

An epistatic mating system model can produce permanent cytonuclear disequilibria in a hybrid zone

(mitochondrial DNA/gametic-phase disequilibrium/*Hyla* tree frogs)

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Communicated by Wyatt W. Anderson, December 11, 1987 (received for review October 12, 1987)

ABSTRACT We examine the evolutionary dynamics of gametic and genotypic disequilibria between a cytoplasmic gene and a nuclear gene under two mating system models relevant to hybrid zones. In the first model, in which female mating preference is determined by an epistatic interaction between the two loci, permanent nonzero cytonuclear disequilibria are possible for a variety of initial genotype frequencies, particularly when rates of assortative mating for the two parental species are high. In contrast, when mating preference is effectively determined by interaction between a cytoplasmic gene and the multilocus nuclear genotypes characteristic of the parental species, all cytonuclear disequilibria, as well as frequencies of pure parentals, rapidly decay to zero unless assortative mating is nearly perfect. Results of the models are applied to the interpretation of observed cytonuclear associations in a hybrid population of *Hyla* tree frogs.

Cytonuclear disequilibria can be defined to measure the statistical associations between a diploid nuclear gene and a haploid uniparentally transmitted cytoplasmic gene. Recently, we (1) introduced and established the interrelationships of four components of cytonuclear disequilibria: the gametic disequilibrium (D), which measures associations between alleles at the two loci; and three genotypic disequilibria (D_1 , D_2 , D_3), which measure associations between two cytotypes and the three genotypes at a diallelic nuclear locus. The evolutionary dynamics of these disequilibria were then examined under three traditional models of the mating system (random mating and positive assortative mating with or without dominance), in each case under the assumption that the mating propensities are influenced solely by the individuals' nuclear genotypes. An important result was that all the cytonuclear disequilibria ultimately decay to zero, although not necessarily in a monotonic fashion.

Here we analyze the dynamic behavior of cytonuclear disequilibria under two "epistatic mating" models, pertinent to hybrid zones, in which mating is assumed to be a function of the individuals' joint cytonuclear genotypes. Of prime concern is whether the mating system itself can, in principle and under some circumstances, generate permanent nonzero cytonuclear associations in outcrossing species. As we will show, the results are particularly relevant to the interpretation of recent empirical findings of nuclear-mitochondrial disequilibria in hybrid populations (2).

Cytonuclear Disequilibria Under an Epistatic Mating Model

As before (1), we consider a diploid population with two alleles, A and a , at a nuclear locus, and two other alleles, M and m , at a cytoplasmic locus such as on mitochondrial DNA (mtDNA) or chloroplast DNA. The six possible cytonuclear genotypes have the frequencies given in Table 1. The gametic

disequilibrium D is defined as frequency of (freq.) (A/M) – freq. (A) freq. (M), or, equivalently, as

$$D = u_1 + \frac{1}{2}v_1 - px, \quad [1]$$

where

$$p = u + \frac{1}{2}v \quad [2]$$

is the gene frequency of allele A at the nuclear locus.

The genotypic disequilibrium for AA/M is $D_1 = \text{freq.}(AA/M) - \text{freq.}(AA) \text{freq.}(M)$, or, equivalently,

$$D_1 = u_1 - ux. \quad [3a]$$

The other genotypic disequilibria are similarly defined as

$$D_2 = v_1 - vx \quad \text{and} \quad D_3 = w_1 - wx. \quad [3b]$$

The gametic and genotypic disequilibria thus measure statistical associations between alleles and genotypes, respectively, at the two loci.

We are interested in the dynamics of these four cytonuclear disequilibria as a function of an epistatic mating pattern in a zone of hybridization between two genetically distinct taxa, denoted as species 1 and species 2. Individuals belonging to species 1 are assumed to be characterized by the AA/M cytonuclear genotype, and those of species 2 are characterized by aa/m . Under this convention, all other joint genotypes (Aa/M , aa/M , AA/m , Aa/m) define hybrid individuals, although clearly the "hybrid" category includes later-generation and backcross hybrids as well as F_1 s.

Other key assumptions of this epistatic mating model are that (i) females of genotype AA/M (species 1) mate assortatively with AA/M males with probability α , and they mate at random (with any of the six cytonuclear genotypes) with probability $1 - \alpha$; (ii) females of genotype aa/m (species 2) mate assortatively with aa/m males with probability β , and they mate at random with probability $1 - \beta$; (iii) all other females mate at random. Note that by incorporating separate assortative mating rates (α , β) for the two species, we have allowed for possible directionality in the interspecific crosses that take place in the hybrid zone.

The remaining assumptions are that there are no viability or fertility differences among the cytonuclear genotypes, the cytoplasmic gene is maternally inherited, the hybrid population is large and closed to outside recruitment so that the effects of drift and migration can be ignored, and the population has discrete nonoverlapping generations.

The recursions for this model can be derived by constructing a mating table showing all possible matings among the six cytonuclear genotypes, together with their frequencies and offspring distribution. The matings involving AA/M females, for instance, are as follows: (i) AA/M ♀ × AA ♂ occurs with frequency $\alpha u_1 + (1 - \alpha)u_1u$ and produces only AA/M off-

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Table 1. Frequencies of nuclear-cytoplasmic genotypes

Cytoplasm	Nuclear genotype			Total
	AA	Aa	aa	
M	u_1	v_1	w_1	x
m	u_2	v_2	w_2	y
Total	u	v	w	1.0

spring; (ii) AA/M ♀ × Aa ♂ occurs with frequency $(1 - \alpha)u_1v$ and produces half AA/M + half Aa/M offspring; (iii) AA/M ♀ × aa ♂ occurs with frequency $(1 - \alpha)u_1w$ and produces only Aa/M offspring. Note that the cytoplasmic gene carried by the male parent can be ignored since the cytoplasmic locus is strictly maternally inherited.

Matings involving aa/m females are treated similarly. The frequency of matings between hybrid females and males of a given nuclear genotype is simply the product of the genotypic frequencies of the two individuals. The mating Aa/M ♀ × AA ♂, for example, occurs with frequency v_1u and produces half AA/M and half Aa/M offspring.

Combining the offspring distribution from each of the 18 possible matings yields the following recursions for the cytonuclear genotypic frequencies:

$$\begin{aligned}
 u'_1 &= pe_1 + \alpha u_1q & u'_2 &= pe_2 \\
 v'_1 &= pe_3 + qe_1 - \alpha u_1q & v'_2 &= pe_4 + qe_2 - \beta w_2p \\
 w'_1 &= qe_3 & w'_2 &= qe_4 + \beta w_2p, \quad [4]
 \end{aligned}$$

where primes denote the values in the next generation, $q = 1 - p$, and $e_1 = u_1 + 1/2v_1$, $e_2 = u_2 + 1/2v_2$, $e_3 = w_1 + 1/2v_1$, and $e_4 = w_2 + 1/2v_2$ are the frequencies of the allelic combinations A/M, A/m, a/M, and a/m, respectively.

Recursions for the marginal single locus genotypic and gene frequencies are easily derived by using the relations shown in Table 1 together with Eqs. 2 and 4:

$$\begin{aligned}
 u' &= p^2 + \alpha u_1q & p' &= p + 1/2(\alpha u_1q - \beta w_2p) \\
 v' &= 2pq - \alpha u_1q - \beta w_2p & x' &= x. \\
 w' &= q^2 + \beta w_2p \quad [5]
 \end{aligned}$$

Note first that while the cytoplasmic gene frequency is constant, the nuclear gene frequency can change. This is a new feature of the epistatic mating model, not found in any of the mating systems previously considered (1). In particular, the nuclear allele A will increase in frequency if and only if $\alpha u_1q > \beta w_2p$ or, equivalently, if and only if $\alpha(u_1/p) > \beta(w_2/q)$, where u_1/p , for instance, is the fraction of the A alleles that are in AA/M individuals. A simple application of this result shows that if the population begins with just species 1 (AA/M) and species 2 (aa/m) individuals (i.e., $u_1^{(0)} + w_2^{(0)} = 1.0$), the nuclear allele corresponding to the more discriminating species will increase in frequency during the first generation. It should be emphasized that gene frequency changes at the nuclear locus are due to the epistatic mating pattern alone and not to selection. While unequal assortative mating rates for the two species may possibly be interpreted as a type of sexual selection, nuclear changes can also occur when $\alpha = \beta$, provided $u_1/p \neq w_2/q$.

Another observation from Eqs. 4 and 5 is that if females of only one of the parental species mate assortatively, while all other females mate at random, the nuclear allele characteristic of the more discriminating species will monotonically increase to fixation. At equilibrium, the hybrid population will consist of the two cytonuclear genotypes, AA/M and AA/m (or aa/M and aa/m), in the frequencies x and $y (= 1 - x)$, respectively, corresponding to the (constant) frequencies of the two cytotypes. It should also be noted from Eqs. 5 that

whenever AA/M or aa/m females exhibit assortative mating (i.e., $\alpha > 0$ and/or $\beta > 0$) there will be an excess of the corresponding nuclear homozygote and a corresponding deficit of heterozygotes relative to Hardy-Weinberg proportions.

Further points of interest stem from the dynamics of the four cytonuclear disequilibria, whose recursions can be obtained by combining Eqs. 4 and 5 with definitions 1 and 3:

$$\begin{aligned}
 D' &= 1/2D + 1/2(\alpha y u_1q + \beta x w_2p) & D'_1 &= pD + \alpha y u_1q \\
 D'_2 &= (q - p)D - \alpha y u_1q + \beta x w_2p & D'_3 &= -qD - \beta x w_2p. \quad [6]
 \end{aligned}$$

While explicit time-dependent solutions for these trajectories are not evident, several interesting features can nonetheless be deduced. First, in contrast to previous models (1), the epistatic model can produce permanent, nonzero cytonuclear disequilibria. Consider, for example, the set of population states satisfying the joint condition $u_1 = w_2$, $v_1 = v_2$, and $u_2 = w_1$. When $\alpha = \beta$, this set is invariant under Eqs. 4, and on this set $p_t \equiv x \equiv 1/2$, $u_t \equiv w_t$, and $D_2^{(t)} = 0$, while $D^{(t)} \equiv D_1^{(t)} \equiv -D_3^{(t)}$ approaches the nonzero value $\alpha/[4(4 - 3\alpha)]$.

Second, this same example shows that under the epistatic model, a nonzero value for D_2 (the genotypic disequilibrium associated with heterozygotes at the nuclear locus) implies some asymmetry in the population, either due to different initial frequencies of the two parental species, or to directionality to interspecific crosses (i.e., $\alpha \neq \beta$).

Third, in contrast to previous models (1), it is possible to move from a state in which all four cytonuclear disequilibria are zero to one in which all are highly nonzero. This follows directly from Eqs. 6, because $u_1 = ux$ and $w_2 = wy$ when $D_1 = D_3 = 0$.

Fourth, the complete equilibrium structure can be derived from the recursions in Eqs. 4-6 for p , u_1 , w_2 , and D (since these with x suffice to describe the state of the population). This reveals that in addition to the two fixation equilibria, $\hat{p} = 0$ ($\hat{w}_1 = x$, $\hat{w}_2 = 1 - x$) and $\hat{p} = 1$ ($\hat{u}_1 = x$, $\hat{u}_2 = 1 - x$), there may be interior polymorphic equilibria with \hat{p} the root of a cubic equation that depends on α , β , and the initial cytoplasmic frequency x . The remaining equilibrium values can then be obtained from the relations

$$\begin{aligned}
 \hat{u}_1 &= \hat{p}^2x/[1 - \alpha(1 - \hat{p}^2)] & \hat{w}_2 &= \hat{q}^2y/[1 - \beta(1 - \hat{q}^2)] \\
 \hat{D} &= \alpha \hat{u}_1 \hat{q} = \beta \hat{w}_2 \hat{p} & \hat{D}_1 &= (\hat{p} + y)\hat{D} \\
 \hat{D}_2 &= 2(x - \hat{p})\hat{D} & \hat{D}_3 &= -(\hat{q} + x)\hat{D}.
 \end{aligned}$$

This analysis reveals the important fact that exactly three qualitatively distinct patterns are possible at equilibrium: (i) H_R: All four disequilibria zero; (ii) H₂: Only $\hat{D}_2 = 0$ (requires $\hat{p} = x$ at equilibrium); (iii) H_{NR}: All four disequilibria nonzero. The last possibility shows that the mating system alone can produce complete permanent cytonuclear disequilibria (Fig. 1), and this can occur whether or not $\alpha = \beta$.

A final distinguishing feature of the epistatic mating model is that either nuclear variation will be lost and all disequilibria will eventually be zero; or nuclear variation will be maintained, in which case \hat{D} and \hat{D}_1 will be positive and \hat{D}_3 will be negative, while \hat{D}_2 will be either zero, negative, or positive depending on whether the equilibrium nuclear gene frequency for species 1 equals, exceeds, or is less than the frequency of its cytotype.

Numerical Study of the Epistatic Mating Model

The probability that all four cytonuclear disequilibria are nonzero at equilibrium was estimated by computer simulation. For each α and β , 100 initial frequencies of the pure

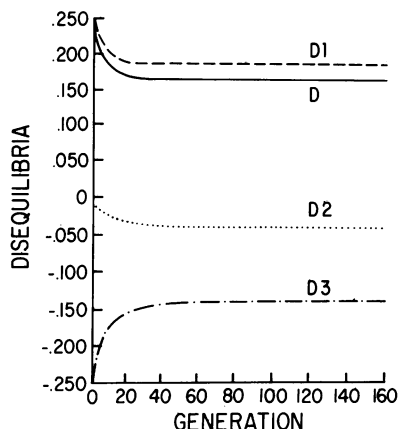


FIG. 1. An example in which gametic and genotypic cytonuclear disequilibria stabilize at nonzero values under the epistatic mating model. Initial pure parental frequencies were $u_1^{(0)} = 0.465$ and $w_2^{(0)} = 0.535$, with $\alpha = 0.94$ and $\beta = 0.86$, respectively.

parental species were examined by selecting $u_1^{(0)} = x$ from $[0, 1]$ using a combination random number generator (3), and setting $w_2^{(0)} = 1 - u_1^{(0)}$. These trajectories were iterated according to Eqs. 4 until the sum of the magnitude of the changes in all four disequilibria was $<10^{-8}$ per generation. The equilibrium was classified as H_{NR} if each of the four disequilibria exceeded 0.01 in magnitude. Values of α and β were varied from 0 to 1 in increments of 0.01. Calculations were performed in double precision in FORTRAN-77 on a PDP-11/34A minicomputer.

Results are summarized in Fig. 2. The equilibrium pattern of cytonuclear disequilibria is strongly dependent on the rates of assortative mating, as well as on the initial frequencies of the parental species in the hybrid population. For example, for a fixed α and β , a population may evolve either to H_R (all disequilibria zero), H_2 (only $\hat{D}_2 = 0$), or H_{NR} (all disequilibria nonzero) depending on the initial frequency condition. H_{NR} is likely to occur only if (i) both assortative mating rates are >0.8 , or (ii) one assortative mating rate is very near 1.0. In our simulations, H_{NR} was found for α as low as 0.04 with $\beta = 0.98$.

The effects of the assortative mating rates and the initial species mix were further explored by determining the equilibrium patterns with $\alpha, \beta = 0, 0.01, \dots, 0.99, 1.0$ for a variety of values for $u_1^{(0)}$ [with $w_2^{(0)} = 1 - u_1^{(0)}$]. In each case, the α, β plane was divided into four regions, with a curve of H_2 equilibria running from $\alpha = \beta = 0$ to $\alpha = \beta = 1$. For large values of α and β near the H_2 curve there are two H_{NR} re-

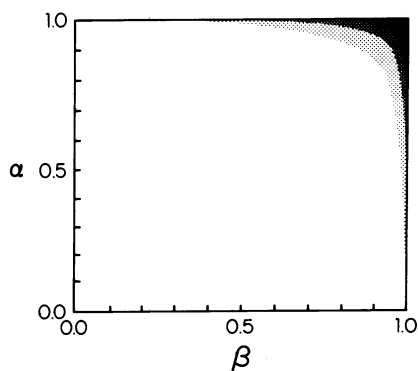


FIG. 2. Smoothed contour plot of the probability that all cytonuclear disequilibria are nonzero at equilibrium (H_{NR}), as a function of α and β . White corresponds to a probability of <0.05 , and successively darker shades correspond to probabilities between 0.05 and 0.15, 0.15 and 0.25, and 0.25 and 0.45.

gions. In the H_{NR} region below the H_2 curve (smaller α), $\hat{D}_2 > 0$, while in the H_{NR} region above the H_2 curve $\hat{D}_2 < 0$. In the remainder of the α, β space, the population goes to fixation for the nuclear allele (A) of species 1 if α is sufficiently greater than β , or for fixation of the nuclear allele (a) of species 2 if β is sufficiently greater than α . As the initial parental species mix is varied, the areas in the four regions grow or shrink as expected. For example, if $u_1^{(0)}$ is increased, the region in which the nuclear allele of species 1 wins increases, and the H_{NR} region with $\hat{D}_2 < 0$ decreases.

A Refinement of the Epistatic Mating Model

The epistatic mating model applies when mating preference is determined by an epistatic interaction between a cytoplasmic gene and a single nuclear gene, in which AA/M and aa/m females preferentially mate with males of their own type. If mating preference is a function of true species status, a modification is necessary because after the first generation some of these AA/M and aa/m genotypes represent offspring of matings involving F_1 or later-generation hybrids. Biologically, such AA/M and aa/m individuals are themselves hybrids. To take this into account the epistatic mating model can be refined to distinguish between these two types of AA/M and aa/m individuals. AA/M , for instance, can be decomposed into hybrids $(AA/M)_h$, with frequency u_{1h} , and pure species $(AA/M)_s$, with frequency u_{1s} , where $u_1 = u_{1s} + u_{1h}$. An analogous decomposition is made for aa/m individuals, with $w_2 = w_{2s} + w_{2h}$. In effect, as shown below, the refined epistatic mating model assumes that female mating propensities are determined by an interaction between cytoplasmic genotype and the multilocus (hybrid versus nonhybrid) nuclear genotype.

The overall cytonuclear genotypic frequencies shown in Table 1 still apply, as do the marginal genotypic frequencies. The frequency of AA individuals is, for instance, $u = u_1 + u_2$, as before. The key difference is that the upper left and lower right cells have been subdivided under the present formulation to reflect the eight possible genetic types now in the population.

Pure species females, $(AA/M)_s$ and $(aa/m)_s$, mate assortatively with their own males with probability α and β , respectively, and mate at random (with the eight types in the population) otherwise. All hybrid females, including the hybrid $(AA/M)_h$ and $(aa/m)_h$ genotypes, mate at random. The matings involving $(AA/M)_s$ females fall into four categories: (i) $(AA/M)_s \text{ } \text{ } \times (AA/M)_s \text{ } \text{ } \delta$ occurs with frequency αu_{1s} and produces only $(AA/M)_s$ progeny; (ii) $(AA/M)_s \text{ } \text{ } \times (AA)_h \text{ } \text{ } \delta$ occurs with frequency $(1 - \alpha)u_{1s}$ ($u_{1h} + u_2$) and produces only $(AA/M)_h$ progeny, where $(AA)_h$ denotes $(AA/M)_h$ or AA/m individuals; (iii) $(AA/M)_s \text{ } \text{ } \times Aa \text{ } \text{ } \delta$ occurs with frequency $(1 - \alpha)u_{1s}v$ and produces half $(AA/M)_h$ + half Aa/M progeny; (iv) $(AA/M)_s \text{ } \text{ } \times aa \text{ } \text{ } \delta$ occurs with frequency $(1 - \alpha)u_{1s}w$ and produces only Aa/M progeny, where aa denotes either $(aa/m)_s$, $(aa/m)_h$, or aa/M individuals. The matings involving $(aa/m)_s$ females are derived similarly.

There are three mating classes for each of the six types of hybrid females, corresponding to the nuclear genotype of the male. The distinction between pure species and hybrid males is not needed in these cases because the progeny necessarily consist of one or more of the six types of hybrids. The mating frequencies are simply the products of the two genotypic frequencies. For example, the mating $(AA/M)_h \text{ } \text{ } \times AA \text{ } \text{ } \delta$ occurs with frequency $u_{1h}u$ and produces only $(AA/M)_h$ progeny, while the mating $Aa/M \text{ } \text{ } \times AA \text{ } \text{ } \delta$ has frequency v_1u and produces half $(AA/M)_h$ + half Aa/M progeny.

Combining the offspring distribution from the 26 possible mating classes produces the recursions for the overall cytonuclear frequencies, the marginal genotypic frequencies, and

the four disequilibria. These are equivalent to those in Eqs. 4–6 for the original epistatic model, with the occurrences of u_1 and w_2 on the right-hand sides replaced by u_{1s} and w_{2s} , respectively. The recursions for the two pure species are

$$u'_{1s} = u_{1s}[\alpha + (1 - \alpha)u_{1s}] \quad \text{and} \quad w'_{2s} = w_{2s}[\beta + (1 - \beta)w_{2s}].$$

Unless the pure species' females only mate assortatively (i.e., $\alpha = 1$ and/or $\beta = 1$), the frequencies of the two pure species monotonically decay to zero at an ever accelerating rate. The rate of loss of a species is faster in each generation the more frequently its females mate at random. Eventually the population will consist only of randomly mating hybrids. Consequently, the disequilibria dynamics reduce asymptotically to those for a completely randomly mating population (1), which means that all four disequilibria ultimately decay to zero.

Based on the original epistatic mating model, one might expect the modified model to nonetheless maintain transient disequilibria over long periods. This hypothesis was tested numerically by running the new model under 27 cases that give rise to complete permanent cytonuclear disequilibria in the original model, setting $u_{1s}^{(0)} = u_1^{(0)}$.

Although the refined model can maintain both nuclear alleles, all four disequilibria always became <0.01 in magnitude within 29–83 generations, with only D_2 temporarily increasing (for 11–27 generations) along the trajectory. It is interesting that D_2 usually had the same sign as, and its maximum magnitude usually exceeded, the corresponding equilibrium value under the epistatic mating model. As in the epistatic model, a nonzero value for D_2 implies some asymmetry in the population.

Discussion

The epistatic mating model demonstrates that permanent nonzero cytonuclear disequilibria are possible in a hybrid population when mating preference is determined by an epistatic interaction between a nuclear gene and a cytoplasmic gene. In contrast, the refined model shows that permanent cytonuclear associations are not possible when mating is based on species status (if assortative mating is less than perfect). These findings have relevance to the biological interpretations placed on cytonuclear associations in hybrid zones.

To illustrate, we will consider the nuclear–mitochondrial genotypic data presented by Lamb and Avise (2) for a hybrid population of tree frogs. At ponds near Auburn, Alabama, extensive introgressive hybridization between *Hyla cinerea* and *Hyla gratiosa* has apparently continued (4) for at least the 30 years since the initial discovery there of frogs exhibiting intermediate morphologies (5). Genetic information for the population, gathered in 1984, consists of mtDNA genotypes, and nuclear genotypes at five unlinked loci, for each of 305 individuals. (The cytoplasmic and nuclear genes assayed normally exhibit fixed allelic differences between the two “pure” species.)

Cytonuclear disequilibria calculated from these data (1) are summarized in Table 2. Are these disequilibria consistent with equilibrium expectations for a realistic set of parameters under the epistatic mating model? Since cytoplasmic gene frequencies always remain constant in this system, the current observed frequencies of the mtDNA types *M* (characteristic of *H. cinerea*) and *m* (characteristic of *H. gratiosa*) can be used as estimates of the original frequencies of the pure parental species at the Auburn site. This yields $u_1^{(0)} = 0.465$ and $w_2^{(0)} = 0.535$. After iterating Eqs. 4–6 under various assortative mating rates, the equilibrium cytonuclear associations (as well as equilibrium genotypic frequencies) when $\alpha = 0.94$ (*H. cinerea*) and $\beta = 0.86$ (*H. gratiosa*) were found, by trial and error, to be in remarkably close accord with observed values for each of the five pairs of nuclear–mitochon-

Table 2. Observed disequilibria in the *Hyla* hybrid population, and values expected at equilibrium under the epistatic mating model with $u_1^{(0)} = 0.465$, $w_2^{(0)} = 0.535$, $\alpha = 0.94$, $\beta = 0.86$

Nuclear locus	Cytonuclear disequilibria			
	D	D_1	D_2	D_3
<i>Alb</i>	0.16	0.19	–0.06	–0.13
<i>Pgi</i>	0.19	0.22	–0.07	–0.15
<i>Ldh</i>	0.18	0.20	–0.05	–0.15
<i>Pep</i>	0.18	0.21	–0.06	–0.15
<i>Mdh</i>	0.17	0.21	–0.07	–0.14
Expected	0.16	0.18	–0.04	–0.14

drial combinations (Fig. 1, Table 2). Furthermore, these α and β values would indicate that while both *H. cinerea* and *H. gratiosa* females tend to mate assortatively with conspecific males, the *H. gratiosa* females are less fidelic. These inferences are qualitatively consistent with the suspected mating behaviors of these species in the hybrid population (2).

Nonetheless, further information can be extracted from a consideration of the multilocus nuclear genotype of each individual in conjunction with its cytotype. The observed numbers of “pure” *H. cinerea* (homozygous for *H. cinerea* alleles at all five nuclear loci and possessing *M* mitochondria), “pure” *H. gratiosa* (homozygous for *H. gratiosa* nuclear alleles and carrying *m* mtDNA), and hybrids (all other genotypes), were $n = 103$, $n = 60$, and $n = 142$, respectively. Thus, a large fraction (53%) of the Auburn *Hyla* population consists of pure parental genotypes. This multilocus approach to an individual's identification provides a more realistic and accurate appraisal of hybrid status than does a single locus approach because, as already noted, some *AA/M* or *aa/m* specimens are themselves of hybrid ancestry. (For example, using mtDNA together with albumin as the sole nuclear gene marker, 19 individuals in the Auburn population would have been misclassified as being pure *H. cinerea* or *H. gratiosa*.)

The refined epistatic mating model, which distinguishes biologically real from illusory parentals, was in fact developed to provide a mathematically tractable analogue to such multilocus assessments of hybrid status. Yet, under the refined epistatic model, pure parental genotypes as well as nonzero cytonuclear disequilibria usually disappear rapidly as the population comes to consist entirely of hybrids. Thus, although the original epistatic mating model can be fitted remarkably well to the observed associations between mtDNA and individual nuclear loci, the multilocus analysis suggests that the single locus agreements are probably spurious. In light of the refined epistatic model, the age of the *Hyla* population together with the presence of pure parentals in high frequency suggests that factors in addition to the mating system are probably involved. Likely possibilities include (i) selection against particular hybrid classes, and/or (ii) continued immigration of pure parentals from surrounding populations. We plan to model these scenarios and their influence on cytonuclear disequilibria elsewhere.

We thank A. Datta for her combination random number generator. This work was supported by National Science Foundation Grants BSR-8420803, BSR-8716804, and BSR-8603775.

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