Sexually Antagonistic Cytonuclear Fitness Interactions in *Drosophila melanogaster*

David M. Rand,* Andrew G. Clark† and Lisa M. Kann*

**Department of Ecology and Evolutionary Biology, Brown University, Providence, Rhode Island 02912 and* †*Department of Biology, Pennsylvania State University, University Park, Pennsylvania 16802*

Manuscript received May 15, 2000
Accepted for publication June 6, 2001

ABSTRACT

Theoretical and empirical studies have shown that selection cannot maintain a joint nuclear-cytoplasmic polymorphism within a population except under restrictive conditions of frequency-dependent or sex-specific selection. These conclusions are based on fitness interactions between a diploid autosomal locus and a haploid cytoplasmic locus. We develop a model of joint transmission of X chromosomes and cytoplasms and through simulation show that nuclear-cytoplasmic polymorphisms can be maintained by selection on X-cytoplasm interactions. We test aspects of the model with a "diallel" experiment analyzing fitness interactions between pairwise combinations of X chromosomes and cytoplasms from wild strains of *Drosophila melanogaster*. Contrary to earlier autosomal studies, significant fitness interactions between X chromosomes and cytoplasms are detected among strains from within populations. The experiment further demonstrates significant sex-by-genotype interactions for mtDNA haplotype, cytoplasms, and X chromosomes. These interactions are sexually antagonistic—*i.e.*, the "good" cytoplasms in females are "bad" in males—analogous to crossing reaction norms. The presence or absence of Wolbachia did not alter the significance of the fitness effects involving X chromosomes and cytoplasms but tended to reduce the significance of mtDNA fitness effects. The negative fitness correlations between the sexes demonstrated in our empirical study are consistent with the conditions that maintain cytoplasmic polymorphism in simulations. Our results suggest that fitness interactions with the sex chromosomes may account for some proportion of cytoplasmic variation in natural populations. Sexually antagonistic selection or reciprocally matched fitness effects of nuclear-cytoplasmic genotypes may be important components of cytonuclear fitness variation and have implications for mitochondrial disease phenotypes that differ between the sexes.

THE nuclear-organelle interactions of eukaryotic cells represent some of the most significant coevolved mutualisms in the history of life. The metabolic processes that are the hallmarks of mitochondria and chloroplasts require the coordinated expression of hundreds of nuclear genes and a few dozen organelle genes (Gillham 1994). Usually, the two genomes involved in this coordinated expression are members of separate domains of life with different genetic codes (Gray *et al.* 1999). Since the phenotypes that emerge from these intergenomic "epistases" are cellular processes central to energy metabolism in higher organisms, there should have been considerable opportunity for natural selection to shape the nature of these interactions. An important component of this cytonuclear coevolution will be macroevolutionary, involving transfer of genes from the endosymbiont to the host nuclear genome and the subsequent modification of these genes for proper expression (*e.g.*, Martin and Herrmann-Reinhold 1998). Once new gene arrangements are stabilized, cytonuclear coevolution will be microevolutionary, where the processes of mutation, recombination, selection, and drift govern the turnover of alleles and haplotypes in both genomes. While the majority of a lineage's history may involve cytonuclear microevolution, this will likely be contingent on the histories of gene transfer and genome rearrangement unique to that lineage.

The distinct rules of transmission for nuclear and cytoplasmic genes provide clear expectations that have motivated models and statistical tests of cytonuclear associations (*e.g.*, Clark 1984; Gregorius and Ross 1984; Asmussen *et al.* 1987; Arnold 1993; Asmussen and Basten 1994; Babcock and Asmussen 1996; Datta *et al.* 1996; Goodisman and Asmussen 1997; Datta and Arnold 1998). Moreover, the uniparental inheritance of most organelle genomes provides a simple tool with which to manipulate cytonuclear genotypes for studies of experimental or natural populations (*e.g.*, Clark 1985; MacRae and Anderson 1988; Scribner and Avise 1994; Hutter and Rand 1995; Cruzan and Arnold 1999). How selection might act jointly on nuclear and cytoplasmic genomes has been a central question for many of these microevolutionary studies, and this becomes all the more important given the recent evidence for nonneutral evolution of both nuclear and mitochondrial genes (Akashi and Kreitman 1995; Eanes 1999; Weinreich and Rand 2000).

Corresponding author: David M. Rand, Department of Ecology and Evolutionary Biology, 69 Brown St., Providence, RI 02912.
E-mail: david_rand@brown.edu

Theoretical studies of nuclear-cytoplasmic fitness interactions have shown that constant viabilities cannot maintain polymorphisms at interacting nuclear and cytoplasmic loci (Clark 1984; Gregorius and Ross 1984). Only under specific conditions of frequency-dependent selection or differential selection in the sexes can a joint polymorphism be maintained (Clark 1984; Gregorius and Ross 1984). Empirical studies of conditional fitnesses in *Drosophila melanogaster* confirmed these theoretical findings (Clark 1985). No nuclear-cytoplasmic (N × C) fitness interactions could be detected among strains of flies from within geographic populations, but N × C interactions were detected among cytonuclear genotypes constructed with strains from diverse geographic origins (Clark and Lyckegaard 1988). These results suggested that fitness variation among cytonuclear genotypes would be removed quickly from within Mendelian populations, but selection might have the additional effect of accentuating cytonuclear fitness differences among populations.

Subsequent population cage experiments revealed a number of cases where mitochondrial (mt)DNA haplotypes showed strong frequency shifts, suggesting that mtDNA was indeed not neutral (MacRae and Anderson 1988; Fos *et al.* 1990; Nigro and Prout 1990; Kambhampati *et al.* 1992; Hutter and Rand 1995; Kilpatrick and Rand 1995). In most of these studies, the strains of insects used were clearly differentiated at nuclear loci so nuclear-mitochondrial fitness interactions were implicated. In one study, when mtDNA haplotypes were competed on essentially homozygous backgrounds, the mtDNAs behaved neutrally, but these same mtDNAs showed clear nonneutral behavior on heterozygous nuclear backgrounds of the two strains (Kilpatrick and Rand 1995). These studies confirmed the importance of nuclear-cytoplasmic interactions in cases of presumed nonneutrality of mtDNA. Since there are hundreds of nuclear-encoded genes that are potential targets of selection for N × C fitness interactions, one might expect that these nuclear loci would be spread more or less randomly around the genome. However, if one considers the distinct transmission patterns of mtDNA, sex chromosomes, and autosomes, some intriguing patterns emerge.

In diploid sexual species where the female is the heterogametic sex and organelle DNA is inherited through the female cytoplasm (*i.e.*, most animals), the patterns of joint nuclear-cytoplasmic chromosomal transmission are different for the X chromosome than for the autosomes. As illustrated in Table 1, a set of male and female parents carry four copies of each autosome but only three copies of the X chromosome. For any autosome, half of the copies are cotransmitted through the female with the organelle genome. For the X chromosomes, however, two-thirds of the copies are cotransmitted through the female with the organelle genome (Table 1).

This difference in the patterns of cotransmission for X chromosomes and autosomes motivated us to reexamine earlier models of nuclear-cytoplasmic fitness interactions that were based on autosomal loci. The important question is whether X-linked cytonuclear fitness interactions also fail to maintain a joint cytonuclear polymorphism within populations (Clark 1984, 1985). Here we extend the earlier models of Clark (1984) to accommodate joint X chromosome and cytoplasm transmission. We then test the model with an empirical study of fitness interactions among all pairwise combinations of X chromosomes and cytoplasms from wild strains of *D. melanogaster*. We explicitly engineered mtDNA haplotype variation into the study so that potential fitness effects of mtDNAs could be tested (recognizing that mtDNA haplotype is not completely independent of other cytoplasmic factors such as Wolbachia). Both the theoretical and empirical results are strikingly different from all previous studies focusing on autosomal-cytoplasm interactions. Our results complement and extend recent theoretical studies of cytonuclear dynamics with differential selection in the sexes (Babcock and Asmussen 1996, 1998) and in haplodiploid species (Goodisman and Asmussen 1997; Goodisman *et al.* 1998). Together, these studies provide strong support for the notion that sex-linked cytonuclear polymorphisms can be maintained within populations and that sexually antagonistic selection is an important component of the dynamics of these systems.

TABLE 1

Patterns of chromosomal transmission

	Chromosomes			
Sex	mtDNA[a]	Y	X	Autosomes
Female (homogametic)	1	0	2	2
Male (heterogametic)	0	1	1	2
Total copies	1	1	3	4
Proportion cotransmitted with mtDNA		0	0.66	0.50

[a] Assuming strict maternal transmission of mtDNA.

MATERIALS AND METHODS

Model of X-linked cytonuclear fitness interactions: Consider an X-linked locus with two alleles (*X* and *x*) following Mendelian transmission and a cytoplasmically transmitted factor with two haplotypes (*M* and *m*). There are six female genotypes (*XXM*, *XXm*, *XxM*, *Xxm*, *xxM*, *xxm*) with frequencies x_1, x_2, x_3, x_4, x_5, x_6, and, with heterogametic males, four male genotypes (*XM*, *Xm*, *xM*, *xm*) with frequencies y_1, y_2, y_3, y_4. Further, let k be the frequency of paternal transmission. Table 2 presents the mating table with the 24 possible mating events and the proportions of offspring genotypes resulting from random mating. The six female and the four male cytogenotypes can be assigned different viabilities, defined as the probability of surviving from zygote to reproductive age. From the mating table and the assigned viabilities, a series of simultaneous recurrence relations were constructed, giving the frequency

TABLE 2

Mating table for the six female and four male sex-linked cytonuclear genotypes

	Female offspring						Male offspring			
Mating	XXM	XXm	XxM	Xxm	xxM	xxm	XM	Xm	xM	xm
XXM × XM	1	0	0	0	0	0	1	0	0	0
Xm	$1-k$	k	0	0	0	0	$1-k$	k	0	0
xM	0	0	1	0	0	0	1	0	0	0
xm	0	0	$1-k$	k	0	0	$1-k$	k	0	0
XXm × XM	k	$1-k$	0	0	0	0	k	$1-k$	0	0
Xm	0	1	0	0	0	0	0	1	0	0
xM	0	0	k	$1-k$	0	0	k	$1-k$	0	0
xm	0	0	0	1	0	0	0	1	0	0
XxM × XM	$\frac{1}{2}$	0	$\frac{1}{2}$	0	0	0	$\frac{1}{2}$	0	$\frac{1}{2}$	0
Xm	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	0	0	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$
xM	0	0	$\frac{1}{2}$	0	$\frac{1}{2}$	0	$^1/_2$	0	$\frac{1}{2}$	0
xm	0	0	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$
Xxm × XM	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	0	0	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$
Xm	0	$\frac{1}{2}$	0	$\frac{1}{2}$	0	0	0	$\frac{1}{2}$	0	$\frac{1}{2}$
xM	0	0	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$	$\frac{1}{2}k$	$\frac{1}{2}(1-k)$
xm	0	0	0	$\frac{1}{2}$	0	$\frac{1}{2}$	0	$\frac{1}{2}$	0	$\frac{1}{2}$
xxM × XM	0	0	1	0	0	0	0	0	1	0
Xm	0	0	$1-k$	k	0	0	0	0	$1-k$	k
xM	0	0	0	0	1	0	0	0	1	0
xm	0	0	0	0	$1-k$	k	0	0	$1-k$	k
xxm × XM	0	0	k	$1-k$	0	0	0	0	k	$1-k$
Xm	0	0	0	1	0	0	0	0	0	1
xM	0	0	0	0	k	$1-k$	0	0	k	$1-k$
xm	0	0	0	0	0	1	0	0	0	1

of the cytogenotypes in the next generation (available from A.G.C. upon request). These equations were coded into a program that iterates the recursion to equilibrium, defined as a maximum genotype frequency change of $<10^{-12}$ in one generation. The behavior of the model was examined through simulation where 10,000 independent sets of 10 random uniformly distributed viabilities were generated for the six female and four male cytogenotypes.

Drosophila strains: Wild lines from three populations were used in the experiment: Australia (Aus 4, 5, and 7); Beijing, China (Bei 1, 2, 7, and 10); and North America (Fayetteville, North Carolina: Fay 11, 12, 13, 15, and 17). The Australia and Beijing lines were obtained from C. F. Aquadro; the Fayetteville lines were collected by Jeff Townsend in July 1993. Sequence polymorphism data from the X chromosome (Begun and Aquadro 1995) and from mtDNA (Rand *et al.* 1994; Rand and Kann 1996; D. Rand, unpublished data) have shown significant genetic differentiation among these continental populations. These lines were chosen from a larger set of lines from each locality. Prior to the use of these lines in the experiment, reciprocal crosses were performed between each pair of lines, and lines exhibiting significant sex ratio or reciprocal cross effects were excluded. Within each population, lines were chosen so that two distinct mtDNA haplotypes were represented. From restriction fragment length polymorphism (RFLP; Hale and Singh 1991) and sequence data (D. M. Rand, unpublished data), three different mtDNA haplotypes were included, here identified as the New World, Old World 1, and Old World 2 haplotypes. The mtDNA haplotypes of the individual lines were as follows: Aus 4 was New World, and Aus 5 and 7 were Old World 1; Bei 1 and 2 were Old World 2, and Bei 7 and 10 were Old World 1; Fay 11 and 12 were Old World 1, and Fay 13, 15, and 17 were New World.

The lines were also checked for the presence of Wolbachia; the three Australia lines carried Wolbachia and the other lines did not. Below we present separate analyses where the Australia/Wolbachia lines have been excluded. While cytoplasmic incompatibility has been reported in *D. melanogaster* (*e.g.*, Hoffmann *et al.* 1998), it is thought to be weaker than the incompatibility typically observed in *D. simulans* (*e.g.*, Poinsot *et al.* 1998).

Extraction of X chromosomes and cytoplasms: Experimental lines were constructed by simultaneously extracting a single X chromosome and cytoplasm from each wild line. The FM7 X chromosome balancer was used, which carries the codominant eye marker, *Bar* (Lindsley and Zimm 1992). Before extracting wild chromosomes, the FM7 balancer was stabilized on a *P* cytotype by 10 generations of backcrosses to females of the Harwich (*P* cytotype) strain of *D. melanogaster*. Bar-eyed males from this balancer stock were then crossed to virgin females from each wild strain. A single F_1 virgin female from each cross (*Bar*/+) was crossed again to FM7 males. Virgin F_2 *Bar*/+ females were again collected and mated to FM7 males; this was continued for 10 generations of backcrossing to place each single wild X chromosome and cytoplasm onto the same genetic background carrying the second, third, and fourth chromosomes of the balancer stock. A final cross between +/Y male and *Bar*/+ female siblings of each extracted line resulted in females homozygous for a single X chromosome in their initial cytoplasm [denoted by $+_i/+_i$ (*i*) following Clark (1985)] and males carrying the same X chromosome and cytoplasm [denoted $+_i$/Y (*i*)]. Sibling males and females carrying FM7 are also generated from this cross, so the lines are maintained by mass culture in vials.

Exchange of cytoplasms and X chromosomes: The X-cytoplasm extraction lines described above were then crossed *inter se* to exchange all X chromosomes with all cytoplasms. For example, with the subscripts *i* and *j* denoting different lines of origin, the exchange crosses were done as follows. First, a $+_i$/FM7 (*i*) female was mated to $+_j$/Y (*j*) males. Second, a $+_j$/*Bar* (*i*) F_1 virgin female offspring was mated with $+_j$/Y (*j*) males, producing females and males with the "exchanged" cytogenotypes $+_j/+_j$ (*i*) and $+_j$/Y (*i*), respectively [$+_j$/FM7 (*i*) females and FM7/Y (*i*) males are produced as well]. Pairwise crosses among the 12 extracted lines produced 144 cytonuclear genotypes, which were assayed for fitness.

Fitness assay and data analysis: Fitness was measured using a chromosome segregation assay in each of the 144 cytogenotypes. For example, a $+_i$/FM7 (*j*) female was crossed to the respective $+_i$/Y (*j*) male. All offspring of this cross will have the *j*th cytoplasm, the females will be either wild-type ($+_i/+_i$) or notch-eyed heterozygotes ($+_i$/FM7), and the males will be either wild ($+_i$/Y) or *Bar* (FM7/Y). Hence, the frequency of the wild *vs.* Bar X chromosome could be scored in each sex across all cytoplasms. Fitness was scored separately for each sex as the number of wild X chromosomes observed in a given sex divided by one plus the total progeny of that sex emerging from a specific nuclear × cytoplasmic cross (Haldane 1956). This avoids a spurious fitness correlation between the sexes. The assay involves both segregation and viability of chromosomes; since this involves more of the life cycle than viability alone we are calling the measure "fitness" even though mating and fecundity are not explicitly incorporated.

Crosses were performed by placing two males and two virgin females into vials and allowing mating and egg laying to take place for 4 days. Each of these crosses was replicated five times with two males and two females of the specific genotypes. Each replicate vial was changed after 4 days so that two broods were scored for fitness from the same set of parents. Some replicates failed to produce offspring, making the data set not perfectly balanced. This was alleviated somewhat by pooling broods across replicates, which was justified statistically as described below. The data structure involved 12 X chromosomes × 12 cytoplasms × five replicates × two broods (or 12 X chromosomes × three mtDNA haplotypes × five replicates × two broods). As intended, X chromosome and cytoplasm are orthogonal effects, but note that due to the maternal inheritance of X chromosomes and cytoplasm, cytoplasm and reciprocal cross were not orthogonal as in the autosomal study by Clark (1985). X chromosome × cytoplasmic (X × C) interactions were tested for significance with analyses of variance, where nuclear chromosome and cytoplasm were random effects and brood was a fixed effect. Similar X chromosome × mtDNA analyses were performed, but mtDNA and cytoplasm are not fully orthogonal. Statistical analyses were done using JMP version 3.2.6 (SAS Institute) and confirmed using Super ANOVA (Abacus Concepts, Berkeley, CA), both on Macintosh computers. Both packages gave the same results with respect to significant and nonsignificant effects, with slight differences in the values reported for sums of squares due to differences in how the two packages handled missing data.

RESULTS

Simulations of the X-cytoplasm model: The dynamics of the X-linked cytonuclear model were examined by generating 10,000 sets of 10 random viabilities for the six female and four male genotypes. Unlike the earlier autosomal models of cytonuclear interactions (Clark 1984; Gregorius and Ross 1984), the X-linked model maintains a joint nuclear and cytoplasmic polymorphism in up to 13.5% of different random sets of the 10 viabilities. Figure 1 shows an example of one set of viabilities

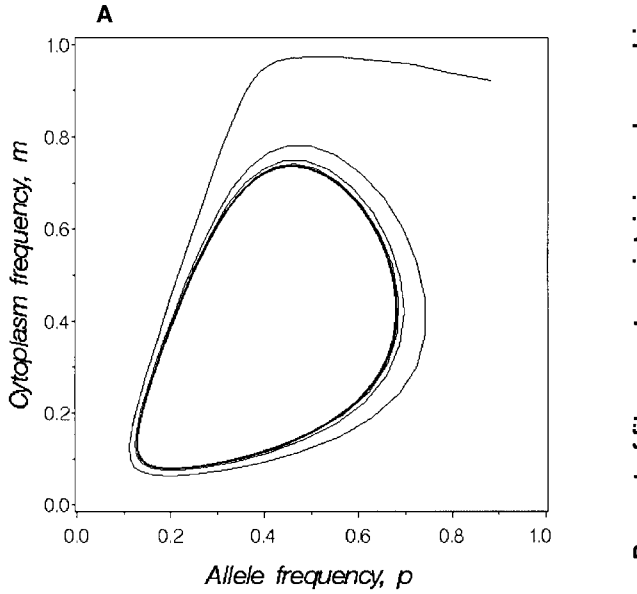

FIGURE 1.—An example of a joint nuclear and cytoplasmic polymorphism. (A) Frequency trajectory of nuclear and cytoplasmic frequencies through time in one simulation. The fitnesses of the six female cytogenotypes were as follows: XXM = 0.5858, XXm = 0.4740, XxM = 0.8027, Xxm = 0.3815, xxM = 0.2805, and xxm = 0.8275, and the four male cytogenotypes were XM = 0.1986, Xm = 0.3774, xM = 0.8427, and xm = 0.0444. (B) The proportion of random fitness sets that maintain joint nuclear and cytoplasmic polymorphisms depends on degree of paternal leakage. Each point is the proportion of random fitness sets (out of 1000 for the given level of paternal leakage) that maintain the joint polymorphism.

(with strict maternal transmission of the organelle genome), which results in a limit cycle for the nuclear and organellar alleles. Figure 1 also shows that the proportion of random viability sets maintaining a joint polymorphism depends on the proportion of paternal contribution of the cytoplasmic genome. Cytonuclear polymorphism can be maintained with either strict uniparental inheritance or nearly biparental inheritance, but some intermediate level of paternal leakage appears to produce the greatest proportion of random fitness sets that maintain polymorphism.

From the 10,000 random viability sets, a sample of 234 sets that maintained cytonuclear polymorphism under no paternal leakage ($k = 0$; hereafter "polymorphic viability sets") was examined for patterns of viability that might suggest important aspects of the behavior of the model. The average viabilities for the 10 genotypes across these 234 polymorphic viability sets are shown in Figure 2. On average there was evidence for heterozygote advantage in the females (shaded bars, Figure 2) and a tendency for female viability to be slightly greater than that of male viability (compare solid *vs.* hatched bars, Figure 2). However, these generalities based on the average across viability sets are not the rule since there are sets with female heterozygote disadvantage that maintain joint polymorphism (*e.g.*, $XXM = 0.966$, $XXm = 0.965$, $XxM = 0.017$, $Xxm = 0.048$, $xxM = 0.570$, $xxm = 0.858$, $XM = 0.538$, $Xm = 0.540$, $xM = 0.335$, and $xm = 0.180$). About 10% of the polymorphic viability sets show female heterozygote disadvantage.

The sample of polymorphic viability sets also shows some interesting correlations among the 10 genotypes (Table 3). For a given pair of male and female nuclear genotypes (*e.g.*, *X vs. XX*), the sign of the correlation changes if the cytoplasmic genotype changes (compare $XM \times XXM$ with $XM \times XXm$ in the lower left block of values in Table 3). If one looks across a given male cytogenotype, the sign of the correlation changes if the nuclear genotype changes (*e.g.*, compare $XM \times XXM$ *vs.* $XM \times xxM$ in Table 3). None of the correlations between male cytogenotypes and the heterozygous female cytogenotypes is significant. These patterns indicate that males and females tend not to favor the same gametic type (Table 3), an observation consistent with recent theory suggesting that differential selection between the sexes is important in conditions that maintain cytonuclear disequilibria (BABCOCK and ASMUSSEN 1996, 1998; GOODISMAN and ASMUSSEN 1997).

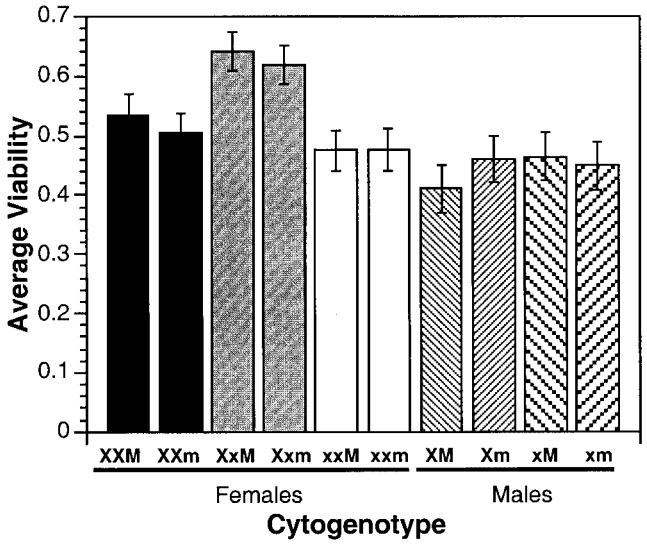

FIGURE 2.—Mean viabilities for 234 random fitness sets that maintain joint nuclear and cytoplasmic polymorphisms with no paternal leakage ($k = 0$). Error bars are 95% confidence limits.

TABLE 3

Correlations between simulated male and female viabilities

	Female genotypes						Male genotypes			
	XXM	XXm	XxM	Xxm	xxM	xxm	XM	Xm	xM	xm
Female genotypes										
XXM	—									
XXm	0.354	—								
XxM	−0.035	0.119	—							
Xxm	0.094	−0.159	0.146	—						
xxM	−0.314	−0.073	−0.047	0.113	—					
xxm	0.122	−0.169	0.140	−0.089	0.137	—				
Male genotypes										
XM	−0.271	0.268	0.074	−0.045	0.363	−0.257	—			
Xm	0.247	−0.257	−0.041	−0.034	−0.125	0.376	−0.468	—		
xM	0.272	−0.158	0.057	−0.085	−0.340	0.231	−0.324	0.438	—	
xm	−0.121	0.236	−0.053	−0.033	0.161	−0.388	0.396	−0.173	−0.369	—

Correlations are among 234 fitness sets that maintain joint X chromosome-cytoplasm polymorphism out of 10,000 random fitness sets examined with no paternal leakage ($k = 0$). All correlations with absolute values >0.13 are significant at the 5% level.

Similar striking changes in the sign of these correlations are observed among genotypes within a sex. For example, the correlation between the female genotypes XXM and XXm is positive, but the correlation between XXM and xxM is negative. In males, the correlations between the "coupling" and "repulsion" genotypes XM × xm and Xm × xM are positive, while the others are negative. All of the male × male correlations are highly significant, 14/24 = 58% of the female × male correlations are significant, and 7/15 = 47% of the female × female correlations are significant. Clearly, some form of viability "matching" occurs between reciprocal cytonuclear genotypes within sexes. Since males do not generally pass on mtDNA, the maintenance of joint X-linked and cytoplasmic polymorphisms involves both sexually antagonistic viabilities as well as intrasexually antagonistic viabilities. It may be that the fitness effects on X chromosomes in males are crucial for the maintenance of the joint X-cytoplasm polymorphism even though males do not pass on the cytoplasm. Since the probability of maintaining a joint X-cytoplasm polymorphism is increased by paternal leakage, this model may apply to a diversity of organisms with both uni- and biparental inheritance of cytoplasmic genomes. A complete analysis of the dynamics of these systems and the stability of the polymorphic equilibria will be presented elsewhere. The goal of the modeling was to answer the question motivated by the differential patterns of chromosomal cotransmission with mtDNA presented in Table 1. These results confirm that sex-linked cytonuclear interactions are different from autosomal cytonuclear systems and strongly motivate an empirical study that examines the nature of these interactions.

Fitness assay of X-linked cytonuclear genotypes: A total of 47,522 flies were scored. The segregation scores (hereafter "fitnesses" or "fitness scores") from the two broods from each set of parents were highly significantly correlated ($P < 0.0001$), their means did not differ significantly, nor were there any X chromosome × brood or cytoplasm × brood interaction effects. This was true for both male fitness and female fitness (all P values > 0.25; data not shown). Thus, data for the two broods were pooled for all subsequent analyses. Analyses using arcsine-square root transformed data were qualitatively indistinguishable from analyses with untransformed data, so only the latter are presented.

The mean fitness scores for males and females, respectively, were 0.59 (95% confidence interval (C.I.) = 0.585–0.607) and 0.433 (95% C.I. = 0.443–0.424). The significantly higher score in males is most likely due to deleterious mutations on the FM7 balancer that are expressed in hemizygous males. While deleterious alleles are expected on wild X chromosomes as well, the density of such mutations is expected to be much lower than on a nonrecombining balancer that has been maintained in lab culture for many years. Also note that the X chromosomes in this study are not a random sample from nature but are those that successfully yielded isogenic lines. In females, $+_i/+_i$ homozygotes have a slight disadvantage with respect to $+_i$/FM7 heterozygotes (on average), presumably reflecting the unmasking of slightly deleterious alleles in homozygous wild chromosomes relative to $+_i$/FM7 heterozygotes where recessive alleles on both the wild and the balancer chromosomes are masked. Despite these differences, the crossing scheme ensures that the same FM7 balancer and Y chromosome are carried in all experimental genotypes, so that relative comparisons are valid.

Across the entire data set the sex ratio (proportion of males) was 0.447 (95% C.I. = 0.441–0.454). Again,

TABLE 4

Analyses of variance for cytonuclear interactions among diverse lines

Source	d.f.	SSQ	*F* ratio	*P* <
X chromosome and cytoplasm: females (all lines, $n = 563$ crosses; Australia/Wolbachia excluded, $n = 326$ crosses)				
X chromosome (all)	11	0.3725	4.1358	0.0001
(no Australia/Wolbachia)	8	0.1926	3.3983	0.0031
Cytoplasm (all)	11	0.5680	6.3059	0.0001
(no Australia/Wolbachia)	8	0.2464	4.3478	0.0003
X chromosome × cytoplasm (all)	121	1.8939	1.5770	0.0006
(no Australia/Wolbachia)	64	1.0001	1.7076	0.0023
Males (all, $n = 566$ crosses; Australia/Wolbachia excluded, $n = 326$ crosses)				
X chromosome (all)	11	0.6543	5.0297	0.0001
(no Australia/Wolbachia)	8	0.3506	4.5926	0.0002
Cytoplasm (all)	11	0.5903	4.5372	0.0001
(no Australia/Wolbachia)	8	0.1469	1.9241	0.0775
X chromosome × cytoplasm	121	2.4356	1.4159	0.0067
(no Australia/Wolbachia)	64	1.1845	1.5014	0.0162
X chromosome and mtDNA: females ($n = 563$ crosses; Australia/Wolbachia excluded, $n = 326$ crosses)				
X chromosome (all)	11	0.2569	1.9049	0.0364
(no Australia/Wolbachia)	8	0.1683	1.7687	0.0827
mtDNA (all)	2	0.0811	3.3059	0.0374
(no Australia/Wolbachia)	2	0.0205	0.8631	0.4229
X chromosome × mtDNA (all)	22	0.3149	1.1674	0.2715
(no Australia/Wolbachia)	16	0.1265	0.6643	0.8284
Males (all, $n = 566$ crosses; Australia/Wolbachia excluded, $n = 326$ crosses)				
X chromosome (all)	11	0.6411	3.4631	0.0001
(no Australia/Wolbachia)	8	0.3521	3.1220	0.0021
MtDNA (all)	2	0.0472	1.4027	0.2464
(no Australia/Wolbachia)	2	0.0480	1.7024	0.1840
X chromosome × mtDNA (all)	22	0.3511	0.9485	0.5301
(no Australia/Wolbachia)	16	0.2352	1.0429	0.4110

Note: the first line of each main effect or interaction term reports the analyses for the complete data set, while the second line labeled (no Australia/Wolbachia) reports the analyses excluding all data involving lines Aus 4, 5, and 7, which carried Wolbachia. SSQ, sum of squares.

this slight female bias presumably reflects the deleterious effects of the FM7 balancer in hemizygous males. Sex ratio was subjected to analysis of variance where X chromosome, cytoplasm, and X × cytoplasm interactions were effects, and none were significant. A similar ANOVA with X chromosome, mtDNA, and X × mtDNA interactions revealed no significant effects. There is no correlation between the sex ratio that emerges from a cross and the female fitness scores from that cross. While male fitness scores are significantly positively correlated with sex ratio, this correlation is not high ($r = 0.16$, $P < 0.001$). Moreover, when the relationship between sex ratio and male fitness score is subjected to analysis of covariance, there is no significant effect of X chromosome, cytoplasm, or their interaction, nor is there an effect of mtDNA when these terms are added as covariates. These analyses suggest that the use of ANOVAs to explore fitness interactions between X chromosomes and cytoplasms and X chromosomes and mtDNA is unlikely to be confounded by aspects of the experimental design. These same ANOVAs were done excluding the Australia lines that carried Wolbachia, and the results were qualitatively the same (no significant results became nonsignificant, and all nonsignificant results remained nonsignificant).

Nuclear-cytoplasmic interactions within and between populations: Over the entire data set there were strong X chromosome, cytoplasm, and X chromosome × cytoplasm (hereafter X × C) effects for both males and females (Table 4). As mentioned above, this interpopulation result is expected from previous theory (Clark 1984; Gregorius and Ross 1984) and empirical studies (Clark and Lyckegaard 1988). Note that the exclusion of the Wolbachia-infected lines from Australia alters the significance of the cytoplasm term only in males. Importantly, there were no significant X chromosome × Wolbachia interactions in either males or females when all lines were examined (data not shown).

Of primary interest is whether X × C effects can be observed among the lines from within each of the three

TABLE 5

Analyses of variance for cytonuclear interactions within populations

Source	d.f.	Sum of squares	*F* ratio	*P* <
Australia females ($n = 36$)				
X chromosome	2	0.0117	0.7207	0.4955
Cytoplasm	2	0.1727	10.6768	0.0004
X chromosome × cytoplasm	4	0.0956	2.9562	0.0379
Australia males ($n = 36$)				
X chromosome	2	0.0491	3.3980	0.0483
Cytoplasm	2	0.0408	2.8226	0.0771
X chromosome × cytoplasm	4	0.0527	1.8236	0.1534
Beijing females ($n = 61$)				
Nuclear	3	0.0024	0.1718	0.8427
Cytoplasm	3	0.1551	11.3189	0.0001
X chromosome × cytoplasm	9	0.0336	0.6132	0.7621
Beijing males ($n = 61$)				
Nuclear	3	0.0310	1.9273	0.1571
Cytoplasm	3	0.0160	0.9937	0.3780
X chromosome × cytoplasm	9	0.0753	1.1697	0.3377
Fayetteville females ($n = 106$)				
X chromosome	4	0.0508	1.2076	0.3140
Cytoplasm	4	0.2752	6.5368	0.0001
X chromosome × cytoplasm	16	0.2471	1.4675	0.1328
Fayetteville males ($n = 106$)				
X chromosome	4	0.2377	4.2598	0.0035
Cytoplasm	4	0.1676	3.0032	0.0230
X chromosome × cytoplasm	16	0.4700	2.1062	0.0156

geographic samples in our data set (Australia, Beijing, and Fayetteville, North Carolina). Since segregation was scored separately for each sex in these three populations, six two-factor ANOVAs can be performed. In two of these six tests there is a significant X × C effect (Australia females and Fayetteville males; see Table 5). To address the issue of multiple tests, Fisher's combined *P*-value test can be applied, which pools inference across independent experiments testing the same null hypothesis (that the X × C interaction is absent). The combined $P = \text{prob}\{\chi^2_{\text{d.f.} = 6 \text{ tests}} > -2 * \Sigma \ln[P \text{ value}(i)]\}$. When this test is applied to the six X × C terms in Table 5, the null hypothesis is rejected ($P < 0.01$). Thus, the evidence for X chromosome × cytoplasm fitness interactions in the current study is significantly different from no detectable autosome × cytoplasm effect for the comparable intrapopulation experiments involving second chromosomes (Clark 1985; Clark and Lyckegaard 1988). Notably, the experimental power to detect an autosome × cytoplasm effect was considerably greater in those autosomal studies than in the current X chromosome study. These results provide empirical support for the model that X × C interaction effects can maintain fitness variation within populations.

Nuclear × mtDNA interactions? Are mtDNA haplotypes responsible for the fitness interactions? ANOVAs were performed, testing for X chromosome, mtDNA haplotype, and their interaction effects. In the entire data set (all 12 lines among three populations), there were significant main effects of X chromosome in both males and females, a significant mtDNA effect only in females, and no significant X × mtDNA interaction effect in either males or females (see Table 4, bottom half). Excluding the Australia lines with Wolbachia tended to reduce the significance of effects. No significant X × mtDNA interaction effects were detected within any of the three population samples.

In comparison to the X chromosome × cytoplasm analyses, these results indicate that the phenotypic effects of mtDNA cannot be equated with that of the term "cytoplasm." There are many factors inherited through the female cytoplasm in Drosophila that could confound mtDNA fitness effects (Wolbachia, σ, and C viruses and maternally loaded mRNAs; Clark 1985). However, it should be noted that there are 11 d.f. for the X × C test and only 2 d.f. for the X × mtDNA test (see Table 4). It is not clear whether the lower significance for X × mtDNA effects is attributable to other confounding cytoplasmic factors or to a reduced power to detect among-class variation with fewer mtDNA haplotypes. We note that no attempt to remove Wolbachia by tetracycline treatment was made in this study, nor was such an attempt made in the second chromosome studies of Clark (1985) and Clark and Lycke-

TABLE 6

Analysis of variance for sex, mtDNA, and cytoplasm

Source	d.f.	SSQ	*F* ratio	*P* <
Sex (all)	1	6.9502	474.3534	0.0001
(No Australia/Wolbachia)	1	3.1066	239.2179	0.0001
X chromosome (all)	11	0.4877	3.0259	0.0005
(No Australia/Wolbachia)	8	0.3257	3.1349	0.0017
Sex × X chromosome (all)	11	0.6129	3.8028	0.0001
(No Australia/Wolbachia)	8	0.2337	2.2494	0.0225
Sex (all)	1	7.6318	534.0623	0.0001
(No Australia/Wolbachia)	1	3.3813	260.2239	0.0001
Cytoplasm (all)	11	0.1720	1.0941	0.3622
(No Australia/Wolbachia)	8	0.1260	1.2125	0.2888
Sex × Cytoplasm (all)	11	1.3308	8.4663	0.0001
(No Australia/Wolbachia)	8	0.4285	4.1224	0.0001
Sex (all)	1	6.0223	394.5786	0.0001
(No Australia/Wolbachia)	1	3.0905	229.9334	0.0001
mtDNA (all)	2	0.0173	0.5651	0.5685
(No Australia/Wolbachia)	2	0.0762	2.8355	0.0594
Sex × mtDNA (all)	2	0.1351	4.4259	0.0122
(No Australia/Wolbachia)	2	0.0337	1.2525	0.2865

The first line of each main effect or interaction term reports the analyses for the complete data set (all); $n = 1129$ crosses. The second line labeled (no Australia/Wolbachia) reports the analyses excluding all data involving lines Aus 4, 5, and 7, which carried Wolbachia ($n = 652$ crosses).

GAARD (1988), where no mtDNA haplotype effects were detected.

Genotype × sex interactions: The model and simulations presented above show that differential selection in the sexes is an important component of the maintenance of cytonuclear fitness effects. We thus subjected the entire data set to analysis of variance with sex and either X chromosome, cytoplasm, or mtDNA as main effects plus their respective interaction terms (Table 6). For each ANOVA the large effect of sex is expected from the FM7 balancer, as described above. The main effect of mtDNA or cytoplasm is not significant given the large difference in fitness scores between males and females (a large within-class variance in these models). However, there is a significant interaction between sex and X chromosome, between sex and cytoplasm, and between sex and mtDNA, indicating that the rank ordering of fitnesses for genotypes is different between the sexes. There is no significant sex × Wolbachia interaction (data not shown), but the significance of the sex × mtDNA interaction is lost when the Australia/Wolbachia lines are excluded. As shown in Figure 3, the genotypes that have high fitnesses in females tend to have low fitness in males, and vice versa. These data indicate that selection among mtDNAs, cytoplasms, and X chromosomes is different in the two sexes, a result that emerged from the simulations presented above as well as from recent theoretical studies focusing on cytonuclear disequilibria (GOODISMAN and ASMUSSEN 1997; BABCOCK and ASMUSSEN 1998).

Negative fitness correlations between the sexes: The data shown in Figure 3 indicate that the fitness of a genotype can change sign with a change in the sex of its carrier. This is analogous to crossing reaction norms where "sex" is considered a different environment for the genotype in question. Across the entire data set of 12 lines, there is indeed a highly significant negative correlation between the fitness scores for the males and females that emerge from the same cross (Figure 4; $r = -0.285$, $P < 0.0001$). This negative correlation remains significant with the exclusion of the Australia/Wolbachia lines ($r = -0.184$, $P < 0.0009$). This negative correlation is not affected by mtDNA haplotype [when female and male fitnesses are subjected to analysis of covariance using mtDNA haplotype and its interaction as covariates, the result is not significant ($P > 0.2$, data not shown)]. As mentioned above, there is no correlation between the sex ratio that emerges from a cross and the female fitness scores from that cross. While male fitness scores are significantly positively correlated with sex ratio, this correlation is low (Figure 4; $r = 0.16$, $P < 0.001$). Moreover, when the relationship between sex ratio and male fitness score is subjected to analysis of covariance, there is no significant effect of X chromosome, cytoplasm, or their interaction, nor is there an effect of mtDNA when these terms are added as covariates. This suggests that the negative correlation between male and female X chromosome fitness scores does not confound the cytoplasm, mtDNA, or interaction effects described above.

The negative correlation between the male and female fitness scores appears to be largely an interpopulation

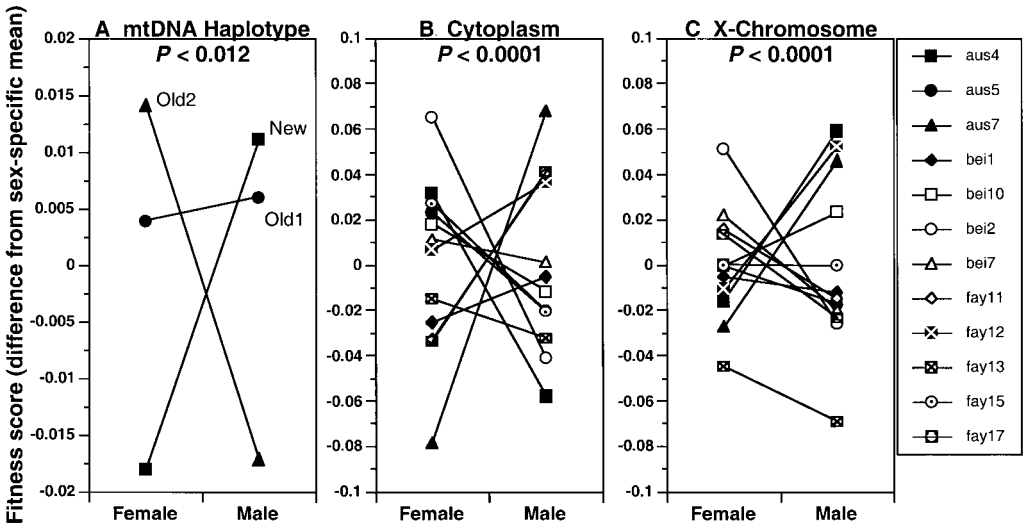

FIGURE 3.—Genotype × sex interactions. (A) mtDNA × sex interactions. Mean fitness score across all lines for each mtDNA haplotype in both sexes. (B) Mean fitness score for cytoplasms (female line) across all lines in both sexes. (C) Mean fitness score for X chromosomes across all lines in both sexes. Fitness scores are plotted as the mean fitness score for a given genotype subtracted from the grand mean of all genotypes. This is done separately for each sex.

phenomenon. Table 7 shows the correlations between male and female fitness scores for crosses involving one strain crossed to all other strains. The data are tabulated for those crosses where the focal strain was the source of the X chromosome (left side of table) or that strain was the source of the cytoplasm (right side of table). For X chromosomes, 10 out of 12 correlations are negative, all significant correlations are negative, and 6 out of 12 are significant and negative. For cytoplasms, 11 out of 12 correlations are negative, all significant correlations are negative, and 6 out of 12 are significant and negative. However, the negative correlation between the sexes is no longer significant when the data set is restricted to crosses between pairs of lines from within a single geographic locality (pooled data for Australia × Australia crosses, Beijing × Beijing crosses, North Carolina × North Carolina crosses; $n = 202$ crosses, $r = -0.1267$, $P = 0.0724$). Note that the sample size for this within-population sample is considerably larger than any of the focal between-population crosses (Table 7).

DISCUSSION

There are hundreds of nuclear-encoded genes that are essential for mitochondrial function (GILLHAM 1994). Nucleotide variation at nuclear and mitochondrial genes is common in all organisms, and ~15% of nuclear data sets and half of mitochondrial data sets show departures from neutral expectations (AKASHI and KREITMAN 1995; NACHMAN 1998; RAND and KANN 1998; EANES 1999; WEINREICH and RAND 2000). Together, these observations would suggest that nuclear-cytoplasmic fitness interactions should be common. This prediction is upheld, but only if one includes studies that have examined populations where some degree of differentiation between two forms is apparent (*e.g.*, MACRAE and ANDERSON 1988; SCRIBNER and AVISE 1994; HUTTER and RAND 1995; CRUZAN and ARNOLD 1999). Perhaps surprisingly, studies reporting cytonuclear fitness interactions within experimental populations (*e.g.*, CLARK and LYCKEGAARD 1988) or cytonuclear disequilibria in samples from natural populations are rare (MAROOF *et al.* 1992). Some cytonuclear disequilibria may be too subtle to detect with reasonable statistical power (*e.g.*, MOYA *et al.* 1993). If the molecular natural history of nuclear and cytoplasmic genomes seems to provide the raw material for abundant cytonuclear fitness interactions, why are they not easier to detect? The present study supports the findings of earlier work (*e.g.*, CLARK 1984, 1985) that the dynamics of haploid selection pro-

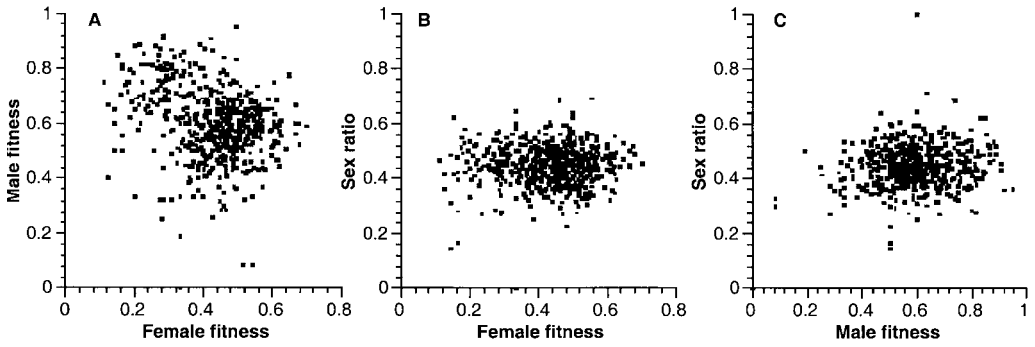

FIGURE 4.—Fitness correlations between the sexes. (A) Negative fitness correlation between males and females of a given cytogenotype. (B) Correlation between female fitness score and sex ratio (proportion of males) for a given cytogenotype. (C) Correlation between male fitness score and sex ratio for a given cytogenotype. All replicates for each cytogenotype are plotted.

TABLE 7

Viability correlations between the sexes among experimental genotypes

	X chromosome			Cytoplasm		
	Correlation	Count	*P*	Correlation	Count	*P*
Aus 4	−0.5667	51	0.0000	−0.0291	51	0.8392
Aus 5	−0.0435	46	0.7742	−0.1784	45	0.2409
Aus 7	−0.4745	40	0.0020	−0.4532	40	0.0033
Bei 1	−0.2422	49	0.0936	−0.3320	55	0.0133
Bei 10	−0.5599	55	0.0000	−0.1820	44	0.2372
Bei 2	0.0189	45	0.9018	−0.1149	50	0.4270
Bei 7	−0.3033	24	0.1497	−0.3628	43	0.0168
Fay 11	−0.3044	50	0.0316	−0.4689	45	0.0012
Fay 12	−0.4805	55	0.0002	−0.3563	49	0.0120
Fay 13	−0.3329	52	0.0159	0.0556	51	0.6982
Fay 15	−0.1751	45	0.2499	−0.0621	43	0.6922
Fay 17	0.0997	50	0.4908	−0.3009	46	0.0422

Correlations were determined for the fitness scores of male and female siblings from the same cross. The crosses were between the focal strain listed in each row of the table and all 12 strains in the experiment (including itself). The left and right sides of the table list the correlation coefficient when the focal strain was the source of the X chromosome or cytoplasm, respectively.

vide the best answer to this question. But when cytonuclear fitness interactions are detected, where in the two genomes might these interactions lie? This study provides both theoretical and empirical evidence that cytonuclear interactions involving sex chromosomes are fundamentally different from those involving autosomes.

Maintaining fitness variation at a nuclear locus is not the problem; there are a number of balancing selection models that can maintain stable polymorphisms (Hartl and Clark 1997, pp. 240–263). The problem lies in the maintenance of polymorphism in the haploid cytoplasmic genome. The conditions for selective maintenance of haploid polymorphism are more restrictive, requiring modulation of fitness by symmetrically balanced frequency-dependent selection, differential selection in the sexes, or selection in multiple niches (Clark 1984; Gregorius and Ross 1984; Babcock and Asmussen 1998). These models suggest that selection on joint nuclear-cytoplasmic polymorphisms would lead to the fixation of alternative cytoplasmic alleles between populations, even if some sort of balancing selection maintained variation at the nuclear locus. Empirical support for this view of the cytonuclear fitness interactions is provided by fitness assays in *D. melanogaster*, where no cytonuclear fitness interactions were detected within geographic populations, but fitness interactions were detected in crosses involving wild strains from distinct geographic populations (Clark 1985; Clark and Lyckegaard 1988).

These initial models and experiments involving cytonuclear genotypes were based on autosomal loci where one need not define uniquely male and female genotypes. For loci on the X chromosome in mammals and insects, or in haplodiploid species (*e.g.*, Goodisman and Asmussen 1997), the rules of chromosomal transmission suggest very different dynamics (Table 1). Simulations of the X-linked model presented here show that constant fitnesses can maintain joint nuclear and cytoplasmic polymorphisms, a result that was not observed in similar models of cytonuclear interactions with autosomes. In our fitness assays, two out of six possible tests detected significant interactions between X chromosomes and cytoplasms among wild strains from within the same geographic populations, and the combined results from all six tests showed a significant X × C effect across the entire experiment (Table 5). Again, this intrapopulation result was not observed in the second chromosome studies of Clark (1985) and Clark and Lyckegaard (1988) even though the power in the latter studies was considerably higher than in the experiments reported here. Thus our theoretical and empirical analyses indicate that opportunities for adaptive nuclear-cytoplasmic interactions are greater for sex chromosomes than for autosomes.

Fitness effects within and between populations: While we detected X chromosome × cytoplasm effects within populations, the strongest X × C effect was that for the entire data set of diverse strains from different populations. Similarly, if we focus on mtDNA haplotypes rather than cytoplasms, our only significant X chromosome × mtDNA effect was among all 144 genotypes from the 12 lines (none of the six intrapopulation tests detected significant X × mtDNA fitness interactions). These results indicate that, like the results for autosomal systems (Clark and Lyckegaard 1988), the between-population component of cytonuclear fitness effects remains an important aspect of cytonuclear fitness interactions. However, a prediction that follows from the model and

results presented above is that a greater proportion of cytonuclear fitness variation would be maintained within populations for the X-cytoplasm system than for the autosome-cytoplasm system. One can attempt to address this prediction by comparing the variance component attributable to the nuclear × cytoplasm fitness effects from Clark and Lyckegaard's (1988) autosomal study to those from the current X-linked study. There are problems with this approach since the studies of Clark and Lyckegaard (1988) were conducted with different strains and balancers from those in the X-linked assays described here and the ANOVA designs were different. Nonetheless, some interesting results emerge.

For the autosomal study the percentages of the variance components attributable to nuclear × cytoplasm fitness effects were 3.66 and 2.76% for two samples from a Pennsylvania population and 5.75% among diverse strains (Table 5 in Clark and Lyckegaard 1988). In our study, males and females were analyzed separately. For females, the X chromosome × cytoplasm variance components were 22.6, 6.2, and 18.2% for the Australia, Beijing, and Fayetteville samples, respectively, and 26.4% among all strains (25.5% excluding the three Australia/Wolbachia lines). For males the values were 13.9, 14.9, and 23.6% for Australia, Beijing, and Fayetteville, respectively, and 24.1% among all lines (24.3% excluding the three Australia/Wolbachia lines). The values for the current X chromosome study are noticeably larger than those for the autosome study by Clark and Lyckegaard (1988), and the exclusion of the Wolbachia-infected lines from Australia has little effect.

Sexually antagonistic selection: The simulations of the model based on the matings shown in Table 2 and the diallel design of the cytonuclear fitness experiment present very different kinds of data, but there are some striking parallels that emerge from both approaches. Among 234 fitness sets that maintained cytonuclear polymorphism in the simulations, it is clear that asymmetry in fitnesses of males and females is a common feature (Figure 2). Similarly, the empirical data reveal significant negative correlations between relative fitnesses of male and female genotypes when tested in alternative cytoplasms (Table 3). This indicates that selection in the two sexes tends not to favor the same gametic types. Our fitness assays also show significant fitness interactions between the sex of the fly and the mtDNA, cytoplasm, or X chromosome carried by that fly (although exclusion of the Wolbachia-infected lines from Australia eliminated the significance of the sex × mtDNA interaction effect). These changes of rank orders of genotypes between the sexes are analogous to crossing reaction norms or sexually antagonistic genotype × environment interactions where the environment is the sex of the fly (Figure 3). This kind of selection may contribute to the maintenance of genetic variation for fitness-related traits (*e.g.*, Wayne *et al.* 1997).

Empirically, the negative fitness correlation between the sexes results from the observation that, when wild-type female offspring have high relative fitness (where +/+ is compared to +/*Bar*), their wild-type brothers (+/Y) are relatively inferior to *Bar*/Y males. Over the entire data set this negative correlation is not affected by cytoplasm, mtDNA haplotype, or Wolbachia, and this is not due to variation in sex ratio (Figure 4). An important aspect of this result is that no such correlations were evident in the second chromosome studies of Clark (1985) and Clark and Lyckegaard (1988). One possible explanation is genetic variation for nondisjunction, which is increased when a wild chromosome is paired with a balancer chromosome (see Zwick *et al.* 1999 and references therein). However, the rates of nondisjunction are sufficiently low that no more than 50 data points in Figure 4 could be influenced by this phenomenon (*cf.* Zwick *et al.* 1999). The negative correlation is still significant if the 25 most extreme points at either end of the correlation are removed (data not shown). Meiotic drive is also unlikely to explain the pattern, since a driving X chromosome would tend to create positive fitness correlations between the sexes in our assay (see above).

The negative fitness correlation between the sexes is also affected by within- *vs.* between-population comparisons. The entire data set shows a very significant negative correlation (Figure 4), but this correlation is no longer significant when the data are restricted to samples from within a geographic locality. This is not a power issue, since smaller samples involving one line crossed to all others tend to show significant negative fitness correlations between the sexes (Table 7). Interestingly, however, the one population where a significant negative correlation is observed between the sexes is Australia (*i.e.*, crosses among Aus4, Aus5, and Aus7; $r = -0.4110$, $P = 0.0128$). Sequence data for the mitochondrial ND5 gene from 10 strains of an Australian sample show six sequences identical to haplotypes found in North America, and 4 strains identical to haplotypes found in Europe and Africa (D. Rand, unpublished data). Moreover, restriction analysis of 150 strains along the eastern coast of Australia show that virtually all wild samples consist of a mixture of two RFLP types in varying frequency (Boussy *et al.* 1998). Since *D. melanogaster* colonized Australia in recent human history, and the mtDNA data suggest two possible sources of colonization, the crosses among Aus4, Aus5, and Aus7 may in fact approximate an interpopulation cross. We acknowledge that the presence of Wolbachia in the Australia lines could affect the negative fitness correlation between the sexes in this population provided there are different cytoplasmic compatibility strains of Wolbachia in these lines. An analysis of the presence and absence of Wolbachia on cytonuclear and sexually antagonistic fitness effects has been initiated and will be reported at a later date.

What is good for the goose is bad for the gander: An

attractive explanation for the negative fitness correlation is that loci on the X chromosome are important targets of sexual selection. As shown by Rice (1996) and Holland and Rice (1999), strong antagonistic sexual selection is a natural component of mating in *D. melanogaster*. Responses to sexual selection are most likely highly polygenic, so it seems quite likely that many loci on the X chromosome could be selected for strong female function/weak male function or vice versa. Even if loci that are direct targets of sexual selection are underrepresented on the X chromosome, there are likely to be many X-linked loci with sexually antagonistic effects that are pleiotropic by-products of sexual selection acting on loci elsewhere in the genome. While our fitness assay did not address sexual selection, we may have uncovered genetic variation for fitness that has been maintained on the X chromosome as a consequence of sexually antagonistic selection. Again, these explanations are attractive in light of the apparent absence of negative fitness correlations between the sexes on second chromosomes (Clark 1985; Clark and Lyckegaard 1988).

The maintenance of X-linked fitness variation by sexually antagonistic selection is an important finding, but how might this kind of "balancing selection" influence the maintenance of cytonuclear and, specifically, mtDNA polymorphism? If a "bad" mtDNA in females is compensated for by being "good" in males, this effect on cytonuclear variation may be nullified by strict maternal inheritance of mtDNA. It would follow that changing the proportion of paternal leakage in cytoplasmic transmission would change the likelihood of maintaining joint cytonuclear polymorphisms. The simulation results certainly suggest this (Figure 1). Paternal leakage in crosses between strains of *D. melanogaster* is virtually undetectable in experimental time (Kondo *et al.* 1990; paternal leakage is detectable in crosses between different species; Kondo *et al.* 1990). Moreover, the simulations show that cytonuclear polymorphisms can be maintained with no paternal leakage (Figure 1). Together these observations suggest that a key component to the maintenance of the joint cytonuclear polymorphism is the change in the fitness interactions between the cytoplasmic locus and the X-linked locus in the two sexes (see Table 3). Thus, sexually antagonistic selection that maintains X chromosome fitness variation could have important consequences for joint cytonuclear polymorphism even with strict maternal transmission of mtDNA (or other cytoplasmic factors such as sigma virus or Wolbachia) and the absence of an mtDNA effect on the negative fitness correlation (Figure 4). Sexual reproduction can create the context for evolutionary conflict (Partridge and Hurst 1998). It may be just this kind of conflict between sex chromosomes that creates evolutionary opportunities with respect to cytonuclear interactions (*e.g.*, Werren and Beukeboom 1998). Since our simulation results indicate that the degree of paternal leakage can alter the probability of maintaining a stable cytonuclear polymorphism, the impact of potential evolutionary conflicts for sex chromosomes on cytonuclear coevolution warrants further attention. A complete analysis of the cytonuclear fitness space and conditions for stability will be presented elsewhere. But these initial simulations and experimental results help focus the stability analyses on this modulation of fitness interactions between the sexes.

The population structure of cytonuclear fitness interactions may have an important connection to sexually antagonistic selection. While the X-linked model and results presented above show that more cytonuclear fitness variation can be maintained within populations than for autosomal systems, this by no means precludes the accumulation of fitness differences between populations (*e.g.*, Clark and Lyckegaard 1988; Asmussen and Arnold 1991; Goodisman and Asmussen 1997; Datta and Arnold 1998; Goodisman *et al.* 1998). Our model shows that ~5% of random fitness sets can maintain joint cytonuclear polymorphisms with strict maternal inheritance (and up to 14% with paternal leakage), so clearly a substantial proportion of fitness sets (~86–95%, depending on paternal leakage) will lead to fixation of alternative cytotypes or nuclear alleles between populations. Thus, fitness divergence among populations for X-linked cytonuclear effects is to be expected. Moreover, since X chromosomes are haploid in males, we would expect more opportunity for fitness differences to accumulate among sex chromosomes as well. As suggested by Rice and Holland (1997), interlocus contest evolution may indeed promote such population differentiation. These experiments strongly motivate a joint fitness assay where X chromosomes, autosomes, and cytoplasms are extracted from the same females (that have been cleared of Wolbachia by tetracycline treatment). In such a design one could partition the differential effects of haploid mtDNA, semihaploid X chromosomes, and diploid autosomes to within- and between-population effects.

In closing we consider some clinical implications of our findings. Many mitochondrial diseases are first detected by a maternal mode of transmission. Our observations of fitness interactions between X chromosomes and mtDNA haplotypes warrant more careful analyses of joint X chromosome/mtDNA genotypes in pedigrees exhibiting disease phenotypes. Moreover, the observations that a good mtDNA (or cytoplasm) in females can be bad in males suggests that the penetrance of mitochondrial disorders in maternal pedigrees might be sex specific. Several mitochondrial disorders have more severe phenotypic effects in males (Frank and Hurst 1996) or are sex limited in their expression (Ruiz-Pesini *et al.* 2000). It is interesting that the sexually antagonistic effects we observed are clearest for the extreme genotypes in either sex and not for the average genotypes (Figure 3), which by definition are not "disease"

genotypes. Thus, evolutionary models of cytonuclear fitness interactions may have an important bearing on the expression of mitochondrial diseases in humans.

We thank Dan Weinreich, Bill Rice, Marjorie Asmussen, and an anonymous reviewer for helpful comments and discussion and Tim Sackton for assistance with some of the fly work. Supported by grants from the National Science Foundation (NSF) to D.M.R. and from the NSF and National Institutes of Health to A.G.C.

LITERATURE CITED

Akashi, H., and M. Kreitman, 1995 Molecular evidence for natural selection. Annu. Rev. Ecol. Syst. **26:** 403–422.

Arnold, J., 1993 Cytonuclear disequilibria in hybrid zones. Annu. Rev. Ecol. Syst. **24:** 521–554.

Asmussen, M. A., and J. Arnold, 1991 The effects of admixture and population subdivision on cytonuclear disequilibria. Theor. Popul. Biol. **39:** 273–300.

Asmussen, M. A., and C. J. Basten, 1994 Sampling theory for cytonuclear disequilbria. Genetics **138:** 1351–1363.

Asmussen, M. A., J. Arnold and J. C. Avise, 1987 Definition and properties of disequilibrium statistics for association between nuclear and cytoplasmic genotypes. Genetics **115:** 755–768.

Babcock, C. S., and M. A. Asmussen, 1996 Effects of differential selection in the sexes on cytonuclear polymorphism and disequilibria. Genetics **144:** 839–853.

Babcock, C. S., and M. A. Asmussen, 1998 Effects of differential selection in the sexes on cytonuclear dynamics. Life stages with sex differences. Genetics **149:** 2063–2077.

Begun, D. J., and C. F. Aquadro, 1995 Molecular variation at the vermilion locus in geographically diverse populations of *Drosophila melanogaster* and *D. simulans*. Genetics **140:** 1019–1032.

Boussy, I. A., I. Masanobu, D. Rand and R. C. Woodruff, 1998 Decay of the eastern Australian latitudinal cline of *P* element-associated properties in *Drosophila melanogaster* populations, and a test of the cline's origins. Genetica **140:** 45–57.

Clark, A. G., 1984 Natural selection with nuclear and cytoplasmic transmission. I. A deterministic model. Genetics **107:** 679–701.

Clark, A. G., 1985 Natural selection with nuclear and cytoplasmic transmission. II. Tests with Drosophila from diverse populations. Genetics **111:** 97–112.

Clark, A. G., and E. M. S. Lyckegaard, 1988 Natural selection with nuclear and cytoplasmic transmission, III. Joint analysis of segregation and mitochondrial DNA in *Drosophila melanogaster*. Genetics **118:** 471–481.

Cruzan, M. B., and M. L. Arnold, 1999 Consequences of cytonuclear epistasis and assortative mating for the genetic structure of hybrid populations. Heredity **82:** 36–45.

Datta, S., and J. Arnold, 1998 Dynamics of cytonuclear disequilibria in subdivided populations. Theor. Biol. **192:** 99–111.

Datta, S., M. Kiparsky, D. M. Rand and J. Arnold, 1996 A statistical test of a neutral model using the dynamics of cytonuclear disequilibria. Genetics **144:** 1985–1992.

Eanes, W. F., 1999 Analysis of selection on enzyme polymorphisms. Annu. Rev. Ecol. Syst. **30:** 301–326.

Fos, M. M., A. Dominguez, A. Latorre and A. Moya, 1990 Mitochondrial DNA evolution in experimental populations of *Drosophila subobscura*. Proc. Natl. Acad. Sci. USA **87:** 4198–4201.

Frank, S. A., and L. D. Hurst, 1996 Mitochondria and male disease. Nature **383:** 224.

Gillham, N. W., 1994 *Organelle Genes and Genomes*. Oxford University Press, New York.

Goodisman, M. A., and M. A. Asmussen, 1997 Cytonuclear theory for haplodiploid species and X-linked genes. I. Hardy-Weinberg dynamics and continent-island, hybrid zone models. Genetics **147:** 321–338.

Goodisman, M. A. D., D. D. Shoemaker and M. A. Asmussen, 1998 Cytonuclear theory for haplodiploid species and X-linked genes. II. Stepping-stone models of gene flow and application to a fire ant hybrid zone. Evolution **52:** 1423–1440.

Gray, M., W. G. Burger and B. F. Lang, 1999 Mitochondrial evolution. Science **283:** 1476–1481.

Gregorius, H. R., and M. D. Ross, 1984 Selection with gene cytoplasm interactions. I. Maintenance of cytoplasmic polymorphisms. Genetics **107:** 165–178.

Haldane, J. B. S., 1956 The estimation of viabilities. J. Genet. **45:** 294–296.

Hale, L. R., and R. S. Singh, 1991 A comprehensive study of genic variation in natural populations of *Drosophila melanogaster*. IV. Mitochondrial DNA variation and the role of history *vs.* selection in the genetic structure of geographic populations. Genetics **129:** 103–117.

Hartl, D. L., and A. G. Clark, 1997 *Principles of Population Genetics*, Ed. 3. Sinauer Associates, Sunderland, MA.

Hoffmann, A. A., M., Hercus and H. Dagher, 1998 Population dynamics of the Wolbachia infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. Genetics **148:** 221–231.

Holland, B., and W. R. Rice, 1999 Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. Proc. Natl. Acad. Sci. USA **96:** 5083–5088.

Hutter, C. M., and D. M. Rand, 1995 Competition between mitochondrial haplotypes in distinct nuclear genetic environments: *Drosophila pseudoobscura vs. D. persimilis*. Genetics **140:** 537–548.

Kambhampati, S., R. Rai and D. Verleye, 1992 Frequencies of mitochondrial DNA haplotypes in laboratory cage populations of the mosquito, *Aedes albopictus*. Genetics **132:** 205–209.

Kilpatrick, S. R., and D. M. Rand, 1995 Conditional hitchhiking of mitochondrial DNA: frequency shifts of *Drosophila melanogaster* mtDNA variants depend on nuclear genetic background. Genetics **141:** 1113–1124.

Kondo, R., Y. Satta, E. T. Matsuura, H. Ishiwa, N. Takahata *et al.*, 1990 Incomplete maternal transmission of mitochondrial DNA in *Drosophila*. Genetics **126:** 657–663.

Lindsley, D. L., and G. G. Zimm, 1992 The genome of *Drosophila melanogaster*. Academic Press, San Diego.

MacRae, A. F., and W. W. Anderson, 1988 Evidence for non-neutrality of mitochondrial DNA haplotypes in *Drosophila pseudoobscura*. Genetics **120:** 485–494.

Maroof, M. A., Q. Zhang, D. B. Neale and R. W. Allard, 1992 Associations between nuclear loci and chloroplast DNA genotypes in wild barley. Genetics **131:** 225–231.

Martin, W., and G. Herrmann-Reinhold, 1998 Gene transfer from organelles to the nucleus: how much, what happens, and why? Plant Physiol. **118:** 9–17.

Moya, A., E. Barrio, D. Martinez, A. Latorre, F. Gonzalez-Candelas *et al.*, 1993 Molecular characterization and cytonuclear disequilibria of two *Drosophila subobscura* mitochondrial haplotypes. Genome **36:** 890–898.

Nachman, M., 1998 Deleterious mutations in animal mitochondrial DNA. Genetica **102/103:** 61–69.

Nigro, L., and T. Prout, 1990 Is there selection on RFLP differences in mitochondrial DNA? Genetics **125:** 551–555.

Partridge, L., and L. D. Hurst, 1998 Sex and conflict. Science **281:** 2003–2008.

Poinsot, D., K. Bourtzis, G. Markakis, C. Savakis, and H. Mercot, 1998 Wolbachia transfer from *Drosophila melanogaster* into *D. simulans*: host effect and cytoplasmic incompatibility relationships. Genetics **150:** 227–237.

Rand, D. M., and L. M. Kann, 1996 Excess amino acid polymorphism in mitochondrial DNA: contrasts among genes from *Drosophila*, mice, and humans. Mol. Biol. Evol. **13(6):** 735–748.

Rand, D. M., and L. M. Kann, 1998 Mutation and selection at silent and replacement sites in the evolution of animal mitochondrial DNA. Genetica **102/103:** 393–407.

Rand, D. M., M. L. Dorfsman and L. M. Kann, 1994 Neutral and non-neutral evolution of Drosophila mitochondrial DNA. Genetics **138:** 741–756.

Rice, W. R., 1996 Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature **381:** 232–234.

Rice, W. R., and B. Holland, 1997 The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. Behav. Ecol. Sociobiol. **41:** 1–10.

Ruiz-Pesini, E., A.-C. Lapeña, C. Díez-Sánchez, A. Pérez-Martos, J. Montoya *et al.*, 2000 Human mtDNA haplogroups associated with high or reduced spermatozoa motility. Am. J. Hum. Genet. **67:** 682–696.

Scribner, K. T., and J. C. Avise, 1994 Population cage experiments

with a vertebrate: the temporal demography and cytonuclear genetics of hybridization in *Gambusia* fishes. Evolution **48:** 155–171.

Wayne, M. L., J. B. Hackett and T. F. C. Mackay, 1997 Quantitative genetics of ovariole number in Drosophila melanogaster: I. Segregating variation and fitness. Evolution **51:** 1156–1163.

Weinreich, D. M., and D. M. Rand, 2000 Contrasting patterns of non-neutral evolution in proteins encoded in nuclear and mitochondrial genomes. Genetics **156:** 385–399.

Werren, J. H., and L. W. Beukeboom, 1998 Sex determination, sex ratios, and genetic conflict. Annu. Rev. Ecol. Syst. **29:** 233–261.

Zwick, M. E., J. L. Salstrom and C. H. Langley, 1999 Genetic variation in rates of nondisjunction: association of two naturally occurring polymorphisms in the chromokinesin *nod* with increased rates of nondisjunction in *Drosophila melanogaster*. Genetics **152:** 1605–1614.

Communicating editor: J. Hey