytonuclear polymorphism increases to 6% and permanent disequilibria are generated roughly 5% of the time. Similar results are found under **reverse hybrid inviability selection.**

**DISCUSSION**

Our study extends previous theoretical investigations of the maintenance of genetic variation (Owen 1953; Haldane 1962; Li 1963; Bodmer 1965; Merat 1969; Kidwell et al. 1977; Clark 1984; Gregorius and Ross 1985) in several ways and confirms that differential selection can play an important role in increasing and maintaining levels of genetic variation. For constant fertility or viability selection models, irrespective of census time, we show that differential nuclear selection within and between sexes will maintain nuclear variation 34% more often than selection without sex differences. More importantly, we demonstrate that differential selection...
between sexes can allow for the maintenance of both cytoplasmic and cytonuclear polymorphism. However, the potential for cytonuclear polymorphism to be maintained is generally low, even in an extensive set of special cases in which we expected it would be greater, and appears to require fairly strong selective differences among genotypes. It is also interesting that while cytonuclear selection provides an opportunity for permanent cytoplasmic variation, it appears to restrict the opportunity for permanent nuclear variation. It should be emphasized that these results are based on the outcome under random fitnesses and initial conditions drawn from a uniform distribution. How often cytoplasmic and cytonuclear variation are actually maintained in natural populations by cytonuclear selection of course depends on how often those cases arise in nature, which we have no way of knowing a priori. The potential is definitely there, however, and provides further motivation for additional experimental tests of cytonuclear fitness interactions.

A secondary goal of this study was to provide a preliminary analytic framework from which to interpret previous experimental reports of often dramatic mtDNA frequency changes in Drosophila cage populations (MacRae and Anderson 1988; Fos et al. 1990; Hutter and Rand 1995; Kilpatrick and Rand 1995). Our results show that for life stages without sex differences, there are exactly two situations in which cytonuclear selection will result in a change in cytoplasmic frequency: either the cytoplasmic gene must affect the female fitnesses directly in at least one nuclear background, or there must be cytonuclear genotypic disequilibria with a nuclear gene that affects the female fitnesses in at least one cytoplasmic background. The latter case can be met by genetic hitchhiking of the cytoplasmic locus on a selected nuclear locus in females, as long as at least two of the nuclear genotypes are nonrandomly associated with the cytoplasmic gene. Although beyond the scope of the present investigation, these results lay the foundation for a detailed examination of the magnitude and degree of such hitchhiking effects which will be presented elsewhere.

The primary goal of this study was to provide the first extensive theoretical investigation of the effects of selection on cytonuclear disequilibria. One important finding is that measurable, permanent cytonuclear disequilibria can be generated de novo by differential selection between sexes; in fact, in most of the cases in which joint cytonuclear variation is maintained, the generated disequilibria are both measurable and permanent. More importantly, we have shown that nonmultiplicative, cytonuclear fitness interactions within females can generate measurable, transient cytonuclear disequilibria de novo. The majority of disequilibria produced under random fitnesses and initial conditions, however, are low in magnitude, of fairly short duration, and have sign patterns that are the same as those produced in a selectively neutral situation. Additionally, we have demonstrated that the likelihood of obtaining measurable transient or permanent disequilibria is directly correlated with the strength of selection. As a result, most disequilibria generated under the assumptions of these models will be difficult to detect in experimental and natural populations.

Our analysis provides a formal theoretical framework

### TABLE 8

Frequency of maintaining genetic variation compared with the frequency of generating permanent disequilibria under nine selection regimes

<table>
<thead>
<tr>
<th>Selection regime</th>
<th>Frequency of polymorphism</th>
<th>Frequency of permanent disequilibria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nuclear</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Random differential selection</td>
<td>0.4056</td>
<td>0.0258</td>
</tr>
<tr>
<td>Hybrid inviability selection</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Reverse hybrid inviability</td>
<td>0.1674</td>
<td>0.0625</td>
</tr>
<tr>
<td>Reverse cytonuclear selection</td>
<td>0.4481</td>
<td>0.0139</td>
</tr>
<tr>
<td>Reverse cytoplasmic selection</td>
<td>0.4388</td>
<td>0.0615</td>
</tr>
<tr>
<td>Reverse directional selection</td>
<td>0.2460</td>
<td>0.0099</td>
</tr>
<tr>
<td>Reverse dir/overdominance</td>
<td>0.5940</td>
<td>0.0589</td>
</tr>
<tr>
<td>Hybrid superiority/overdom</td>
<td>1.0</td>
<td>0.0204</td>
</tr>
<tr>
<td>Reverse overdominance</td>
<td>0.7322</td>
<td>0.0542</td>
</tr>
<tr>
<td>Random reverse over/under</td>
<td>0.4778</td>
<td>0.0852</td>
</tr>
</tbody>
</table>

### TABLE 9

Frequency of generating measurable (magnitude >0.01) transient and permanent disequilibria and the frequency of maintaining cytonuclear polymorphism under different strengths of selection

<table>
<thead>
<tr>
<th>Selection*</th>
<th>Transient disequilibrium</th>
<th>Permanent disequilibrium</th>
<th>Cytonuclear polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>[0.1]</td>
<td>0.543</td>
<td>0.014</td>
<td>0.026</td>
</tr>
<tr>
<td>[0.2, 1]</td>
<td>0.440</td>
<td>0.009</td>
<td>0.022</td>
</tr>
<tr>
<td>[0.5, 1]</td>
<td>0.255</td>
<td>0.004</td>
<td>0.016</td>
</tr>
<tr>
<td>[0.7, 1]</td>
<td>0.072</td>
<td>0.0</td>
<td>0.013</td>
</tr>
<tr>
<td>[0.9, 1]</td>
<td>0.0</td>
<td>0.0</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*Numbers in brackets indicate the range from which random fitnesses are drawn.
from which to interpret and possibly explain the patterns of disequilibrium trajectories recently observed in experimental Drosophila populations (D. M. Rand, personal communication). In cage studies of cytonuclear interactions, D. melanogaster mtDNA variants were competed on nuclear backgrounds heterozygous for the second chromosome, and gene frequencies and disequilibrium values were calculated for four generations. In these cage populations, nonrandom cytonuclear associations were generated \textit{de novo} along with changes in nuclear and cytoplasmic frequencies. Although typically small in magnitude, some of the generated disequilibrium were statistically significant at the 0.05 level and are consistent with the generation of transient disequilibrium \textit{de novo} by strong selection on joint cytonuclear genotypes as predicted by our model.

In summary, we have developed six models of differential selection in a cytonuclear system. The analysis here addressed the population dynamics under the three mathematically identical models for which there are no sex differences at the life stage censused: fertility selection with a zygote or adult census, and viability selection with a zygote census. Although we analyzed these models in the context of fertility selection with a zygote census, the results apply to the model of fertility selection with an adult census if the zygote frequencies are replaced by adult frequencies, and to the model of viability selection with a zygote census if the fertilities are replaced by viabilities. While the conditions maintaining genetic variation pertain to all six models, the dynamics of cytonuclear frequencies and disequilibria are model dependent. The remaining three models differ mathematically and may thus have distinct cytonuclear dynamics. Indeed, preliminary analysis of disequilibrium produced under constant viability selection with an adult census, shows that following a single generation of selection, the disequilibria generated are two to four times greater in initial magnitude than the results presented in this study. The complexity of the disequilibrium recursions also suggests there is a potential for distinctive disequilibrium sign patterns under this scenario. These initial results indicate that one may be most likely to detect disequilibria generated by viability selection with an adult census of populations and motivate the ongoing investigations of both this and X-linked models of differential selection in order to provide theoretical guidelines for deducing the operation of cytonuclear selection in empirical studies.

We are particularly grateful to W. W. Anderson for suggesting this investigation of differential selection and to two anonymous reviewers for their valuable comments including the suggested fitness reparameterization used in Appendix A and the investigation of the role of selection strengths. We thank M. Arnold, J. Avise, J. McDonald, L. Miller and members of the Asmussen lab for helpful discussions during the preparation of this manuscript and C. Basten for the random number generator. This work was supported by National Institutes of Health (NIH) training Grant T32GM-07103 (C.S.B.) and NIH grant GM-48528 (M.A.A.).

LITERATURE CITED


Cytonuclear Disequilibria


Communicating editor: A. G. Clark.

APPENDIX A

The conditions for generating disequilibria *de novo* are readily derived via the following reparameterization of the cytonuclear fitnesses. Denote the fitnesses of the nuclear genotypes by \(W_{aa} = 1\), \(W_{ab} = 1 + d - \gamma_2\), \(W_{bb} = 1 - s\) (with \(d\) reflecting the degree of dominance and \(s\) reflecting additive effects) and the fitnesses of the cytoplasts by \(W_c = 1\), \(W_t = 1 - t\). The fitness of each joint cytonuclear genotype, \(nm\), is then defined as \(W_{nm} = W_n W_m + x(1 - W_n)(1 - W_m)\), with \(x = x_1\) for genotype \(aa/c\) and \(x = x_2\) for genotype \(aa/c\), reflecting the nonmultiplicative fitness interaction between the loci. All sets of cytonuclear fitnesses can be written uniquely in this way. Under this parameterization, the conditions in Equation 13 for generating disequilibria *de novo* reduce to the requirement that at least one of the following be nonzero:

\[
x_2 sf 
eq 0 \quad (A1)
\]

\[
x_2 sf (1 + d - \gamma_2 + x_1(d - \gamma_2)(1 - s) 
eq 0. \quad (A3)
\]

Since the third of these expressions is a linear combination of the first two, it is evident that at least two of the inequalities in (13) must be met and that disequilibria will be generated *de novo* if and only if either (A1) or (A2) holds. It then follows that disequilibria will be generated *de novo* if and only if (1) \(t\) is nonzero; and (2) either \(x_2\) and \(s\) are nonzero, or \(x_1\) is nonzero and \(d\) does not equal \(\gamma_2\). Condition 1 requires that the cytoplasmic marker affect fitness, while condition 2 requires that the nuclear locus affect fitness and have a nonmultiplicative fitness interaction with the cytoplasmic marker. As a byproduct, this parameterization demonstrates that the additive and dominance effects at the nuclear locus have no bearing on the generation of disequilibria beyond their determination of selective differences among nuclear genotypes.

APPENDIX B

At an edge equilibrium \(\bar{x} = 1, 0 < \bar{p} < 1\), the equilibrium nuclear allele frequencies in adult males (\(\bar{p}^m\)) and females (\(\bar{p}^f\)) satisfy the two simultaneous nonlinear equations:

\[
\begin{align*}
\hat{p}^m &= \frac{(\phi_0^m - \phi_1^m)(\hat{p}^m)^2 + (\phi_2^m - \gamma_2/2\phi_0^m)\hat{p}^m}{(2\phi_2^m - \phi_1^m - \phi_0^m)(\hat{p}^m)^2 - \phi_0^m} \quad (B1) \\
\hat{p}^f &= \frac{(\phi_2^f - \phi_0^f)(\hat{p}^f)^2 + (\phi_1^f - \gamma_2/2\phi_0^f)\hat{p}^f}{(2\phi_2^f - \phi_1^f - \phi_0^f)(\hat{p}^f)^2 - \phi_0^f} \quad (B2)
\end{align*}
\]

(Kidwell et al. 1977). Substituting the expression for \(\hat{p}^m\) into (B1), we obtain a quintic polynomial in \(\hat{p}^f\), which can be reduced to the following cubic:

\[
\begin{align*}
(a^2g - ace + \phi_0^f\phi_2^f)(\hat{p}^f)^3 \\
+ (a^2g - a^2d + 2abg - ace - bg - \phi_0^f\phi_2^f)(\hat{p}^f)^2 \\
+ (a^2g + b^2g - a^2d - 2abd + 2abg - bee) \\
- \phi_2^f ag + 2\phi_2^f\phi_0^f\phi_2^f\hat{p}^f + (a + b)^2(g - d) \\
- \phi_2^f (ae + bg + ag) = 0
\end{align*} \quad (B3)
\]

where

\[
\begin{align*}
a &= 2(\phi_0^f - \phi_1^f) \\
b &= 2\phi_1^f - \phi_0^f \\
c &= 2(2\phi_2^f - \phi_0^f - \phi_1^f) \\
d &= 2(\phi_0^m - \phi_1^m) \\
e &= 2\phi_2^m - \phi_0^m
\end{align*}
\]

The roots of (B3) yield up to three polymorphic equilibria for \(\hat{p}^f\), from which the corresponding solutions for \(\hat{p}^m\) can be calculated from (B2).