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CYTONUCLEAR THEORY FOR HAPLODIPLOID SPECIES AND X-LINKED GENES. II. STEPPING-STONE MODELS OF GENE FLOW AND APPLICATION TO A FIRE ANT HYBRID ZONE

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Abstract.—We develop cytonuclear, hybrid zone models for haplodiploid species or X-linked genes in diploid species using a stepping-stone framework of migration, in which migration rates vary with both direction and sex. The equilibrium clines for the allele frequencies, cytonuclear disequilibria, and frequencies of pure parental types are examined for species with diagnostic markers, under four important migration schemes: uniform migration of both sexes in both directions, greater migration of both sexes from one direction, greater migration of females, and greater migration of males. Of the three cytonuclear variables examined, the allele frequency clines are the most informative in differentiating among the various migration patterns. The cytonuclear disequilibria and the frequency of the pure parental types tend to be useful only in revealing directional asymmetries in migration. The extent of hybrid zone subdivision has quantitative but not qualitative effects on the distribution of cytonuclear variables, in that the allele frequency clines become more gradual, the cytonuclear disequilibria decrease in magnitude, and the frequencies of pure parentals decline with increasing subpopulation number. Also, the only major difference between the X-linked and haplodiploid frameworks is that a higher frequency of pure parentals is found when considering haplodiploids, in which male production does not require mating. The final important theoretical result is that censusing after migration yields greater disequilibria and parental frequencies than censusing after mating. We analyzed cytonuclear data from two transects from a naturally occurring hybrid zone between two haplodiploid fire ant species, *Solenopsis invicta* and *S. richteri,* using our stepping-stone framework. The frequency of *S. invicta* mtDNA exceeds the frequency of the *S. invicta* nuclear markers through much of this hybrid zone, indicating that sex differences in migration or selection may be occurring. Maximum-likelihood estimates for the migration rates are very high, due to an unexpectedly large number of pure parental types in the hybrid zone, and differ substantially between the two transects. Overall, our model does not provide a good fit, in part because the *S. invicta–S. richteri* hybrid zone has not yet reached equilibrium.

Key words.—Cytonuclear, disequilibria, fire ants, haplodiploid, hybrid zone, *Solenopsis invicta, Solenopsis richteri*, X-linked.

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Hybridization between two genetically differentiated populations may result in the formation of allele frequency clines, and the size and shape of these clines may serve as an indicator of the evolutionary forces that are operating within a hybrid zone (Bazykin 1969; Endler 1973, 1977; Slatkin 1973; May et al. 1975; Nagylaki 1975, 1976; Felsenstein 1976; Moore 1977; Barton 1979a,b, 1983; Barton and Hewitt 1985, 1989; Mallet and Barton 1989; Barton and Clark 1990; Harrison 1990; Mallet et al. 1990; Barton and Gale 1993). Studies of these clines can be particularly informative if data are obtained from both cytoplasmic and nuclear genomes of the same individuals because the uniparental inheritance of cytoplasmic markers accompanied by the biparental inheritance of nuclear markers allows for the detection of evolutionary forces that affect the sexes differently, such as differential dispersal of, or selection in, males or females (Avise et al. 1990; Forbes and Allendorf 1991; Paige et al. 1991; Arnold 1993; Cruzan and Arnold 1993, 1994; Moya et al. 1993; Scribner and Avise 1993; Abernethy 1994; Asmussen and Basten 1994; Oldroyd et al. 1995; Babcock and Asmussen 1996, 1998; Hare and Avise 1996; Sites et al. 1996). To aid in the analysis of such data, mathematical models and statistical methodology have been developed that allow for the interpretation of cytonuclear frequencies and disequilibria (nonrandom associations between nuclear and cytoplasmic loci) both within hybrid zones (Arnold et al. 1988; Asmussen et al. 1989; Asmussen and Arnold 1991; Arnold 1993; Dean and Arnold 1996; Avise et al. 1997; Gill 1997; Harrison and Bogdanowicz 1997) and in other evolutionary contexts (Clark 1984; Asmussen et al. 1987; Asmussen and Schnabel 1991; Fu and Arnold 1991, 1992; Schnabel and Asmussen 1992; Cellino and Arnold 1993; Asmussen and Basten 1994, 1996; Datta and Arnold 1996; Datta et al. 1996a,b; Basten and Asmussen 1997).

However, the existing theoretical framework is not appropriate for the analysis of all types of hybrid zones. For example, most previous cytonuclear theory has dealt with autosomal, nuclear loci in diploid organisms and has not allowed for important situations where males are haploid and females diploid, as is the case in haplodiploid organisms or when considering sex-linked loci in many animals (but see Owen [1986] for a discussion of autosomal genes in haplodiploids). Such models may be particularly important in light of the perceived significance of the sex chromosomes in the speciation process (Coyne and Orr 1989; Coyne 1992, 1994; Sperling 1994), the frequent lack of concordance between clines for X-linked and nuclear markers (Moran 1979; Hewitt et al. 1988; Shaw et al. 1988; Hagen 1990; Sperling and Spence 1991; Tucker et al. 1992; Dod et al. 1993), and the ecological and evolutionary importance of haplodiploid organisms (LaSalle and Gauld 1993; Crozier and Pamilo 1996;

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TABLE 1. Frequencies of joint cytonuclear genotypes in females.

Cytotype	Nuclear genotype *AA*	*Aa*	*aa*	Total
C	$u_1 = ux_f + D_1$	$v_1 = vx_f + D_2$	$w_1 = wx_f + D_3$	x_f
c	$u_2 = uy_f - D_1$	$v_2 = vy_f - D_2$	$w_2 = wy_f - D_3$	y_f
Total	u	v	w	1.0

Jervis and Kidd 1996). We have recently taken a first step in addressing this shortcoming by developing the basic cytonuclear framework for haplodiploid and sex-linked systems and determining the baseline dynamics of their sex-specific, cytonuclear frequencies and disequilibria under Hardy-Weinberg conditions (Goodisman and Asmussen 1997). In this initial study, we also formulated a model of unidirectional gene flow and hybridization that allows for assortative mating of pure species individuals and differential migration by the sexes. However, this and previous cytonuclear, hybrid zone models (Asmussen et al. 1989) have assumed a simple continent-island, population structure whereby genetically differentiated individuals migrate into a single area of hybridization. Such formulations cannot account for the frequent observation of clinal variation for both nuclear and cytoplasmic markers (Shaw et al. 1988; Baker and Davis 1989; Dowling and Hoeh 1991; Paige et al. 1991; Arnold et al. 1992; Latorre et al. 1992; Cruzan and Arnold 1993; Dod et al. 1993; Larruga et al. 1993; Scribner and Avise 1993; Abernethy 1994; Hare and Avise 1996; Sites et al. 1996; Gill 1997; Harrison and Bogdanowicz 1997; Jaarola et al. 1997).

Here, we expand on our previous work in an attempt to account for the observation of population structure within hybrid zones. Our focus is on genetic systems where males are haploid and females are diploid, as is the case with haplodiploid organisms or sex-linked, nuclear loci in diploid organisms. We extend our continent-island framework (Goodisman and Asmussen 1997) into a one-dimensional, stepping-stone model, whereby gene flow is allowed only between adjacent subpopulations within the hybrid zone. We then investigate numerically the equilibrium clines of key cytonuclear variables under a series of biologically important migration schemes in which migration rates may vary with direction or sex. Two separate formulations, corresponding to the two possible census schemes (after migration and before mating vs. before migration and after mating), are analyzed, as well as the distinctions that arise when considering X-linked loci in diploid species versus autosomal loci in haplodiploid species. Finally, we examine the distribution of cytonuclear variables across a naturally occurring area of hybridization between two fire ant species, *Solenopsis invicta* and *S. richteri*, and fit our stepping-stone model to these data to determine to what extent differential migration by sex and by direction can account for the observed cytonuclear structure.

CYTONUCLEAR SYSTEM

Our two-locus, cytonuclear framework follows that of Goodisman and Asmussen (1997) and is applicable to haplodiploid species and X-linked nuclear loci in diploid species. However, for ease of discussion, we will describe the parameterization in the context of haplodiploids. Females receive half of their nuclear complement from their mother and half from their father, whereas their haploid, cytoplasmic genome is strictly maternally inherited. Males receive their single allele at each nuclear and cytoplasmic locus from their mother. We assume two alleles at both the nuclear locus (*A, a*) and the cytoplasmic locus (*C, c*). The frequencies of the six cytonuclear genotypes in the diploid females are as shown in Table 1, along with their marginal nuclear genotype and cytoplasmic (cytotype) frequencies. From these, the marginal frequencies of the two nuclear alleles in females are calculated as $p_f = \text{freq}(A) = u + \frac{1}{2}v$ and $q_f = 1 - p_f = \text{freq}(a) = w + \frac{1}{2}v$, where freq denotes the frequency in females. It will also be convenient to define the four female cytonuclear diallelic combinations (Table 2), whose frequencies represent those of female gametes if the cytoplasmic marker is maternally (or biparentally) inherited and no selection occurs (Asmussen and Basten 1994). Formally, $p_1^f = \text{freq}(A/C)$, for example, is defined as the probability that a random female from the population has cytotype *C* and that a randomly sampled allele at her nuclear locus is *A*. These variables provide useful analogs to the male genotype frequencies.

As in our previous study (Goodisman and Asmussen 1997), we consider four measures of cytonuclear disequilibrium in females. The three female genotypic disequilibria: $D_1 = \text{freq}(AA/C) - \text{freq}(AA)\text{freq}(C) = u_1 - ux_f$, $D_2 = \text{freq}(Aa/C) - \text{freq}(Aa)\text{freq}(C) = v_1 - vx_f$, and $D_3 = \text{freq}(aa/C) - \text{freq}(aa)\text{freq}(C) = w_1 - wx_f$ quantify the nonrandom associations between the cytotypes and each of the three, female nuclear genotypes, whereas the female allelic disequilibrium, $D_f = \text{freq}(A/C) - \text{freq}(A)\text{freq}(C) = p_1^f - p_f x_f$, measures the nonrandom association between the nuclear and cytoplasmic alleles in females. The four female cytonuclear disequilibria are related by $D_f = D_1 + \frac{1}{2}D_2$ and $D_1 + D_2 + D_3 = 0$. Although they represent only two independent statistics, we consider all four measures of female cytonuclear disequilibria, because their joint sign patterns can be valuable markers of evolutionary processes (Asmussen et al. 1989).

The haploid males have only four possible joint genotypes whose frequencies are given in Table 3, along with the marginal allele frequencies at both loci. Males have only a single,

TABLE 2. Frequencies of cytonuclear diallelic combinations in females.

Cytotype	Nuclear allele *A*	*a*	Total
C	$p_1^f = u_1 + \frac{1}{2}v_1 = p_f x_f + D_f$	$q_1^f = w_1 + \frac{1}{2}v_1 = q_f x_f - D_f$	x_f
c	$p_2^f = u_2 + \frac{1}{2}v_2 = p_f y_f - D_f$	$q_2^f = w_2 + \frac{1}{2}v_2 = q_f y_f + D_f$	y_f
Total	$p_f = u + \frac{1}{2}v$	$q_f = w + \frac{1}{2}v$	1.0

TABLE 3. Frequencies of joint cytonuclear genotypes in males.

Cytotype	Nuclear allele: A	Nuclear allele: a	Total
C	$p_1^m = p_m x_m + D_m$	$q_1^m = q_m x_m - D_m$	x_m
c	$p_2^m = p_m y_m - D_m$	$q_2^m = q_m y_m + D_m$	y_m
Total	p_m	q_m	1.0

allelic disequilibrium, defined as D_m = freq(A/C) − freq(A)freq(C) = $p_1^m - p_m x_m$, where freq now denotes the frequency in males.

HYBRID ZONE MODEL

We assume that individuals from two genetically differentiated source populations of constant genetic composition continuously migrate into an area where mating occurs. Inside the area of hybridization, all individuals have equal fitness, population size is large enough to preclude the effects of drift, there is no mutation, mating occurs at random within subpopulations, and generations are discrete and nonoverlapping.

Hybrid Zone Structure

In contrast to our previous continent-island models of hybridization (Asmussen et al. 1989; Goodisman and Asmussen 1997), we now assume a more complex, one-dimensional stepping-stone formulation (Kimura and Weiss 1964; Maruyama 1970a,b, 1971). As shown in Figure 1, the hybrid zone is divided into n subpopulations that lie along a straight line (i.e., a transect). Each subpopulation is separated from its nearest neighbors by the distance that an individual can disperse in a single generation, and migration only occurs between adjacent subpopulations (or the adjacent subpopulation and source population in the case of subpopulations 1 and n). Although our model is more general, we focus our analysis on situations where the two hybridizing species are fixed for alternate alleles at both their nuclear and cytoplasmic loci. Under this scenario, source population 1 contains pure species females and males of genotype *AA/C* and *A/C*, respectively, whereas source population 2 contains only pure females and males of genotypes *aa/c* and *a/c*.

We assume that every generation a constant fraction, $m_f^{(1)}$, of females within any given subpopulation in the hybrid zone are migrants from the adjacent (sub)population in the direction of source population 1, and a constant fraction of females, $m_f^{(2)}$, are migrants from the adjacent (sub)population in the direction of source population 2. The remaining fraction of females, $1 - m_f^{(1)} - m_f^{(2)}$, are offspring produced by random mating among the previous residents in that subpopulation. Males may migrate at different rates than the females, with the corresponding male migration rates denoted as $m_m^{(1)}$ and $m_m^{(2)}$. This migration scheme thus assumes that each subpopulation acts as an island exchanging migrants with the two adjacent (sub)populations, with the exception of the two source populations, which do not receive migrants from the hybrid zone.

We denote the value of a given cytonuclear variable z in subpopulation i as $z^{(i)}$, and the value in the combined, incoming migrant pool for that subpopulation as $\bar{z}^{(i)}$. For a frequency variable, $\bar{z}^{(i)}$ is simply the average of the corresponding frequencies in the two neighboring (sub)populations weighted by the appropriate sex-specific migration rates. Formally, the cytotype frequency of females migrating into subpopulation i, for example, is given by $\bar{x}_f^{(i)} = (m_f^{(1)} x_f^{(i-1)} + m_f^{(2)} x_f^{(i+1)})/(m_f^{(1)} + m_f^{(2)})$, where for subpopulation 1, $x_f^{(i-1)}$ is the female cytotype frequency in source population 1, and for subpopulation n, $x_f^{(i+1)}$ is the female cytotype frequency in source population 2. If there are fixed differences between the two source populations then $x_f^{(i-1)} = 1$ for subpopulation 1 and $x_f^{(i+1)} = 0$ for subpopulation n. The cytonuclear disequilibria in the migrant pool can be calculated directly from the definitions (e.g., $\overline{D}_1^{(i)} = \bar{u}_1^{(i)} - \bar{u}^{(i)}\bar{x}_f^{(i)}$) or via the admixture formulae for haplodiploid and X-linked cytonuclear systems (Goodisman and Asmussen 1997).

Pure Species Status

Within the hybrid zone, individuals that possess two-locus homospecific allelic combinations may be pure parental individuals or they may be hybrids that look like pure parental individuals at the two loci. As in previous formulations (Arnold et al. 1988; Asmussen et al. 1989; Goodisman and Asmussen 1997), it is useful to divide such homoallelic individuals into two different classes based on their pure species

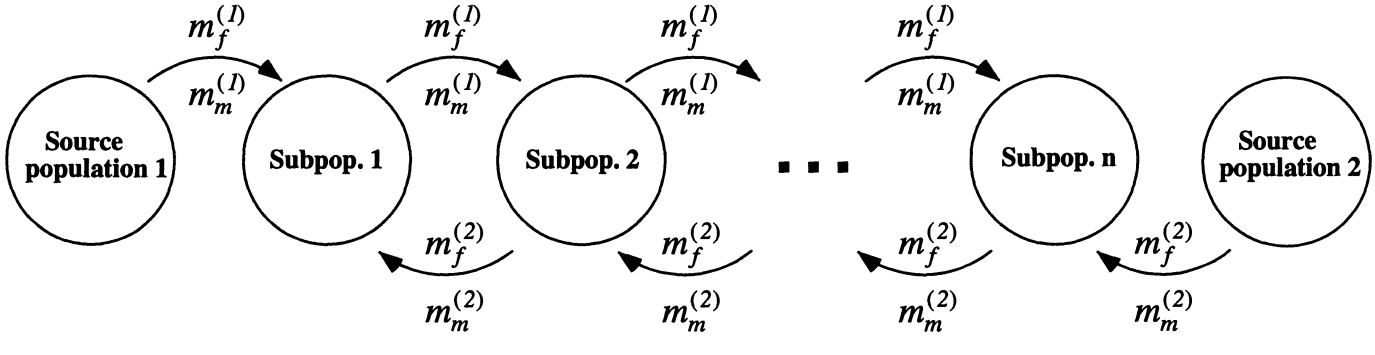

FIG. 1. Stepping-stone model of migration with n subpopulations and two source populations. Each generation, a constant fraction of females and males in each subpopulation come from the adjacent (sub)population in the direction of source population 1 ($m_f^{(1)}$ and $m_m^{(1)}$) and 2 ($m_f^{(2)}$ and $m_m^{(2)}$). The remaining fraction of females ($1 - m_f^{(1)} - m_f^{(2)}$) and males ($1 - m_m^{(1)} - m_m^{(2)}$) are the offspring of previous residents in the subpopulation.

status. The frequency of *AA/C* females in subpopulation *i*, for instance, is decomposed as $u_1^{(i)} = u_{1s}^{(i)} + u_{1h}^{(i)}$, where the subscript *s* represents a pure species individual and the subscript *h* represents a hybrid with the same two-locus genotype. Similarly, we may divide the other two-locus, homoallelic classes to yield the corresponding divisions, $w_2^{(i)} = w_{2s}^{(i)} + w_{2h}^{(i)}$, $p_1^{m(i)} = p_{1s}^{m(i)} + p_{1h}^{m(i)}$, and $q_2^{m(i)} = q_{2s}^{m(i)} + q_{2h}^{m(i)}$. In practice, these subclasses may be distinguished from one another with high accuracy through the examination of multiple diagnostic nuclear markers in conjunction with a diagnostic cytoplasmic marker. Our previous study has revealed that pure type individuals have different dynamics in haplodiploid and diploid, X-linked models (Goodisman and Asmussen 1997). These distinctions arise because of the differing modes of male production in the two systems. Haplodiploid males are produced parthenogenetically; therefore all the sons of a pure type female will be pure type males. This is not true in the diploid case, where a pure species son is produced if and only if a pure species female mates with a conspecific male. We will first consider the case of haplodiploids; the differences that arise when considering X-linked genes in diploid organisms will be addressed in a subsequent section.

Numerical Analysis

The complex nature of the stepping-stone model precludes solving analytically for the equilibria of the cytonuclear frequencies and disequilibria within each subpopulation, so we have investigated their behavior numerically. We assume that the hybrid zone is initiated with pure species, with the *n*/2 subpopulations closest to source population 1 initially containing only individuals of species 1 and the *n*/2 subpopulations closest to source 2 initially containing only individuals of pure species 2. In practice, however, the starting conditions do not matter; examination of simulated hybrid zones initiated with varying genotype frequency arrays indicates that the final frequencies of the individuals within each subpopulation depend only on the migration rates.

After the simulated hybrid zone has been initiated with the starting genotype array, we allow for random mating within, and migration between, adjacent subpopulations. Because census time can significantly affect the results (Asmussen et al. 1989; Asmussen and Arnold 1991; Goodisman and Asmussen 1997), we consider the two census schemes shown in Figure 2: censusing after migration and before mating (census 1) or before migration and after mating (census 2). We will first focus on results obtained under census 1, and the effects of census time will be addressed in a subsequent section. In either case, the joint cytonuclear genotype frequencies within any subpopulation change from one generation to the next according to the recursions for the appropriate continent-island model of hybridization for haplodiploid species (Goodisman and Asmussen 1997; Appendices A and B). We allow mating and migration to continue until the frequencies of all cytonuclear genotypes in each subpopulation do not change by more than 10^{-9} in a single generation. At this point the hybrid zone is defined as being at equilibrium.

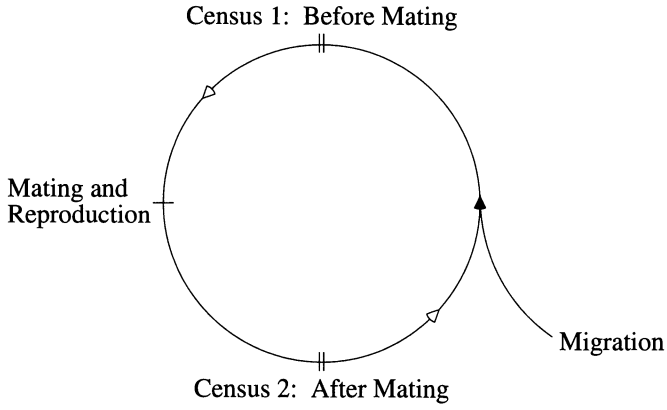

FIG. 2. Census times of individuals within hybrid zones. Censusing may occur after migration and before mating (census 1) or after mating and before migration (census 2).

CASE STUDIES

We turn now to a characterization of the equilibrium clines for the nuclear and cytoplasmic alleles, the cytonuclear disequilibria, and the pure parental types under a series of migration schemes including uniform migration from each direction and sex, greater migration of both sexes from one direction, greater female migration from one or both directions, and greater male migration from one or both directions. We will initially consider a hybrid zone composed of $n = 10$ subpopulations and will discuss the effect of the number of subpopulations later.

Allele Frequencies

We begin with the basic issue of how these four major patterns of migration affect the equilibrium clines for the sex-specific nuclear and cytoplasmic allele frequencies across the hybrid zone.

Uniform Migration ($m_f^{(1)} = m_f^{(2)} = m_m^{(1)} = m_m^{(2)} = m$).—This first, simple case with equal migration of both sexes in both directions provides a useful baseline from which the effects of the more complex migration scenarios can be interpreted. Figure 3A shows the equilibrium nuclear and cytoplasmic allele frequencies in both sexes when $m = 0.1$, and reflects the expected effect of neutral diffusion of alleles across the hybrid zone. All four allele frequency clines coincide and form a straight line. Increasing the migration rate does not alter the patterns or values of the allele frequencies across the hybrid zone.

Greater Migration from One Direction ($m_f^{(1)} = m_m^{(1)} = m^{(1)} > m_f^{(2)} = m_m^{(2)} = m^{(2)}$).—The effects of a direction-based asymmetry in the migration rates of both sexes are shown in Figure 3B for the case where $m^{(1)} = 0.2$ and $m^{(2)} = 0.1$. In contrast to the case of uniform migration (Fig. 3A), the hybrid zone is now dominated by the more mobile, species 1 alleles until near source population 2. Furthermore, although all four clines still coincide, the common cline is shifted toward source population 2, with its start farther into the hybrid zone, and it is concave down rather than linear. Not surprisingly, a larger difference between the two migration rates, $m^{(1)}$ and

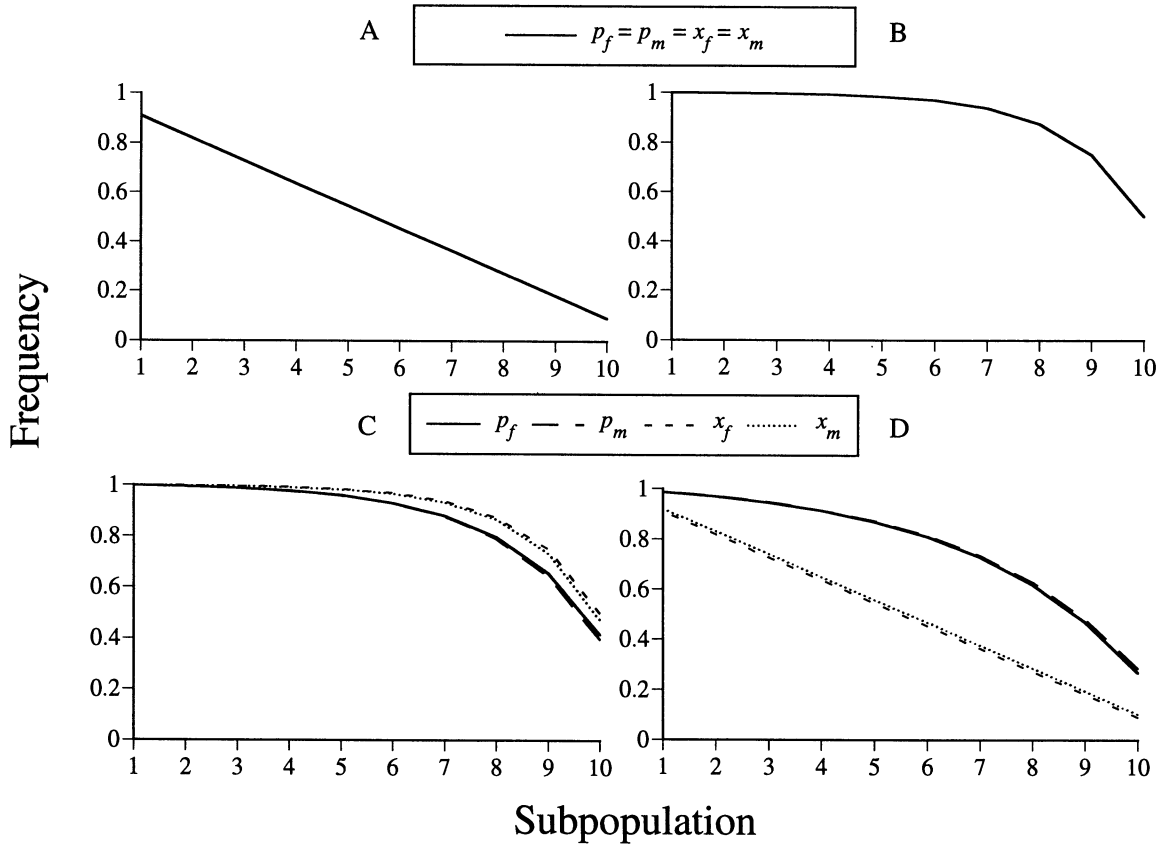

FIG. 3. Female and male nuclear (p_f and p_m) and cytoplasmic (x_f and x_m) allele frequencies across a haplodiploid hybrid zone composed of 10 subpopulations for (A) uniform migration rates with $m_f^{(1)} = m_f^{(2)} = m_m^{(1)} = m_m^{(2)} = 0.1$; (B) greater migration from one direction (source population 1) with $m_f^{(1)} = m_m^{(1)} = 0.2 > m_f^{(2)} = m_m^{(2)} = 0.1$; (C) greater female migration from one direction (source population 1) with $m_f^{(1)} = 0.2 > m_f^{(2)} = m_m^{(1)} = m_m^{(2)} = 0.1$; and (D) greater male migration from one direction (source population 1) with $m_m^{(1)} = 0.2 > m_f^{(1)} = m_f^{(2)} = m_m^{(2)} = 0.1$.

$m^{(2)}$, results in an even higher frequency of species 1 alleles, and a correspondingly greater shift of the common cline.

Greater Female Migration.—If the migration rates of females are uniformly greater than those of males ($m_f^{(1)} = m_f^{(2)} = m_f > m_m^{(1)} = m_m^{(2)} = m_m$), then the allele frequency clines in the two sexes are the same as those for the baseline model with $m = m_f$ (Fig. 3A) whether or not there is any male migration ($m_f > m_m \geq 0$). This scenario is indistinguishable from neutral diffusion since each sex migrates equally from each direction (i.e., $m_f^{(1)} = m_f^{(2)}$ and $m_m^{(1)} = m_m^{(2)}$), and thus the rates within each sex effectively oppose one another.

If, instead, the migration rate of females is greater from one direction only ($m_f^{(1)} > m_f^{(2)} = m_m^{(1)} = m_m^{(2)}$), then new distinctive patterns arise. In this case, all four clines are distinct and show a hierarchical equilibrium relationship within any subpopulation of $p_m < p_f \ll x_m < x_f$, with higher female than male frequencies and higher cytoplasmic than nuclear frequencies (Fig. 3C). The shape of each cline resembles the single cline seen in the corresponding case of greater migration from one direction by both sexes (Fig. 3B), with the species 1 alleles carried by the more mobile females predominating well into the zone. The female cytotype frequencies are, in fact, identical in both these cases, because x_f is governed solely by the female migration rates. A larger disparity between $m_f^{(1)}$ and the other migration rates leads to a greater frequency of species 1 alleles in the hybrid zone, and a closer correspondence between the four clines. Surprisingly, if $m_f^{(1)}$ is sufficiently different from the other migration rates, the two inner clines for the female nuclear allele frequency, p_f, and the male cytotype frequency, x_m, may reverse order in the equilibrium hierarchy; however, the female cytotype frequency, x_f, and the male nuclear allele frequency, p_m, always remain as the maximum and minimum values, respectively.

Greater Male Migration.—When males migrate at a uniformly greater rate than females in both directions ($m_m^{(1)} = m_m^{(2)} = m_m > m_f^{(1)} = m_f^{(2)} = m_f$), the two rates for each sex again cancel and yield the neutral diffusion clines found in the baseline case (Fig. 3A) and uniformly greater female migration, provided that there is some female migration ($m_m > m_f > 0$). The case of male migration only ($m_m > m_f = 0$) is degenerate in that, without female movement, the female cytotype frequency in any subpopulation will not change (because only females transmit cytoplasmic alleles), and the cytoplasmic and joint cytonuclear clines will thus depend on the initial conditions of the hybrid zone.

Higher male migration from one direction only ($m_m^{(1)} >$

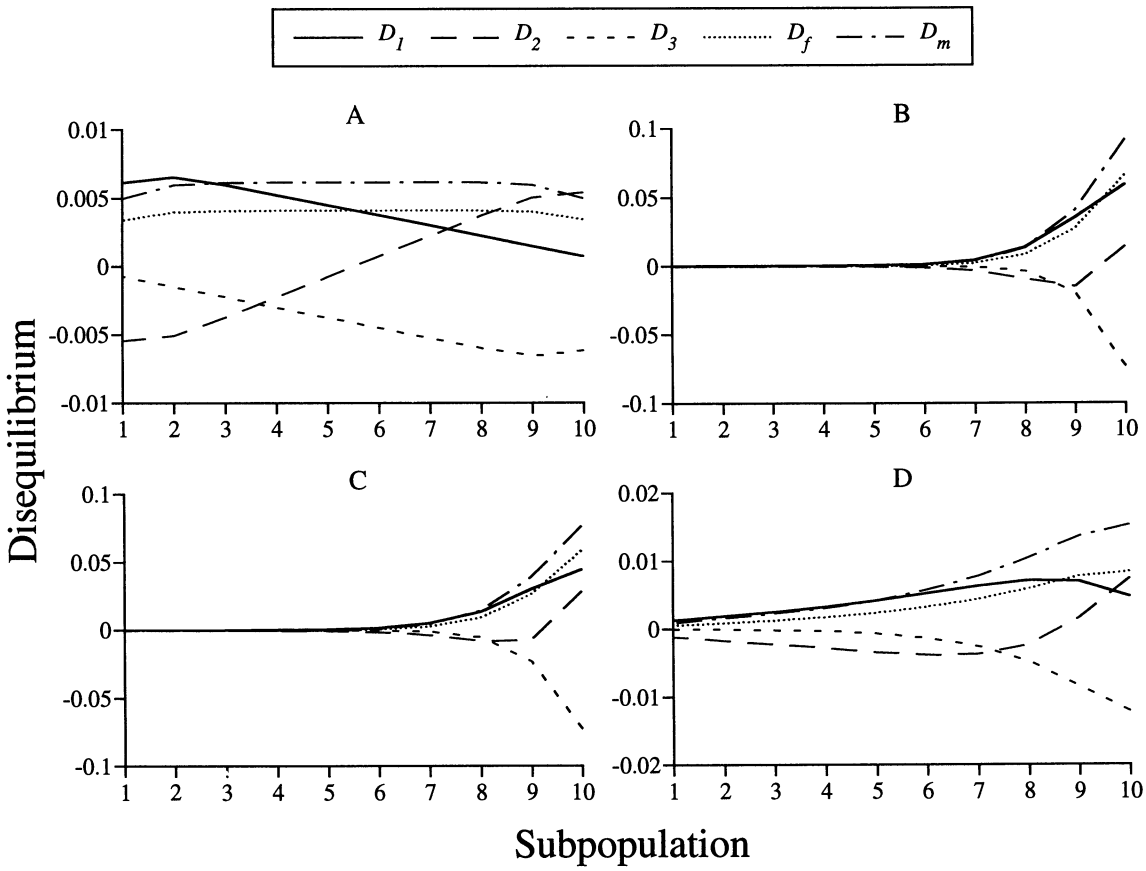

FIG. 4. Female genotypic (D_1, D_2, and D_3), and female and male allelic (D_f and D_m) cytonuclear disequilibria across a haplodiploid hybrid zone composed of 10 subpopulations for (A) uniform migration rates with $m_f^{(1)} = m_f^{(2)} = m_m^{(1)} = m_m^{(2)} = 0.1$; (B) greater migration from one direction (source population 1) with $m_f^{(1)} = m_m^{(1)} = 0.2 > m_f^{(2)} = m_m^{(2)} = 0.1$; (C) greater female migration from one direction (source population 1) with $m_f^{(1)} = 0.2 > m_f^{(2)} = m_m^{(1)} = m_m^{(2)} = 0.1$; and (D) greater male migration from one direction (source population 1) with $m_m^{(1)} = 0.2 > m_f^{(1)} = m_f^{(2)} = m_m^{(2)} = 0.1$. Note the difference in scale across panels.

$m_f^{(1)} = m_f^{(2)} = m_m^{(2)}$) has striking differences from the corresponding female case. As shown in Figure 3D, within any subpopulation, we find the reverse equilibrium order, $x_f < x_m \ll p_f < p_m$, with the sex with the higher migration rate again having the higher allele frequencies, but the nuclear frequencies now exceeding the cytotype frequencies throughout the hybrid zone. Furthermore, the difference between the two markers is now much more pronounced, and the shapes of the clines are substantially altered. The unidirectional increase in gene flow by the haploid males yields lower nuclear clines that begin substantially closer to source population 1, whereas the cytotype frequencies are virtually unaffected by the asymmetrical male migration and thus show neutral diffusion. When the difference between the higher male migration rate, $m_m^{(1)}$, and the other three migration rates becomes larger, the hybrid zone is increasingly dominated by the nuclear alleles of species 1. If $m_m^{(1)}$ is sufficiently greater than the other migration rates, the start of the male cytotype cline will also be shifted farther into the hybrid zone, although not to the extent of the nuclear clines, whereas the female cytotype frequency remains unaffected by the increased male migration. At the same time, we find that the female nuclear allele frequency, p_f, and the male cytotype frequency, x_m, may reverse order in the equilibrium hierarchy, as in the corresponding female case, although the male nuclear allele frequency, p_m, and female cytotype frequency, x_f, always remain as the outer bounds.

Cytonuclear Disequilibria

We now examine the clines for the steady-state cytonuclear disequilibria in the two sexes.

Uniform Migration ($m_f^{(1)} = m_f^{(2)} = m_m^{(1)} = m_m^{(2)} = m$).—All five disequilibrium clines have distinctive patterns in the baseline case. For example, as shown in Figure 4A, the female homozygote disequilibrium, D_1, remains positive throughout the hybrid zone but declines steadily in magnitude (after a slight increase from subpopulation 1 to 2) as one moves farther from source population 1. The value of the alternate homozygote disequilibrium, D_3, is always negative and forms a perfect reflection of D_1 through the center of the graph. The female heterozygote disequilibrium, D_2, is also symmetrical through the midpoint of the graph, increases continuously in value, and changes sign in the middle of the hybrid zone; its magnitude is thus greatest near the two source populations and declines to zero toward the center of the hybrid zone. The two allelic associations, D_f and D_m, show yet another pattern. Each increases to a plateau in the interior

of the hybrid zone and remains positive throughout, with the value of D_m being approximately 1.5 times the value of D_f in any given subpopulation. The general trend of the male allelic disequilibrium being greater than the female value is consistent with previous results from the continent-island model (Goodisman and Asmussen 1997); in haplodiploid and X-linked systems, female genomes have undergone one extra round of mating relative to those of the males (who are a reflection of their mothers), and their allelic disequilibrium is therefore below that of the males.

Although the baseline, disequilibrium patterns are interesting, it should be noted that with $m = 0.1$, their magnitudes are negligible (< 0.01) and they would therefore be difficult to detect experimentally (Asmussen and Basten 1994; Basten and Asmussen 1997). A higher migration rate, m, will lead to greater values of cytonuclear disequilibria that are more readily detectable, although the general patterns of the clines will remain the same. For example, if $m = 0.3$, then there is an approximately fivefold increase over the associations when $m = 0.1$, whereas a migration rate of $m = 0.02$ leads to an approximately fivefold decrease in the disequilibria.

Greater Migration from One Direction ($m_f^{(1)} = m_m^{(1)} = m^{(1)} > m_f^{(2)} = m_m^{(2)} = m^{(2)}$).—The disequilibrium patterns with a directional asymmetry are very different from those in the baseline model. For example, for the case of $m^{(1)} = 0.2$ and $m^{(2)} = 0.1$, we see from Figure 4B that the disequilibria now all attain substantial values near source population 2, where the allele frequencies become more intermediate (Fig. 3B). The female homozygote disequilibria, D_1 and D_3, are now imperfect reflections of one another about the zero disequilibrium axis, rather than the center of the graph, with D_1 still always positive and D_3 negative. Furthermore, D_2 no longer shows symmetry about the midpoint of the graph and is negative except near source population 2. However, as with uniform migration, D_m is always about 1.5 times as large as D_f, and both allelic associations are always positive. The overall pattern of the four disequilibria is intensified by larger differences between $m^{(1)}$ and $m^{(2)}$. Because there is then an even higher frequency of species 1 alleles near source population 1, the magnitudes of the disequilibria become smaller in that part of the hybrid zone, although they take on even larger values near source population 2. Furthermore, in some cases, D_2 may be negative throughout the hybrid zone.

Greater Female Migration.—When the migration rates of females are uniformly greater than those of males ($m_f^{(1)} = m_f^{(2)} = m_f > m_m^{(1)} = m_m^{(2)} = m_m$), the steady-state female disequilibria are remarkably similar to the baseline case (Fig. 4A) with $m = m_f$; in fact, for a given value of m_f, the female values are the same, regardless of the magnitude of m_m. The main difference is that the greater the disparity in the female and male migration rates, the closer the allelic disequilibria in the two sexes will be, culminating in the extreme case of no male migration, for which the male allelic disequilibrium equals that of females.

If, instead, female migration is higher from one direction only ($m_f^{(1)} > m_f^{(2)} = m_m^{(1)} = m_m^{(2)}$), we see from Figure 4C that the clines are very similar to those when the two sexes have an equal directional bias (Fig. 4B). Paralleling the latter case, increasing the migration differential leads to smaller cytonuclear disequilibria in most of the hybrid zone, even higher values near source population 2, and occasionally negative female heterozygote disequilibria (D_2).

Greater Male Migration.—In general, when the migration rates of males are uniformly greater than those of females ($m_m^{(1)} = m_m^{(2)} = m_m > m_f^{(1)} = m_f^{(2)} = m_f$), the overall disequilibrium patterns are similar to those in both the baseline case and uniformly greater female migration, but the magnitudes of the disequilibria are now reduced. For example, when $m_f = 0.1$ and $m_m = 0.2$, there is a twofold reduction in the disequilibria relative to those when $m_f = 0.2$ and $m_m = 0.1$. Furthermore, as the female migration rates approach (but do not equal) zero, the female disequilibria vanish. This magnifies the sex differences because continued male migration allows for positive and measurable male disequilibria provided that $m_m > 0.3$.

Figure 4D shows the cytonuclear disequilibria when male migration is higher from one direction only ($m_m^{(1)} > m_f^{(1)} = m_f^{(2)} = m_m^{(2)}$). The shapes of the disequilibrium clines are somewhat like those with a corresponding, unidirectional bias in female migration (Fig. 4C), but again with elevated male migration all the disequilibria are much smaller; the values of the five disequilibria are negligible (< 0.01) in virtually every subpopulation, with the exception of D_3 and D_m, which reach measurable levels near source population 2. In accord with the other cases shown in Figure 4, D_1, D_f, and D_m are always positive, D_3 is always negative, and the sign of D_2 varies from negative to positive as one moves in the direction of source population 2. As with the other forms of directional bias, a greater difference between $m_m^{(1)}$ and the other migration rates yields cytonuclear disequilibria that are smaller in magnitude near source population 1 and greater near source population 2, with possibly negative values of D_2 throughout the hybrid zone.

Frequencies of Pure Species

The last important equilibrium distributions we will examine are those of the four, pure type individuals.

Uniform Migration ($m_f^{(1)} = m_f^{(2)} = m_m^{(1)} = m_m^{(2)} = m$).—As shown in Figure 5A, the pure species frequencies within each sex (e.g., u_{1s} and w_{2s}) are reflections of each other about the midpoint of the transect, and, as in the simpler, continent-island framework (Goodisman and Asmussen 1997), the frequencies of the two pure type males are consistently larger than those of the corresponding females (i.e., $p_{1s}^m > u_{1s}$, $q_{2s}^m > w_{2s}$). The frequencies of the pure types are high near their corresponding source populations, but they fall off rapidly, and are negligible in the middle of the zone. Higher gene flow does not alter the pattern but does increase the frequency of the pure type individuals in the hybrid zone. For instance, when $m = 0.35$, approximately 50% of each sex in subpopulations 1 and 10 are pure types, and all 10 subpopulations have measurable frequencies (> 0.03) of at least one of the two pure species males, where a measurable frequency is defined as having a 95% chance of finding at least one such individual given a sample size of 100.

Greater Migration from One Direction ($m_f^{(1)} = m_m^{(1)} = m^{(1)} > m_f^{(2)} = m_m^{(2)} = m^{(2)}$).—Figure 5B shows the frequencies of the four pure type individuals in the hybrid zone when $m^{(1)} = 0.2$ and $m^{(2)} = 0.1$. As expected, the clines of the two pure

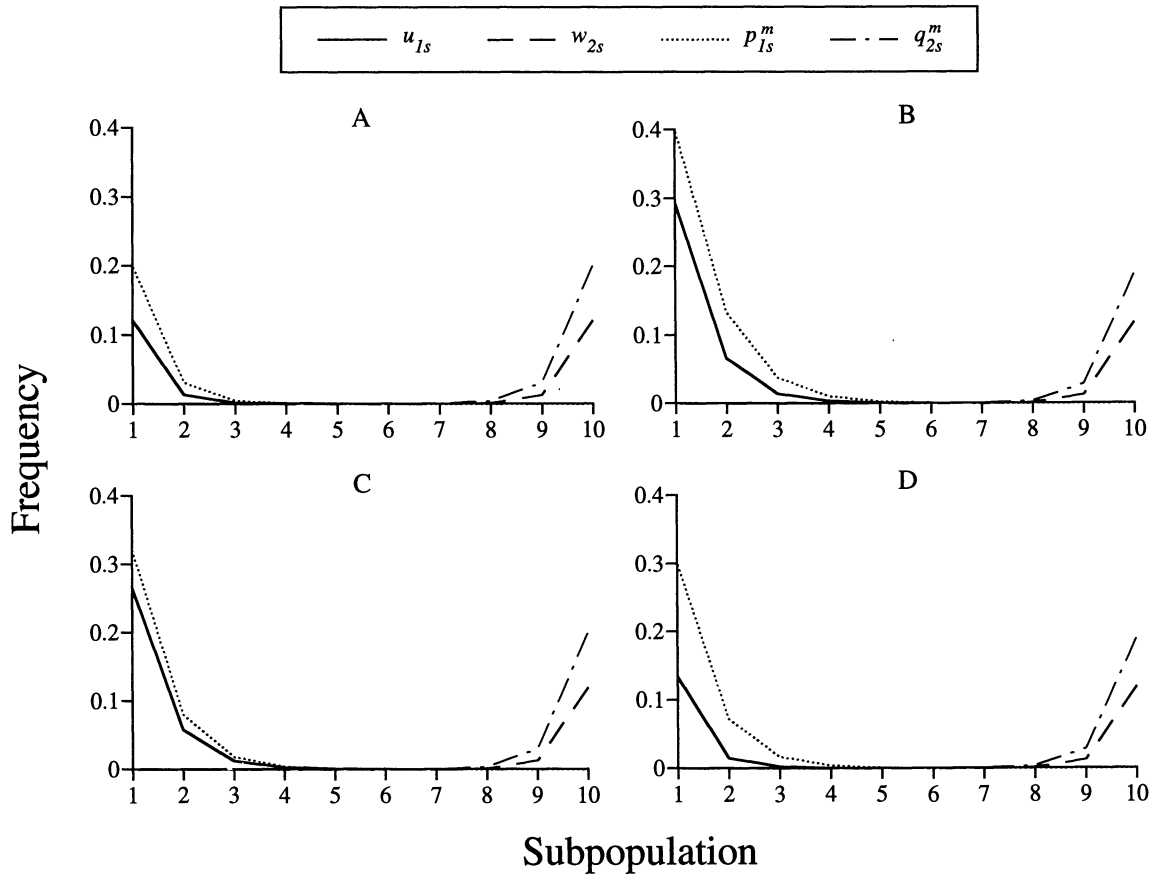

FIG. 5. Frequency of female (u_{1s} and w_{2s}) and male (p^m_{1s} and q^m_{2s}) pure type individuals across a haplodiploid hybrid zone composed of 10 subpopulations for (A) uniform migration rates with $m_f^{(1)} = m_f^{(2)} = m_m^{(1)} = m_m^{(2)} = 0.1$; (B) greater migration from one direction (source population 1) with $m_f^{(1)} = m_m^{(1)} = 0.2 > m_f^{(2)} = m_m^{(2)} = 0.1$; (C) greater female migration from one direction (source population 1) with $m_f^{(1)} = 0.2 > m_f^{(2)} = m_m^{(1)} = m_m^{(2)} = 0.1$; and (D) greater male migration from one direction (source population 1) with $m_m^{(1)} = 0.2 > m_f^{(1)} = m_f^{(2)} = m_m^{(2)} = 0.1$.

type individuals in each sex (e.g., u_{1s} and w_{2s}) are no longer symmetrical, because the more mobile, pure species 1 individuals are found at higher frequencies and farther into the hybrid zone, whereas pure species 2 individuals are found at nearly identical frequencies to those seen in the baseline case. Increasing $m^{(1)}$ to as high as 0.4 intensifies these patterns, with pure species 1 males, for instance, being found at measurable frequencies as far into the hybrid zone as subpopulation 7.

Greater Female Migration.—When females migrate at a uniformly greater rate than males ($m_f^{(1)} = m_f^{(2)} = m_f > m_m^{(1)} = m_m^{(2)} = m_m$), the main difference from the baseline model is that the frequencies of the corresponding pure type males and females converge. This phenomenon reaches its extreme when there is no male migration, in which case the frequencies of the pure type males equal the corresponding frequencies in females (e.g., $u_{1s} = p^m_{1s}$). This same effect was seen for the allelic disequilibria and is not surprising because males are produced parthenogenetically and thus reflect the genetic composition of their mothers.

Higher female migration from one direction only ($m_f^{(1)} > m_f^{(2)} = m_m^{(1)} = m_m^{(2)}$) also leads to convergent clines for pure species females and males (Fig. 5C). However, in this case, the frequency of pure species 1 individuals is greater than those from uniformly higher female migration, whereas the frequency of pure species 2 individuals is lower. As the difference between $m_f^{(1)}$ and the other migration rates becomes larger, the hybrid zone becomes increasingly dominated by pure species 1 individuals, although the frequency of pure species 2 individuals remains the same.

Greater Male Migration.—The pure species patterns under uniformly higher male migration ($m_m^{(1)} = m_m^{(2)} = m_m > m_f^{(1)} = m_f^{(2)} = m_f$), are similar to those with uniformly higher female migration. The main difference is that elevated male migration leads to lower frequencies of pure species individuals, particularly pure species females. This again reflects the weaker effect that the haploid males have on the cytonuclear structure of the hybrid zone. In the limiting case, with only male migration, pure type females completely disappear, and because pure males are produced parthenogenetically by pure females, there is a concomitant decrease in the frequency of pure males.

The effects of greater unidirectional male migration ($m_m^{(1)} > m_f^{(1)} = m_f^{(2)} = m_m^{(2)}$) are seen in Figure 5D. The patterns are nearly identical to those under a unidirectional bias in female migration (Fig. 5C), except that, again, the

frequencies of pure species 1 females are substantially lower. Increasing the difference between $m_m^{(1)}$ and the other migration rates leads to greater frequencies of pure species 1 individuals, but, as with the other asymmetrical migration schemes, not to substantially lower frequencies of pure species 2 individuals.

Effect of Hybrid Zone Subdivision

Variation in the number of subpopulations does not lead to major, qualitative differences in the patterns of the cytonuclear variables, but it does lead to a few distinctions. For the four major migration schemes above, the clines in the allele frequencies become more gradual as the number of subpopulations increases (assuming that the dispersal distance per generation of an individual is the unit of distance and this remains constant). For example, the formula for the slope of the common allele frequency cline under uniform migration (e.g., Fig. 3A) for the general case of n subpopulations is given by $(n + 1)^{-1}$, which decreases in value as n increases. The magnitudes of the cytonuclear disequilibria also decrease with increasing subdivision of the hybrid zone. For instance, as shown in Figure 4A, when both sexes migrate into a hybrid zone composed of 10 subpopulations at a uniform rate of $m = 0.1$, the cytonuclear disequilibria are unlikely to be detected (< 0.01 in value), whereas in the extreme case of 1 subpopulation (i.e., the continent-island model), the allelic disequilibria in both sexes and the homozygote disequilibria in females are all greater than 0.08, while the female heterozygote disequilibrium is zero (Goodisman and Asmussen 1997). Surprisingly, increasing hybrid zone subdivision has very little effect on the pure species individuals. Rather, the frequencies of pure species 1 and 2 individuals in a given subpopulation are directly related to their distance from sources 1 and 2, respectively. For example, when comparing two hybrid zones composed of different numbers of subpopulations, the frequency of pure species 1 individuals will be the same in subpopulation 1 of both of the hybrid zones, whereas that of pure species 2 individuals will be the same in the last subpopulation of each of the hybrid zones.

Effect of Census Time

Under the alternate census scheme (census 2, Fig. 2), sampling occurs after mating but before migration. To determine the effects of sampling at the differing censuses we repeated the computer simulations described above using the genotypic recursions for haplodiploids under census 2, which are given in Appendix B. Unlike our initial, continent-island model (Goodisman and Asmussen 1997), a simple, analytical ordering between the values of the cytonuclear variables in any subpopulation under the two census schemes is not apparent in the stepping-stone model. Nonetheless, some general conclusions may be drawn from our simulations. Most importantly, the overall, steady-state patterns of the cytonuclear variables are qualitatively the same under both censuses. In fact, the frequencies of the nuclear and cytoplasmic alleles are virtually identical for all of the migration patterns discussed above. The values of the cytonuclear disequilibria, however, are always greater under census 1. For example under the baseline model of equal migration of both sexes in both directions with $m = 0.1$, the value of each disequilibrium statistic in any subpopulation under census 1 is at least twice as great as that under census 2.

The frequencies of pure type individuals are affected in a similar manner, so that there will always be a greater frequency of pure species individuals when censusing occurs after migration rather than before. For the baseline case with $m = 0.1$, the frequency of pure females in any subpopulation under census 1 is at least five times that under census 2 and the frequency of pure males is greater by a factor of at least 1.5. These results are in accord with those found for the continent-island model (Goodisman and Asmussen 1997) and logically follow from the effects of random mating and migration. In general, mating tends to reduce cytonuclear disequilibria and eliminate pure species individuals, whereas migration of individuals from source populations that are fixed for alternate alleles and composed only of pure species individuals will augment disequilibria and the frequency of pure types (Asmussen et al. 1989; Goodisman and Asmussen 1997). Thus, to maximize the detection of nonrandom associations within a hybrid zone, sampling should be conducted under census 1, when the disequilibria will be higher.

X-Linked Genes

In contrast to the parthenogenetic mode of male production in haplodiploid species, in most diploid species males can only be produced by mating. This difference leads to distinct dynamics for the pure species individuals (recursions shown in Appendix C). Specifically, pure types will be found at a higher equilibrium frequency when considering haplodiploids rather than diploids (Goodisman and Asmussen 1997). For instance, continuing with the baseline case of uniform migration with $m = 0.1$ as an example, we find that within any subpopulation there will be a 5% greater frequency of pure type females and a 75% greater frequency of pure type males in haplodiploids. Unlike the frequencies of the pure parental types, the allele frequencies and cytonuclear disequilibria are the same under both the haplodiploid and X-linked formulations, as they are in the continent-island model with random mating (Goodisman and Asmussen 1997).

Application to a Natural Hybrid Zone

We now apply our stepping-stone model to a naturally occurring hybrid zone that has formed between two haplodiploid species. The red imported fire ant *S. invicta* and the black imported fire ant *S. richteri* were introduced to North America earlier this century, and have since spread throughout the southeastern United States (Lofgren 1986; Vinson and Greenberg 1986). Although the ants do not regularly hybridize in their native habitat (Ross and Trager 1990), in their introduced range they have formed a large hybrid zone, that spans much of northern Alabama, Mississippi, and Georgia (Vander Meer et al. 1985; Ross et al. 1987; Diffie et al. 1988; Vander Meer and Lofgren 1989; Shoemaker et al. 1994, 1996). The area of hybridization (several hundred kilometers) is significantly larger than the distance which a fire ant queen can naturally traverse in a single generation (maximum 1–10 km; Markin et al. 1971), and thus application of our stepping-stone model, rather than the corresponding continent-

island model (Goodisman and Asmussen 1997), is appropriate.

Collection of Data

The details of the sampling scheme and the assay of the nuclear genotypes are provided in Shoemaker et al. (1996). To briefly summarize, an alate (winged) queen was collected from approximately 30 nests within each of 44 relatively evenly spaced sites (subpopulations) along two transects (T1 and T2). The transects are each approximately 200 km long, span the area of hybridization, and begin in an area of pure *S. invicta* and end in an area of pure *S. richteri*. All of the ants were genotyped at three diallelic, nuclearly encoded isozyme loci, *Est-2, Gpi*, and *Odh*, which are diagnostic, with fixed differences between the two species in North America (Shoemaker et al. 1994). Individuals sampled from T1 were additionally scored at a diagnostic, codominant RAPD marker, *UBC 105*, for which *S. invicta* and *S. richteri* populations are fixed for alternate alleles differing in size. Thus, in contrast to the majority of RAPDs, which are dominant markers, *UBC 105* behaves as a monomeric isozyme, with heterozygotes displaying two bands (Shoemaker et al. 1994).

All of the ants were also subsequently genotyped at a diagnostic, cytoplasmic marker. Briefly, an approximately 1-kb portion of the mtDNA was PCR-amplified using the primers DDS-COII4 (Ross and Shoemaker 1997) and COI-RLR (Simon et al. 1994). PCR was performed as in Ross and Shoemaker (1997) with the exceptions that the cycling parameters were 1 min at 94°C, 1 min at 50°C, 1.5 min at 72°C for 35 cycles, and $MgCl_2$ concentration was 1.5 mM. PCR products were digested with *Taq*I and separated electrophoretically in 1.5% agarose gels, stained with ethidium bromide, and visualized under UV light. Pure *S. invicta* females were polymorphic for two restriction fragment profiles, with digestion yielding either two fragments of approximately 350 bp and 650 bp, or two fragments of approximately 400 bp and 600 bp, whereas pure *S. richteri* females invariably displayed three fragments of approximately 425 bp, 290 bp, and 285 bp. Thus, the presence of two versus three mtDNA restriction fragments was diagnostic for *S. invicta* and *S. richteri*, respectively.

The joint cytonuclear genotypes of the ants were used to define the spatial limits of the area of hybridization. Specifically, we considered the hybrid zone to be the area between the two outermost subpopulations that display alleles of both species. Under our definition, T1 consisted of 17 hybrid subpopulations separated by an average distance of 4.9 km, and T2 consisted of 20 hybrid subpopulations separated by an average distance of 8.2 km. Outside of the area of hybridization only pure types are found, and these outer regions thus represent the source populations of our model, with pure *S. invicta* and *S. richteri* individuals comprising source populations 1 and 2, respectively. As in previous studies (Moran 1979; Ferris et al. 1983; Lamb and Avise 1986; Asmussen et al. 1989; Baker and Davis 1989; Cruzan and Arnold 1993; Shoemaker et al. 1996; Sites et al. 1996; Harrison and Bogdanowicz 1997), individuals inside the hybrid zone that were fixed for all of the alleles of either pure species at all loci assayed were considered to be pure types, although this procedure will likely overestimate the actual number of pure types within the hybrid zone.

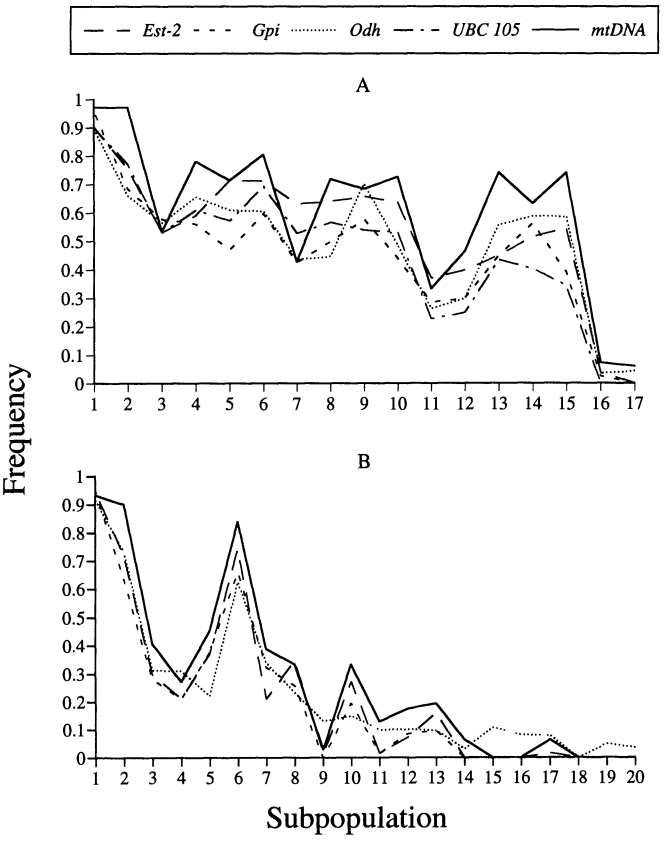

FIG. 6. Frequency of *Solenopsis invicta* alleles at (A) four nuclear markers and one mitochondrial marker across transect T1, and (B) three nuclear markers and one mitochondrial marker across transect T2, of a hybrid zone between *S. invicta* and *S. richteri*. Note the difference in subpopulation number.

Allele Frequencies, Cytonuclear Disequilibria, and Pure Parental Types

Figure 6A and 6B show the frequency of the *S. invicta* alleles for the females in the hybrid subpopulations of T1 and T2. Overall, the clines of all the markers are highly concordant within each transect. However, in contrast to expectations from our model, the clines through this hybrid zone are not smooth. Another striking feature of both transects is that the cline for the *S. invicta* mtDNA is uppermost in the majority of the subpopulations. Specifically, in 11 of the 17 subpopulations of T1 and 10 of the 19 subpopulations of T2 where *S. invicta* alleles were present, the *S. invicta* mtDNA allele frequency was greater than that of the corresponding nuclear markers. The two-tailed probability that the mtDNA marker would display such a pattern if the actual order of the allele frequencies were random is $P = 0.0002$ and $P = 0.0178$ for T1 and T2, respectively. Thus, the frequency of the *S. invicta* mitochondrial marker is higher, on average, than the *S. invicta* nuclear markers.

The clines for the female cytonuclear disequilibria across T1 and T2 are shown in Figures 7 and 8, and like those of

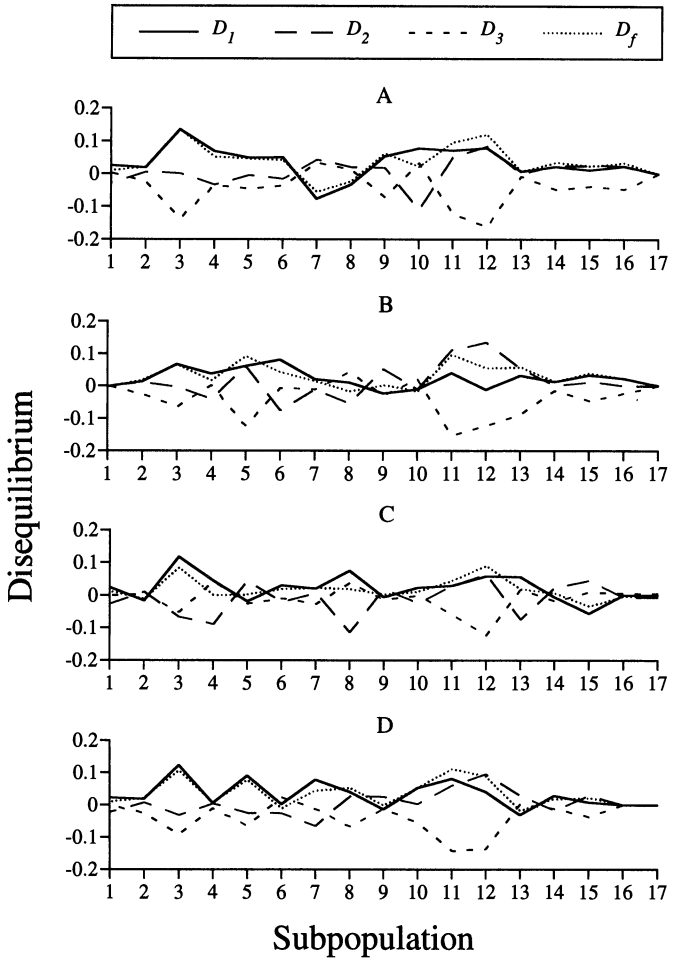

FIG. 7. Female genotypic (D_1, D_2, and D_3) and allelic (D_f) cytonuclear disequilibria through transect T1 of a hybrid zone between *Solenopsis invicta* and *S. richteri*, calculated using a single mitochondrial marker in conjunction with the four nuclear markers (A) *Est-2*; (B) *Gpi*; (C) *Odh*; and (D) *UBC 105*.

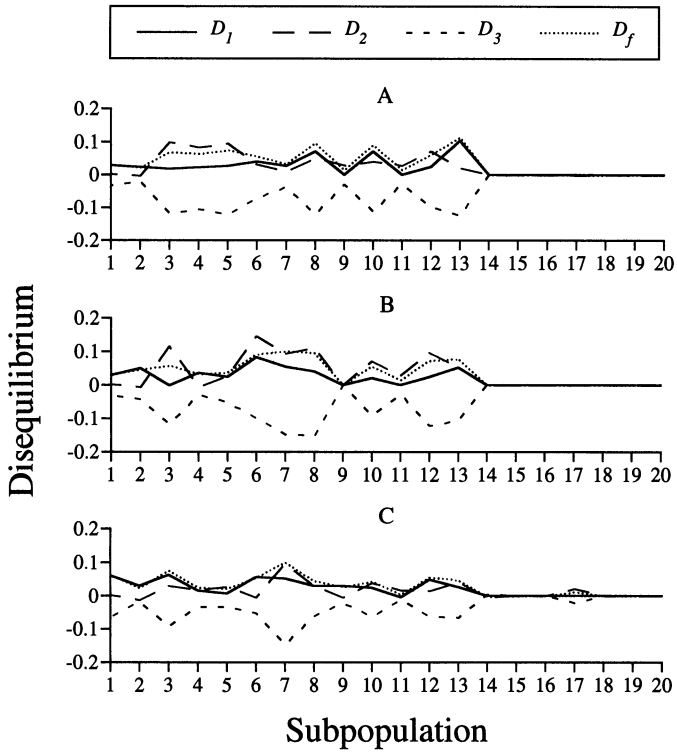

FIG. 8. Female genotypic (D_1, D_2, and D_3) and allelic (D_f) cytonuclear disequilibria through transect T2 of a hybrid zone between *Solenopsis invicta* and *S. richteri*, calculated using a single mitochondrial marker in conjunction with the three nuclear markers (A) *Est-2*; (B) *Gpi*; and (C) *Odh*.

the allele frequencies (Fig. 6), the clines for the disequilibria are not smooth. Although the magnitudes of the disequilibria are often large, the relatively small sample sizes within each subpopulation, combined with the problem of correcting significance values when making multiple, statistical comparisons (Rice 1989), make it unlikely that many of the cytonuclear disequilibria will prove to be significantly different from zero using established methods (Asmussen and Basten 1994; Basten and Asmussen 1997). However, we may look for expected trends in the disequilibria across subpopulations. Specifically, from our theoretical studies (see Case Studies section above), we have found that D_1 and D_f are always expected to be positive, whereas D_3 should always be negative. Table 4 shows the number of subpopulations across each of the two transects where these three associations are positive, along with the corresponding, one-tailed P-values. Significance was determined using a one-tailed binomial test with a null hypothesis that 50% of the statistics should be less than zero and 50% should be greater than zero. Although the disequilibria within a transect are not independent, all the trends are in the predicted direction, and the majority of the P-values are quite small; thus we conclude that the signs of

TABLE 4. Number of subpopulations in a *Solenopsis invicta–S. richteri* hybrid zone in which the female cytonuclear disequilibria, D_1, D_3, and D_f, are greater than zero, over the total number of subpopulations with nonzero values for those statistics. Calculations are shown for each of the nuclear loci scored in each of the two transects (T1 and T2).

	T1			T2		
Nuclear marker	D_1	D_3	D_f	D_1	D_3	D_f
Est-2	11/15	3/15*	13/15**	11/11***	1/14***	13/14***
Gpi	14/16**	2/16**	15/16***	11/12**	0/12***	12/12***
Odh	11/15	7/17	12/17	12/13**	1/15***	14/15***
UBC 105	11/14*	0/14***	11/14*	—	—	—

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

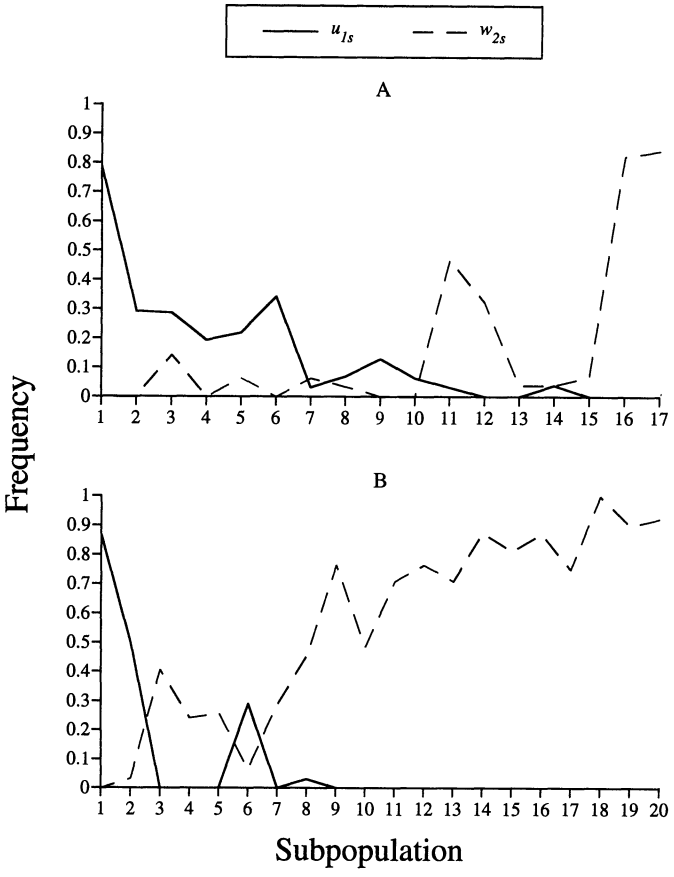

FIG. 9. Frequency of *Solenopsis invicta* and *S. richteri* pure type females (u_{1s} and w_{2s}) through transects (A) T1 and (B) T2 of a hybrid zone between the two species of fire ants. Note the difference in subpopulation number.

the nonrandom associations are consistent with the expectations from our model.

Finally, the frequencies of the pure species females are shown in Figure 9A and 9B for T1 and T2, respectively. Once again, contrary to expectations from our model, the clines for the pure types are not smooth. Furthermore, pure *S. invicta* and *S. richteri* both extend unexpectedly deep into the hybrid zone, particularly pure *S. richteri* in T2.

Estimation of Migration Rates

Our stepping-stone model was combined with maximum-likelihood techniques (Asmussen et al. 1989; Edwards 1992; Sites et al. 1996) to estimate gene flow through this fire ant hybrid zone. Although the fundamental assumptions of our model (i.e., equal migration between and random mating within discrete subpopulations bounded by two pure parental populations) may not be fully met in this zone of admixture, comparisons of the observed allele frequencies, cytonuclear disequilibria, and frequencies of pure parental types to those expected under our best-fitting model may yield substantial information concerning the factors that influence the distribution of cytonuclear genotypes in this fire ant hybrid zone.

One million sets of migration rates ($m_f^{(1)}$, $m_f^{(2)}$, $m_m^{(1)}$, and $m_m^{(2)}$) were chosen at random and used with the numerical methods above to calculate the expected frequencies of the joint cytonuclear genotypes at equilibrium. For each parameter set, the expected frequencies and observed counts of the eight classes of females in each subpopulation were used to calculate the composite, log-likelihood value $S = \sum_{i=1}^{n} \sum_{j=1}^{8} N_{ij} \log \hat{f}_{ij}$, where N_{ij} is the number of individuals observed in female class j in subpopulation i, and $\hat{f}_{ij}$ is the expected frequency of such individuals at equilibrium (female classes 1–8 correspond to pure *AA/C*, hybrid *AA/C*, *Aa/C*, . . . hybrid *aa/c*, and pure *aa/c*). The random migration rates that gave the highest log-likelihood value, *S*, were judged to be the maximum-likelihood estimates (MLEs). The expected number in each female genotypic class in each subpopulation under the MLEs was then determined, and compared via a χ^2-statistic to the observed counts to assess the statistical fit of the model. To ensure that the expected numbers were not too low, the eight classes of females within each subpopulation were lumped into two classes of moderate sizes before calculating the χ^2-value.

Ninety-five-percent confidence intervals for the migration rates were obtained by bootstrapping the two datasets 1000 times and removing the top and bottom 25 estimates for each migration rate. Due to constraints on computer time, recursions for the bootstrapping procedure were iterated until no genotype frequency changed by more than 10^{-3}, rather than 10^{-9}, and only 10^4 sets of migration rates were examined per bootstrap, rather than 10^6. We have found these modifications do not significantly alter our results, and, in general, these changes are conservative in that they will tend to lead to wider confidence intervals.

Until recently, it was unclear whether our queen samples had been obtained under census 1 or census 2 (Fig. 2), because mating and migration essentially occur at the same time in fire ants (Markin et al. 1971). However, recent studies in *S. invicta* have helped resolve the order of these fundamentally important processes (C. J. DeHeer, M. A. D. Goodisman, and K. G. Ross, unpubl. data). It now appears that on their nuptial flights, queens first migrate widely and then mate locally. Under this scenario, the queens we collected are the progeny of females who migrated in from neighboring (sub)populations, and then mated and reproduced within the subpopulations where they landed. Therefore we use the model that assumes our samples were collected before migration but after mating and reproduction (census 2).

The best-fitting migration rates of the fire ants through the hybrid zone are given in Table 5. The first striking feature of the MLEs is that they are almost all very high, with the overall migration rate of each sex, $m_f = m_f^{(1)} + m_f^{(2)}$ and $m_m = m_m^{(1)} + m_m^{(2)}$, approximately equal to one. This result, if true, would mean that almost all individuals within subpopulations are being replaced by migrants each generation. These estimates reflect the presence of many pure parental types in the middle of the hybrid zone (Fig. 9), which is not expected under the stepping-stone model unless migration rates are very high. A second notable feature is that the estimates from each of the nuclear markers for a given transect show strong agreement. This result is consistent with the coincident clines of nuclear allele frequencies within each transect (Fig. 6).

TABLE 5. Best-fitting migration rates of females ($m_f^{(1)}$, $m_f^{(2)}$) and males ($m_m^{(1)}$, $m_m^{(2)}$) through two transects (T1 and T2) of a *Solenopsis invicta–S. richteri* hybrid zone. Estimates are based on joint cytonuclear genotypes of females for multiple nuclear markers, and censusing is assumed to have occurred before migration and after mating (census 2). Values in parentheses delimit the 95% confidence intervals for the estimates. In all cases, the observed genotypic counts showed highly significant deviations ($P < 0.0001$) from those expected as judged by a χ^2 goodness-of-fit test.

	Nuclear Marker	$m_f^{(1)}$	$m_f^{(2)}$	$m_m^{(1)}$	$m_m^{(2)}$
T1	*Est-2*	0.51 (0.47, 0.54)	0.47 (0.42, 0.49)	0.48 (0.27, 0.51)	0.51 (0.35, 0.57)
	Gpi	0.52 (0.47, 0.53)	0.48 (0.42, 0.49)	0.44 (0.24, 0.47)	0.53 (0.35, 0.59)
	Odh	0.52 (0.47, 0.53)	0.49 (0.42, 0.49)	0.44 (0.26, 0.48)	0.54 (0.35, 0.58)
	UBC 105	0.51 (0.47, 0.53)	0.48 (0.42, 0.49)	0.44 (0.22, 0.46)	0.56 (0.36, 0.60)
T2	*Est-2*	0.10 (0.03, 0.13)	0.89 (0.76, 0.95)	0.14 (0.01, 0.33)	0.85 (0.25, 0.94)
	Gpi	0.11 (0.03, 0.13)	0.88 (0.74, 0.94)	0.10 (0.01, 0.32)	0.89 (0.31, 0.92)
	Odh	0.08 (0.03, 0.12)	0.91 (0.78, 0.95)	0.17 (0.03, 0.32)	0.80 (0.34, 0.91)

We next consider the distinctive features of each transect. Turning first to T1, we see that the estimated female migration rate from the *S. invicta* direction, $m_f^{(1)}$, and the estimated male migration rate from the *S. richteri* direction, $m_m^{(2)}$, are always greater than the other two migration rates. These findings are consistent with the frequency of the *S. invicta* mtDNA being higher than the frequency of the *S. invicta* nuclear markers through most of the hybrid zone (Fig. 6), which, in general, is expected when $m_f^{(1)}$ or $m_m^{(2)}$ is greater than the other migration rates. However, if we consider the MLEs together with their corresponding confidence intervals, the estimates for the four rates of gene flow by females and males from both directions are relatively similar, which is not surprising given the near equality of the overall frequencies of *S. invicta* and *S. richteri* alleles in the T1 transect.

We find a very different pattern in T2. In this transect, estimates of both migration rates from the *S. richteri* direction, $m_f^{(2)}$ and $m_m^{(2)}$, are significantly greater than those from the *S. invicta* direction, $m_f^{(1)}$ and $m_m^{(1)}$. (Here, two migration rates are considered to be significantly different from one another if, and only if, the point estimate of each falls outside of the 95% confidence interval of the other.) In general, the greater migration rates of individuals from the *S. richteri* direction reflect the preponderance of *S. richteri* alleles in T2 (in contrast to T1). The estimated migration rates, however, appear to be at odds with another critical feature of this transect: As in T1, the overall frequency of the *S. invicta* mtDNA is significantly greater than that of the *S. invicta* nuclear markers (Fig. 6). Based on the simple migration schemes analyzed earlier, we might predict that $m_f^{(1)}$ or $m_m^{(2)}$ would thus be greater than the other migration rates. However, the MLEs in T2 clearly do not reflect this simple pattern. In this case, the elevated *S. invicta* mtDNA allele frequencies are likely to be a result of a more complex migration scheme or combination of other evolutionary processes.

The MLEs for the sex-specific migration rates through this hybrid zone must be interpreted with extreme caution. The equilibrium genotype frequencies under the best-fitting stepping-stone models deviate significantly from the observed data ($P < 0.0001$), due to the high frequency of pure parental types (Fig. 9) and the frequent reversals in genotype frequencies through the hybrid zone (as indicated by the reversals in allele frequencies in Fig. 6). Thus, we must conclude that our initial stepping-stone models do not provide a good fit to this fire ant hybrid zone. Possible reasons for this lack of fit are taken up in the Discussion.

Estimation of Migration Parameters from Partial Datasets

A noticeable property of our MLEs is that the 95% confidence intervals for the female estimates are much smaller than those for the male estimates. This result may stem from the absence of males from our dataset, which motivated us to test the efficiency of our estimation program in recovering the correct migration patterns when data are available from only one of the sexes. To do this, we first generated the equilibrium genotype frequencies for our baseline case of uniform migration with $m_f^{(1)} = m_f^{(2)} = m_m^{(1)} = m_m^{(2)} = 0.1$ under both censuses 1 and 2. The expected frequencies for the females and males in each subpopulation under this migration scheme were multiplied by 100 and then rounded to the nearest integer, thus providing us with a simulated dataset with 100 females and 100 males in each of 10 subpopulations. Next, we used our estimation program to obtain MLEs for the migration rates, given female data only, male data only, or data from both sexes. Due to constraints on computer time, recursions for calculating the log-likelihood equations were iterated until they changed by no more than 10^{-3} rather than 10^{-9}. The estimation procedure was conducted 100 times for each of the three datasets. Ninety-percent confidence intervals for the MLEs for each scenario were obtained by ranking the migration rates and then removing the top and bottom five estimates.

We found that the MLEs never differed significantly from the expected values, as judged by the fact that the 90% confidence intervals overlapped the true value of 0.1 (data not shown). Moreover, the confidence intervals for the female

migration rates were always smaller than those for the males, reflecting the importance of the diploid females in establishing the distribution of genotypes in the hybrid zone. Under census 1, the accuracy of each sex-specific migration estimate depended on whether data were present from the corresponding sex. That is, the confidence intervals around the female and male estimates tended to be smaller when female or male data were present, respectively. However, this trend was not observed under census 2, where the confidence intervals were approximately the same regardless of the type of data used. The effects of partial datasets on estimating sex-specific migration are thus somewhat equivocal, but, in general, the more independent classes of data that are available, the better the estimates.

Discussion

We have expanded our cytonuclear, hybrid zone framework (Goodisman and Asmussen 1997) for haplodiploid species and X-linked genes in diploid species to incorporate situations where the area of hybridization is greater than the distance that an individual can migrate in a single generation. In these cases a stepping-stone system of migration is appropriate rather than the previous continent-island formulation (Goodisman and Asmussen 1997). A main difference between this and other hybrid zone models is that we do not assume that hybrid genotypes are selected against inside the hybrid zone (Bazykin 1969; Endler 1977; Moore 1977; Barton 1979b; Barton and Hewitt 1985, 1989; Barton and Gale 1993; Arnold and Hodges 1995; Arnold 1997) or that pure species individuals mate assortatively (Arnold et al. 1988; Asmussen et al. 1989). Rather, the main factor operating in this initial, baseline formulation is differential migration by the two sexes from the directions of two genetically constant source populations, with random mating within subpopulations. Thus, our models provide null hypotheses against which hybrid zone, cytonuclear data from haplodiploid species, or X-linked genes may be compared.

Inferences from Theoretical Clines

Our focus has been on situations where the two hybridizing species show fixed differences at both nuclear and cytoplasmic markers. In these cases, the presence of population structure within the hybrid zone can lead to clines in allele frequencies, cytonuclear disequilibria, and pure parental frequencies, which may be informative in determining the patterns of migration of females and males through the hybrid zone. We tested the utility of such clines by examining the equilibrium distribution of these cytonuclear variables under four important migration schemes: uniform migration of both sexes, greater migration of both sexes from one direction, greater migration of females, and greater migration of males.

The allele frequency clines are most informative in cases where there is greater migration of either males or females from one direction only because, in these situations, the four clines are distinct. If females migrate at a greater rate from the direction of one of the source populations, then the frequency of the female cytotype found in that source population (x_f) will always be greater than the frequency of both the corresponding male cytotype (x_m) and female nuclear allele (p_f), whereas the male nuclear allele frequency (p_m) will always be the smallest ($p_m < p_f$, $x_m < x_f$). However, the pattern reverses if males migrate at a greater rate from the direction of the same source population, so that the male nuclear allele from that source population has the highest frequency, and the female cytotype, the lowest ($x_f < x_m$, $p_f < p_m$). The allele frequency clines do not allow detection of uniformly greater female or male migration because, without directional biases by either sex, the nuclear and cytotype frequencies of both sexes form identical, linear clines. However, greater migration of both sexes from one direction is distinguishable because, although the clines for the four markers still coincide, the hybrid zone is now dominated by the more mobile species' alleles.

In general, the cytonuclear disequilibrium clines convey less information about the patterns of migration than those of the allele frequencies, although they can be useful in certain cases. For example, directional asymmetries in migration can generate large disequilibria in areas of the hybrid zone where the allele frequencies are intermediate, which, in these instances, will be the subpopulations closest to the source population from which the less mobile individuals originate. In these cases, a joint analysis of the allele frequency and disequilibrium clines can be particularly useful; although greater female migration from source 1 and greater male migration from source 2 lead to similar orders in the allele frequency clines, detectable disequilibria are apt to be generated only by the asymmetric female movement. If neither sex has a directional bias in migration, however, the disequilibria will be small in magnitude and relatively difficult to detect.

Another notable point regarding the equilibrium cytonuclear associations is that their sign patterns do not vary with the different migration schemes considered here. In each instance, we find that, as in previous nuclear (Barton and Clark 1990; Barton and Gale 1993) and cytonuclear (Asmussen et al. 1989; Goodisman and Asmussen 1997) frameworks, homospecific allelic combinations occur at a greater than random frequency in the hybrid zone. Here, this is reflected in the signs of the female homozygote disequilibria, D_1 and D_3, which are always positive and negative, respectively, and in the signs of the female and male allelic disequilibria, D_f and D_m, which are always positive. Also, under this framework, D_m is always expected to be greater than D_f, reflecting the fact that male genomes have undergone one less round of random mating than those of females.

The equilibrium frequencies of the pure parental types are not as useful for inferring patterns of gene flow as the other cytonuclear variables. In most cases, pure species individuals are not found very far into the hybrid zone, because their frequencies decay rapidly under random mating. More importantly, the distribution of pure types is relatively robust to changes in migration rates. However, a general observation is that the more common species in the hybrid zone usually has the higher migration rates from the direction of its source population. Surprisingly, the only difference between the haplodiploid and X-linked frameworks considered here relates to the frequencies of pure parental types. We find that the frequency of pure parentals will be greater under the haplodiploid model, because mating is not required to pro-

duce pure males in haplodiploid species, whereas it is in diploids (Goodisman and Asmussen 1997).

A noteworthy, although not unexpected, result from our numerical analyses is that the females, who carry two copies of the nuclear markers and are solely responsible for transmitting the cytoplasmic marker, have a much greater impact on the genetic structure of the hybrid zone than the males. For instance, a unidirectional bias in female migration will affect all four of the allele frequency clines (Fig. 3C); however, in the corresponding male case, the female cytotype cline can be virtually unchanged from that under uniform migration of the two sexes, and both nuclear clines are less affected than in the female case (Fig. 3D). The significance of females is also seen when comparing uniformly greater female migration to uniformly greater male migration. Both the cytonuclear disequilibria and frequency of pure parental types are greater when females have the higher migration rate.

Two other practical discoveries are that the degree of hybrid zone subdivision and the census time (before mating and after migration vs. after mating and before migration) can have large effects on the magnitudes but not the patterns of the cytonuclear variables. For instance, the clines in allele frequencies become more gradual and the cytonuclear disequilibria and frequency of pure types decrease as the number of subpopulations increases. The trends in the disequilibria and pure types can be both explained by the same mechanism: greater hybrid zone subdivision allows more area for random mating, which tends to break apart the nonrandom associations introduced by the constant input of pure parentals from the two source populations. The effect of census time under our stepping-stone formulation is the same as in the continent-island model with random mating (Goodisman and Asmussen 1997). The magnitude of the disequilibria and the frequency of the pure parental types are lower when censusing occurs after mating and before migration, because random mating within the zone tends to reduce disequilibria and the frequency of pure parental types. Therefore, to maximize the probability of detecting disequilibria, samples should be obtained under census 1.

In addition to providing useful qualitative predictions, the expected distributions of cytonuclear genotypes under our models can also be used to obtain formal MLEs for migration rates of females and males through hybrid zones (Asmussen et al. 1989; Sites et al. 1996). Ideally, the data would consist of the cytonuclear genotypes of individuals collected from several sites along one or more transects through a hybrid zone, with the distance between these sites equaling the distance that an individual can migrate in a single generation. If data have been collected continuously through the transect, then discrete sites could be created by grouping individuals in proximity. In this way, our stepping-stone model can still yield approximate expected clines in allele frequencies, cytonuclear disequilibria, and pure parental types, even though the hybrid zone consists of a single continuous population. The expected genotypic clines that accompany the MLEs for the migration rates can then be compared to the observed data by way of a goodness-of-fit test to determine the fit of the model.

Application to a Fire Ant Hybrid Zone

This study was originally motivated by the need to interpret new data generated from a hybrid zone between two haplodiploid species. Pre-reproductive females were collected from two transects (T1 and T2) through an area of hybridization between two imported fire ant species, *S. invicta* and *S. richteri*, and were genotyped at four (T1) or three (T2) diagnostic, nuclear markers (Shoemaker et al. 1996). Subsequently, these individuals were genotyped at a single diagnostic mitochondrial marker. The clines of all the nuclear markers are highly concordant, indicating that genomewide forces are likely to be involved in determining the distribution of genotypes through this hybrid zone, as opposed to selection or other forces acting solely on the individual loci assayed. However, through both transects, the cline for the *S. invicta* mtDNA marker is higher than those of the corresponding nuclear markers. This finding is consistent with other studies that have noted differences in the movement of cytoplasmic and nuclear markers in hybridizing species (reviewed in Rieseberg and Wendel 1993; Arnold 1997), and, theoretically, may be explained by a directional bias in migration of one of the sexes or differential selection in the sexes. The cytonuclear disequilibria through the transects are often large in magnitude and show the expected homospecific associations, with D_1 and D_f positive and D_3 negative in the majority of subpopulations. Similar sign patterns in the cytonuclear disequilibrium statistics have been observed in other studies, and have been attributed to the continuous gene flow of pure parental types, assortative mating, and/or selection acting against hybrid phenotypes (Asmussen et al. 1989; Avise et al. 1990, 1997; Cruzan and Arnold 1993, 1994; Abernethy 1994; Sites et al. 1996; Harrison and Bogdanowicz 1997).

We combined our model with maximum-likelihood methods to estimate the rates of gene flow through this naturally occurring hybrid zone (Asmussen et al. 1989; Sites et al. 1996). The estimated migration rates are qualitatively consistent with the observed genotype and allele frequencies through the hybrid zone. For example, the extremely high migration rate estimates are presumably due to the large numbers of pure parentals observed in the middle of the hybrid zone. Also, the estimated migration rates of individuals from the two source populations parallel the allele frequencies of the two species through the transects. Specifically, in T1, where the overall allele frequencies of the two species are approximately the same, the estimated migration rates in the two directions are about equal, whereas in T2, where there is an excess of *S. richteri* alleles, the estimated migration rates for individuals from the *S. richteri* direction are greater than those from the *S. invicta* direction.

Despite these interesting results, the expected genotype frequencies under the best-fitting migration rates deviate significantly from those observed. There are several reasons why our model may fail to explain the distribution of genotypes through this hybrid zone. For instance, hybrid fire ants may show reduced fitness relative to the parental species, which would not be accounted for under the initial structured model here. Hybrid ants exhibit relatively high levels of fluctuating asymmetry in morphological characters as compared to pure *S. invicta* and *S. richteri*; moreover, hybrids display abnormal wing venation more frequently than the pure types. These morphological deviations are believed to be caused by a breakdown in parental coadapted gene complexes and may

be associated with reduced hybrid fitness (Ross and Robertson 1990). Further evidence of fitness differences among *Solenopsis* genotypes is given by the near absence of *S. richteri* alleles from the southernmost range of the fire ants in North America, where *S. richteri* was extirpated by *S. invicta* earlier in this century (Lofgren 1986; Shoemaker et al. 1996). This process implies that the *S. invicta* phenotype (and genotype) confers a fitness advantage under some conditions.

A second and more important reason why our model may not fit the fire ant data is that the *S. invicta–S. richteri* hybrid zone is probably no more than 60 years old and has been moving over that period of time (Lofgren 1986; Ross et al. 1987; Shoemaker et al. 1994, 1996) and, therefore, is probably not at equilibrium. Finally, much of the movement of the ants in this area presumably has occurred by human means (Lofgren 1986). Thus, at this time, the distribution of fire ants in the southeastern United States is probably a result of stochastic and historical factors (Shoemaker et al. 1996), rather than of a steady-state process of mating and migration of the two parental species.

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APPENDIX A

Census 1 for Haplodiploids

Under the haplodiploid model for census 1 (before mating and after migration) the frequencies of the two pure type females in subpopulation $i = 1, 2, \ldots, n$ are given by

$$(u_{1s}^{(i)})' = m_f \bar{u}_{1s}^{(i)} + (1 - m_f) u_{1s}^{(i)} p_{1s}^{m(i)}$$

and

$$(w_{2s}^{(i)})' = m_f \bar{w}_{2s}^{(i)} + (1 - m_f) w_{2s}^{(i)} q_{2s}^{m(i)}, \tag{A1}$$

whereas those of the six basic cytonuclear genotypes in females are

$$(u_1^{(i)})' = m_f \bar{u}_1^{(i)} + (1 - m_f) p_m^{(i)} (p_f^{(i)} x_f^{(i)} + D_f^{(i)})$$

$$(v_1^{(i)})' = m_f \bar{v}_1^{(i)} + (1 - m_f)[(p_f^{(\iota)} q_m^{(i)} + q_f^{(i)} p_m^{(i)}) x_f^{(i)} + (q_m^{(i)} - p_m^{(i)}) D_f^{(i)}]$$

$$(w_1^{(i)})' = m_f \bar{w}_1^{(i)} + (1 - m_f) q_m^{(i)} (q_f^{(i)} x_f^{(i)} - D_f^{(i)})$$

$$(u_2^{(i)})' = m_f \bar{u}_2^{(i)} + (1 - m_f) p_m^{(\iota)} (p_f^{(i)} y_f^{(i)} - D_f^{(i)})$$

$$(v_2^{(i)})' = m_f \bar{v}_2^{(i)} + (1 - m_f)[(p_f^{(i)} q_m^{(\iota)} + q_f^{(i)} p_m^{(\iota)}) y_f^{(i)} - (q_m^{(i)} - p_m^{(i)}) D_f^{(i)}]$$

and

$$(w_2^{(i)})' = m_f \bar{w}_2^{(i)} + (1 - m_f) q_m^{(i)} (q_f^{(i)} y_f^{(i)} + D_f^{(i)}), \tag{A2}$$

where $\bar{z}^{(i)}$ is the value of variable z in the migrants entering subpopulation i, as defined in the Hybrid Zone Model section. Similarly, the recursions for the two pure type males are

$$(p_{1s}^{m(i)})' = m_m \bar{p}_{1s}^{m(i)} + (1 - m_m) u_{1s}^{(i)}$$

and

$$(q_{2s}^{m(i)})' = m_m \bar{q}_{2s}^{m(i)} + (1 - m_m) w_{2s}^{(i)} \tag{A3}$$

and those for the four basic genotypes in males are

$$(p_1^{m(i)})' = m_m \bar{p}_1^{m(i)} + (1 - m_m) p_1^{f(i)}$$

$$(p_2^{m(i)})' = m_m \bar{p}_2^{m(i)} + (1 - m_m) p_2^{f(i)}$$

$$(q_1^{m(i)})' = m_m \bar{q}_1^{m(i)} + (1 - m_m) q_1^{f(i)}$$

and

$$(q_2^{m(i)})' = m_m \bar{q}_2^{m(i)} + (1 - m_m) q_2^{f(i)} \tag{A4}$$

(Goodisman and Asmussen 1997).

APPENDIX B

Census 2 for Haplodiploids

Under census 2 sampling occurs after mating but before migration. It is convenient to define the value of any frequency variable in subpopulation $i = 1, 2 \ldots n$ directly after migration as $\tilde{z}^{(i)} = m\bar{z}^{(i)} + (1 - m)z^{(i)}$, where m is the sex-specific migration rate, $z^{(i)}$ is the frequency before migration, and $\bar{z}^{(i)}$ is the frequency in the migrants into subpopulation i, as defined in the Hybrid Zone Model section. The recursions for the two pure type females are then given by

$$(u_{1s}^{(i)})' = \tilde{u}_{1s}^{(i)} \tilde{p}_{1s}^{m(i)} \quad \text{and} \quad (w_{2s}^{(i)})' = \tilde{w}_{2s}^{(i)} \tilde{q}_{2s}^{m(i)}, \tag{B1}$$

whereas those for the composite female cytonuclear genotypes are

$$(u_1^{(i)})' = \tilde{p}_1^{f(i)} \tilde{p}_m^{(i)} \qquad (u_2^{(\iota)})' = \tilde{p}_2^{f(\iota)} \tilde{p}_m^{(i)}$$

$$(v_1^{(i)})' = \tilde{p}_1^{f(i)} \tilde{q}_m^{(i)} + \tilde{q}_1^{f(i)} \tilde{p}_m^{(\iota)} \qquad (v_2^{(i)})' = \tilde{p}_2^{f(i)} \tilde{q}_m^{(i)} + \tilde{q}_2^{f(i)} \tilde{p}_m^{(i)}$$

$$(w_1^{(i)})' = \tilde{q}_1^{f(i)} \tilde{q}_m^{(i)} \quad \text{and} \qquad (w_2^{(i)})' = \tilde{q}_2^{f(i)} \tilde{q}_m^{(\iota)}. \tag{B2}$$

The recursions for the two pure species males are simply

$$(p_{1s}^{m(i)})' = \tilde{u}_{1s}^{(i)} \quad \text{and} \quad (q_{2s}^{m(i)})' = \tilde{w}_{2s}^{(i)}, \tag{B3}$$

whereas those of the composite male genotypes are

$$(p_1^{m(i)})' = \tilde{p}_1^{f(i)} \qquad (p_2^{m(i)})' = \tilde{p}_2^{f(i)}$$

$$(q_1^{m(i)})' = \tilde{q}_1^{f(i)} \quad \text{and} \quad (q_2^{m(i)})' = \tilde{q}_2^{f(i)} \tag{B4}$$

(Goodisman and Asmussen 1997).

APPENDIX C

Frequency of Pure Parentals for Diploid, X-Linked System

Under census 1, the recursions for the pure species males in subpopulation $i = 1, 2 \ldots n$ are given by

$$(p_{1s}^{m(i)})' = m_m \bar{p}_{1s}^{m(i)} + (1 - m_m) u_{1s}^{(i)} p_{1s}^{m(i)}$$

and

$$(q_{2s}^{m(\iota)})' = m_m \bar{q}_{2s}^{m(i)} + (1 - m_m) w_{2s}^{(i)} q_{2s}^{m(i)}, \tag{C1}$$

whereas under census 2, the recursions are identical to those of the corresponding pure species females, so that

$$(p_{1s}^{m(i)})' = \tilde{u}_{1s}^{(i)} \tilde{p}_{1s}^{m(i)} \quad \text{and} \quad (q_{2s}^{m(\iota)})' = \tilde{w}_{2s}^{(i)} \tilde{q}_{2s}^{m(i)}, \tag{C2}$$

where $\tilde{z}^{(i)} = m\bar{z}^{(i)} + (1 - m)z^{(i)}$ with m replaced by m_f for $z^{(i)} = u_{1s}^{(i)}$ and $w_{2s}^{(i)}$ and m replaced by m_m for $z^{(i)} = p_{1s}^{m(i)}$ and $q_{2s}^{m(i)}$.