Mitochondrial Genotype Affects Fitness in *Drosophila simulans*

Avis C. James[1] and J. William O. Ballard

Department of Biological Sciences, University of Iowa, Iowa City, Iowa 52242-1324

Manuscript received November 5, 2002
Accepted for publication January 16, 2003

ABSTRACT

Drosophila simulans is known to harbor three distinct mitochondrial DNA (mtDNA) haplotype groups (*si*I, -II, and -III) with nearly 3.0% interhaplotypic divergence but <0.06% intrahaplotypic diversity. With the large amount of genetic variation in this system, the potential power to detect intraspecific fitness differences in fly lines that carry distinct haplotypes is great. We test three life-history traits on fly lines with known sequence differences in the mtDNA genome after controlling the nuclear genome by backcrossing. We find that flies with the *si*I haplotype are fastest developing and have the lowest probability of surviving to three experimental periods (2–6, 12–17, and 34–39 days of age). Wild-type males with *si*III mtDNA were more active while disruption of specific coadapted nucleo-mitochondrial complexes caused a significant decrease in activity. These results are discussed in the context of the geographic distribution of each haplotype.

IN humans, more than 50 inherited diseases of metabolism are known to involve mitochondria. Defects in mitochondrial function have also been linked to many of the common diseases of aging, including Alzheimer's dementia, Parkinson's disease, and Huntington's disease (Scheffler 2001). Given the demographic realities of the world, where more individuals are living longer and the mean age of the individuals making up the population is steadily increasing, a better understanding of the role mitochondria play in regulating fitness and well-being becomes increasingly critical.

This study tests the fitness of three divergent mitochondrial genotypes in Drosophila, in which evidence has been found for mitochondrial genotype or nucleo-mitochondrial interactions that influence fly fitness traits (Hiraizumi 1985; Clark and Lyckegaard 1988; MacRae and Anderson 1988; Fos *et al.* 1990; Hutter and Rand 1995; Kilpatrick and Rand 1995; García-Martínez *et al.* 1998). This report uses *Drosophila simulans* to investigate mitochondria and fitness. *D. simulans* is an ideal system to study mitochondrial DNA (mtDNA) fitness effects because it is known to harbor three distinct haplotype groups (*si*I, -II, and -III) with nearly 3.0% interhaplotypic divergence (Solignac and Monnerot 1986; Satta *et al.* 1987; Baba-Aïssa *et al.* 1988) but <0.06% intrahaplotypic diversity (Ballard 2000). Flies carrying these three haplotype groups have a distinct geographical subdivision but are not distinct in chosen makers of nuclear DNA [*alcohol dehydrogenase related* (Ballard 2000), *NADH: ubiquinone reductase 75 kD subunit precursor* (Ballard *et al.* 2002), and the *period* locus (M. D. Dean, K. J. Ballard, A. Glass and J. W. O. Ballard, unpublished results)]. Furthermore, they cannot be distinguished by genital arch morphology and they mate randomly (Ballard *et al.* 2002). The geographical subdivision of mitochondrial but not nuclear DNA suggests that cytoplasmic factors influence the distribution of the three mitochondrial haplotypes.

A mitochondrial DNA mutation may be positively selected if it causes a net increase in the fitness of the molecule in a specific local environment. Thus, if there is no recombination, the selective fixation of any mutation in the mtDNA will lead to the fixation of all variants in that genome by genetic hitchhiking (Maynard Smith and Haigh 1974; Kaplan *et al.* 1989). Patterns of mtDNA variation in *D. simulans* are not consistent with neutral models of evolution. Ballard (2000) compared 22 *D. simulans* and 2 *D. melanogaster* mitochondrial genomes (minus the A/T-rich region). A maximum-likelihood sliding-window analysis identified regions of the mitochondrial genome with distinct substitution patterns. In addition, Ballard found differences in patterns of polymorphisms (in particular the ratio of nonsynonymous to synonymous substitutions) specific to the distinct lineages, indicating differential selection in the subdivided populations.

Evidence of differential fitness of *D. simulans* haplotypes comes from micro-injection studies among eggs carrying *si*I, *si*II, and *si*III mtDNA (de Stordeur 1997). After micro-injection, the fate of the foreign injected mtDNA was assayed to detect which haplotypes could be accepted by the foreign cells and, if heteroplasmic flies were created, which haplotype went to fixation in

[1]*Corresponding author:* Department of Biological Sciences, 200 Old Biology Bldg., University of Iowa, Iowa City, IA 52242-1324. E-mail: avis-james@uiowa.edu

the fly line. When the *si*I haplotype was injected into each of the two other hosts (*si*II and *si*III), heteroplasmic offspring were never detected. When the *si*II cytoplasm was injected into *si*I eggs the frequency of *si*II mtDNA increased in 37 out of 38 lines. When the *si*II cytoplasm was injected into *si*III eggs, the frequency of the *si*II haplotype tended to increase. When *si*III mtDNA was micro-injected into *si*I flies the frequency of the foreign mtDNA increased in 9 of 10 fly lines and the resident *si*I mtDNA was lost after three to eight generations. When *si*III mtDNA was micro-injected into *si*II flies the foreign *si*III mtDNA was systematically lost in all lines analyzed. Thus the relative fitnesses of the three haplotypes inferred from this assay are *si*II > *si*III > *si*I.

Additional evidence for fitness differences among *D. simulans* mtDNA haplotypes comes from a combination of micro-injections and population cage competition studies. Nigro (1994) included three lines in her study. The first line had the *si*II mitochondrial DNA, and the second line had *si*III mitochondrial DNA. The third line had been created by micro-injection; the *si*II haplotype had replaced the mitochondria in what was originally a *si*III line. In cages where competition was between lines having the original cytonuclear associations, the frequency of the *si*II mtDNA increased. In cages where the line with *si*III mtDNA competed against the transplasmic line, the frequency of the *si*III mtDNA increased. These data show that the mtDNA haplotype and the nuclear background in which it resides can influence the frequency of fly haplotypes in competition studies.

While the three haplotypes are not equally fit in microinjection or population cage competition studies, it is quite possible that mitochondrial fitness differences were not causally involved in the divergence of the haplotypes. *D. simulans* is infected with the α-proteobacteria Wolbachia that can induce cytoplasmic incompatibility. In the simplest case incompatibility occurs when an uninfected female mates with an infected male, causing a reduction in the egg hatch rate (see Hoffmann and Turelli 1997 for review). *D. simulans* carries at least four strains of Wolbachia. Sweeps of Wolbachia through populations due to incompatibility will "pull" a mitochondria variant with it, as both the Wolbachia and the mitochondria are maternally inherited. Our laboratory is currently testing the alternate hypotheses that Wolbachia have caused a "pruning" of internal branches in the mtDNA genealogy and that Wolbachia infection caused an increase in the mitochondrial mutation rate.

In this study, we test whether specific known sequence differences in the mtDNA genome of the three distinct *D. simulans* genotypes confer fitness differences after controlling the nuclear genome by backcrossing. The prediction is that life-history trait differences can be attributed to the mitochondrial genotype only if the effect occurs in flies with both native and introgressed nuclear genomes. Because of the large amount of genetic variation in this system the potential power to distinguish fitness between carriers of these three major haplotypes is considerable. In contrast, the genetic variation in *D. melanogaster* is nearly an order of magnitude lower than that in *D. simulans*.

We considered three measures of fitness. First, egg-to-adult development time was measured. Rapid preadult development time increases fitness, as these flies develop in ephemeral resources and are vulnerable to deterioration of their food and attack by predators and parasitoids. Second, we measured the activity level of males at three ages. The demonstrated role of mitochondria in the production of 90% of the energy our cells need to function makes activity levels particularly relevant when considering fitness. Third, we measured adult longevity. Specific mitochondrial haplotypes have been found at high frequencies in a population of older humans (de Benedictis *et al.* 2000), implying that haplotype may influence aging. We find that fly lines that carry the *si*I haplotype are exceptional in development time and survivorship and that wild-type *si*III males are more active.

MATERIALS AND METHODS

Construction of introgression lines: To disrupt any nucleo-mitochondrial gene complexes we introgressed the nuclear background from isofemale fly lines that carry each mitochondrial type into lines that carried each haplotype. The *si*I (TT01T) line was originally captured in Papeete, Tahiti, in July of 1998. The *si*II (MD106T) and *si*III (RU07T) lines were collected in Antsirabe, Madagascar, and Salazie, Reunion, respectively, in March of 1998. The mitochondrial genomes of these lines (excluding the A + T-rich region) are included in Ballard (2000). These lines were chosen because they were recently collected and because they are all close in latitude (18–20° S); thus clinal variation found in a number of species is controlled. All lines were tetracycline treated prior to backcrossing to ensure they were not infected with Wolbachia. Subsequent PCR confirmed all lines were Wolbachia uninfected.

Virgin females from all lines were collected and mated individually to males from each of the other two lines. Virgin F_1 females were collected and mated to males from the same line as their fathers. This was repeated until 12 generations of backcrossing were complete. In theory, 99.975% of the nuclear genome was replaced. However, it is unlikely that this level of replacement was achieved. Dermitzakis *et al.* (2000) crossed *D. sechellia* males with *D. simulans* females in 221 independent lines and found that some parts of the *D. sechellia* genome were never detected in the F_1 hybrids. This could be attributed to incompatibilities of epistatic genes and, in particular, genes that are associated from the *D. simulans* mitochondrial and the *D. sechellia* nuclear genomes. Our 12 generations of backcrossing may break coadapted nucleo-mitochondrial gene complexes. Alternatively, it may be that separating such complexes by backcrossing is biologically impossible, making these lines particularly interesting for investigating such genes.

Two independent, replicate introgression lines per nuclear

background and mitochondrial haplotype were employed for the experiments described here. The mtDNA of each line was tested and confirmed according to the assay presented at http://www.biology.uiowa.edu/ballard.

Production of experimental animals: All lines were maintained at 25°. New cultures were started from flies that were <6 days old (Hercus and Hoffmann 2000). Parents of the experimental animals were placed in "laying pots" and allowed to acclimate for 2–3 days. Egg-laying periods lasted for 4 hr, and 22 hr after the egg lay first instar larvae were collected and placed in fly media vials in groups of 30. Sample sizes and summary statistics of the life-history trait experiments (by mitochondrial haplotype) are listed in Table 1.

Statistical analyses: In an attempt to show the main effects clearly we analyze these data using a stepwise approach considering a probability level of 0.05 as significant. First we consider only the major difference between these lines, the mitochondrial haplotype (model 1 in Tables 2–4). This is biologically reasonable because there is no evidence of nuclear subdivision associated with the mitochondrial haplotypes (Ballard *et al.* 2002). Then we relax this assumption and consider the effects of the mitochondrial haplotype, the nuclear background, and the interaction between the two (model 2 in Tables 2–4). We consider this important because it is possible that coadapted nucleo-mitochondrial interactions exist.

We assume that the lines we used are representative of the lines within each haplogroup. This is valid, as there is little within-haplogroup divergence [the most divergent haplotype is *si*I, and Ballard (2000) found the most dissimilar sequences to differ by 9/14,958 bp] but great between-haplogroup divergence [on average 150/14,958 bp (Ballard 2000)]. On the other hand, any significant differences due to nuclear variation could be a general result or a line effect.

Development time: Times to pupariation and eclosion were recorded every 6 hr on 20 vials per line (but only 9 were collected for one introgression line). Larval development periods were estimated by subtracting the midpoint of the egg-laying period from the average time of pupation for each vial. Egg-to-adult times were determined by subtracting the midpoint of the egg lay from the average eclosion time for each sex. Pupal periods were calculated by subtracting the larval period from the egg-to-adult development time, per vial and sex. Development times were calculated per vial and averaged over each replicate.

Activity: Virgin males were collected over a 12-hr period. Activity of replicate males was monitored on consecutive days 2–6, 12–17, and 34–39 after virgins were collected.

Activity was monitored with the "*Drosophila* Activity Monitoring System" (TriKinetics). With this system, flies are put in individual 10-mm tubes with food in one end and cotton in the other. The tubes are placed on a rack that has an infrared beam that bisects the middle of each tube. Computer software counts each time the fly breaks the beam by passing it.

On the day each male was to be tested, they were immobilized with ice and placed in the tubes between 11:30 a.m. and noon. At 2 p.m., the tubes were placed in the rack, and data collection began at 4 p.m. Thus the activity of each male was monitored for 22 hr, from 4 p.m. one day until 2 p.m. the next. On the consecutive days of data collection, tubes with each fly line were rotated (in groups of eight) to offset any bias due to location within the rack.

Data were collected as the total number of movements recorded per male in each monitoring session and analyzed per line over the males/days.

Longevity: Virgin males that eclosed in a 12-hr period were collected and placed in new vials. Vials were kept at constant

TABLE 1

Sample sizes and summary statistics of life-history trait experiments

	*si*I			*si*II			*si*III		
	Control	Introgressed	Combined	Control	Introgressed	Combined	Control	Introgressed	Combined
				Development time (days ± 95% confidence intervals)					
No. vials	20	80	100	20	69	89	20	80	100
LDT	5.69 ± 0.02	5.56 ± 0.02	5.59 ± 0.02	5.74 ± 0.03	5.68 ± 0.02	5.69 ± 0.02	5.62 ± 0.02	5.76 ± 0.02	5.73 ± 0.02
FDT	8.38 ± 0.10	8.24 ± 0.06	8.27 ± 0.06	8.58 ± 0.13	8.37 ± 0.08	8.42 ± 0.07	8.22 ± 0.08	8.46 ± 0.05	8.41 ± 0.05
MDT	9.76 ± 0.06	9.66 ± 0.05	9.68 ± 0.04	9.82 ± 0.06	9.73 ± 0.06	9.75 ± 0.05	9.76 ± 0.09	9.77 ± 0.04	9.77 ± 0.03
				Activity (no. movements ± 95% confidence intervals)					
No. males	27	37	64	33	31	64	32	44	76
Activity	114.7 ± 11	109.1 ± 13	111.4 ± 10	134.2 ± 11	157.6 ± 13	145.5 ± 10	1570.0 ± 12	140.8 ± 13	147.6 ± 10
				Longevity (% surviving to three time periods)					
No. males	85	218	303	102	289	391	58	325	383
Average longevity	89 ± 2	77 ± 2	80 ± 2	85 ± 2	87 ± 1	86 ± 1	89 ± 3	87 ± 1	87 ± 1

LDT, larval development time; FDT, egg-to-adult development time of females; MDT, egg-to-adult development time of males.

TABLE 2

ANOVAs on larval development time

Source of variation	d.f.	SS	*F*	*P*
	Model 1			
Haplotype	2	1.05	17.89	<0.001
	Model 2			
Haplotype	2	0.58	13.36	<0.001
Nuclear background	2	1.24	28.38	<0.001
Haplotype by nuclear background	4	0.87	9.96	<0.001

SS, sum of squares.

TABLE 3

ANOVAs on activity

Source of variation	d.f.	SS	*F*	*P*
	Model 1			
Haplotype	2	39,757.85	2.36	0.09
Age	2	33,923.06	2.02	0.13
Haplotype by age	4	18,970.52	0.56	0.69
	Model 2			
Haplotype	2	38,010.00	2.46	0.09
Age	2	32,050.98	2.07	0.13
Haplotype by age	4	22,504.29	0.72	0.57
Nuclear background	2	101,782.39	6.5	<0.01
Haplotype by nuclear background	4	9,796.27	0.32	0.86
Age by nuclear background	4	36,060.46	1.17	0.33
Haplotype by age by nuclear background	8	66,099.33	1.07	0.39

temperature (25°). Deaths were recorded between 5 and 7 p.m. every other day, and vials were changed every 4 days.

RESULTS

Development time: The larval period differed between haplotypes (Table 2, Figure 1a). Larvae harboring the *si*I haplotype developed faster than those with the *si*II or *si*III haplotype [*a posteriori* contrasts (*t*-tests on the least mean squares to detect which pairs are significantly different) of *si*I comparisons with *si*II and *si*III were $t = 4.16$ and 5.77, respectively, both $P < 0.001$, while *si*II and *si*III haplotypes were not significantly different].

In the extended model, the larval period showed significant variation due to the mitochondrial haplotype, the nuclear background, and the interaction between them (Table 2). Flies with the *si*I haplotype developed faster than those with *si*II and *si*III (*a posteriori* contrasts, $t = 4.11$ and 4.18, respectively, both $P < 0.001$). Flies with *si*II and *si*III did not differ in larval development time (*a posteriori* contrast, $t = 0.49$, $P = 0.63$).

The nuclear effect in the extended model was a result of flies with the RU07T nuclear background developing significantly more quickly than ones with either the MD106T or the TT01T backgrounds (*a posteriori* contrasts, $t = 7.16$ and 5.74, respectively, both $P < 0.001$). Flies with the MD106T and TT01T nuclear background did not differ from each other (*a posteriori* contrasts, $t = 1.46$, $P = 0.15$). This is likely a line-specific result.

Relationships between the *si*I and *si*III haplotypes and their nuclear backgrounds were responsible for the significant interaction term. When the *si*I haplotype was placed in the nuclear background of a fly line that was originally *si*III, the larvae developed significantly faster than the original *si*I line or lines introgressed with the *si*II line's nuclear background (*a posteriori* contrasts: $t = 6.11$ and 7.44, respectively, $P < 0.001$ in both cases). In contrast, when the *si*III haplotype was placed in the background of a fly line that was originally *si*I, development was slowed relative to the original *si*III line or lines introgressed with the *si*II line's nuclear background (*a posteriori* contrasts: $t = 5.35$ and 4.45, respectively, $P < 0.001$ in both cases), while there was no difference when the *si*III haplotype was placed in the nuclear background of MD106T (*a posteriori* contrasts: $t = 1.71$, $P = 0.09$). There were no differences when the *si*II haplotype was introgressed.

The pupal period was longer for males than for females ($F_{1, 568} = 7990.18$, $P < 0.001$), but there was no difference between haplotypes ($F_{2, 568} = 1.39$, $P = 0.25$) or in the interaction between haplotype and sex ($F_{2, 568} = 2.24$, $P = 0.11$).

Egg-to-adult development time is simply the larval

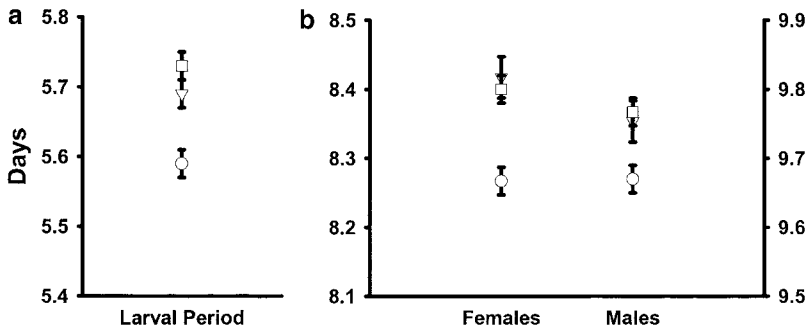

FIGURE 1.—Development time traits by mitochondrial haplotype (means and 95% confidence limits). (a) Egg to pupariation. (b) Egg to eclosion of males and females. Circles are *si*I fly lines, triangles are *si*II lines, and squares are *si*III lines. Each haplotype is represented by four introgressed lines and one control line.

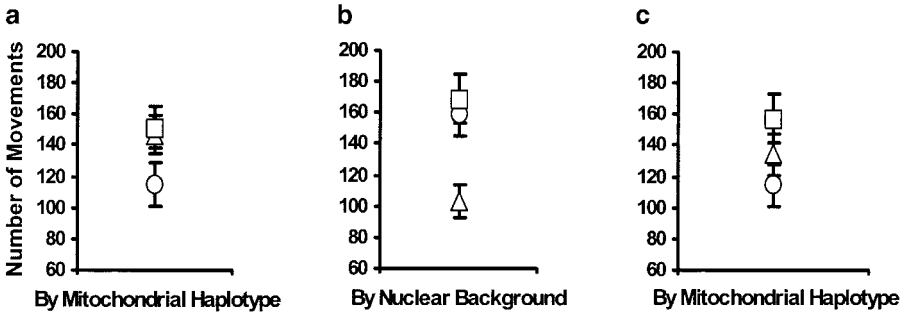

FIGURE 2.—Activity of males averaged over three time periods (means and 95% confidence limits). (a) By mitochondrial haplotype. Circles are *si*I fly lines, triangles are *si*II lines, and squares are *si*III lines. Each haplotype is represented by four introgressed lines and one control line. (b) By nuclear background. Circles are lines derived from TT01T, triangles are derived from MD106T, and squares are derived from RU07T. Each nuclear background is represented by four introgressed lines and one control line. (c) Wild-type flies. Circles are three *si*I fly lines, triangles are three *si*II lines, and squares are three *si*III lines.

and pupal periods combined and is not independent of either. Analyses of this trait tell us if the patterns in the larval period are strong enough to be apparent after adding on the pupal times that do not show pattern. Indeed the relationships of the characters show the same trend as the larval period (Figure 1b).

Activity: Activity measurements of males were not statistically different between haplotypes and age classes or in the interaction between the two (Table 3, model 1). However, *a posteriori* contrasts of the combined data indicated that *si*III flies moved more than *si*I flies ($t = 2.07$, $P = 0.04$); all other contrasts were not significant.

In the extended model, when the nuclear background and associated interaction terms were added, only the nuclear background showed significant differences (Table 3, model 2). The mitochondrial and nuclear effects are almost exclusively the result of low activity of lines with *si*I mtDNA introgressed into the MD106T nuclear background (Figure 2, a and b). As each of these introgressed lines showed low activity we suggest that these data demonstrate a clear fitness reduction caused by the disruption of coadapted nucleo-mitochondrial complexes rather than by a specific line effect.

To further investigate the activity of males we extended our data by testing two additional recently collected nonintrogressed controls from each haplotype. When data from these lines are included with the data from the three other wild-type lines the activity of males harboring each mtDNA haplotype did not differ significantly ($F_{2,89} = 2.11$, $P = 0.12$). However, males with *si*III mtDNA moved more than males with *si*I mtDNA (*a posteriori* contrasts: $t = 2.04$, $P = 0.04$, Figure 2c); all other contrasts were not significant.

Longevity: In the longevity study, survivorship curves were significantly different among fly lines with different haplotypes (Figure 3; Wilcoxon test for homogeneity between groups, $\chi^2 = 15.86$, $P < 0.001$). Comparisons of haplotypes were as follows: *si*I and *si*II, $\chi^2 = 4.65$, $P = 0.03$; *si*I and *si*III, $\chi^2 = 7.56$, $P = 0.006$; and *si*II and *si*III, $\chi^2 = 0.67$, $P = 0.41$. Thus, the survivorship curve of *si*I flies is exceptional.

We compared the percentage surviving to each of the three age classes in our activity study (2–6, 12–16, and 34–38 days of age). ANOVA on arcsine-transformed data shows that the mitochondrial haplotype and age class influence the percentage surviving (Table 4). The age effect was simply due to the fact that as flies got older, survival decreased. There was no significant haplotype-by-age effect; thus the age classes can be collapsed (Figure 4). Fewer flies with the *si*I haplotype than fly lines with the *si*II or *si*III haplotypes survived to the time periods ($t = 3.83$ and 4.12, respectively, $P < 0.001$ in each case), while fly lines with *si*II and *si*III did not differ from each other ($t = 0.26$, $P = 0.80$). The activity data showed that *si*I flies were least active. One possible explanation for this result is that *si*I flies were less active because they were physiologically older than *si*II or *si*III flies at each experimental period. This hypothesis is rejected because the activity measurements of males were not statistically different between age classes.

Our extended model including the nuclear background shows these haplotypic results were robust (Table 4). There was no effect of the nuclear background, but there was an interesting haplotype-by-nuclear background interaction. Flies from *si*I lines survive signifi-

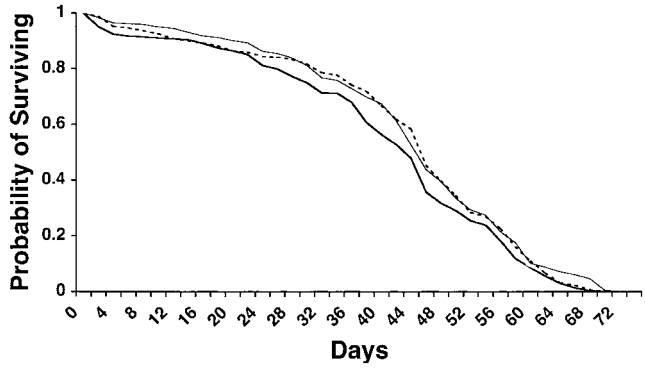

FIGURE 3.—Survivorship curves of fly lines by haplotype. The solid line is *si*I fly lines, dashed line is *si*II, and shaded line is *si*III. Each haplotype is represented by four introgressed lines and one control line.

TABLE 4

ANOVAs on percentage surviving

Source of variation	d.f.	SS	*F*	*P*
	Model 1			
Haplotype	2	0.28	10.39	<0.001
Age	2	3.88	142.01	<0.001
Haplotype by age	4	0.09	1.69	0.15
	Model 2			
Haplotype	2	1.16	3.16	<0.05
Nuclear background	2	0.64	2.05	0.12
Haplotype by nuclear background	4	1.67	2.69	<0.05

cantly more successfully to our time periods if associated with their own nuclear background than with the nuclear background from the *si*II or *si*III line (*a posteriori* contrast with *si*II, $t = 3.01$, $P < 0.01$; with *si*III, $t = 2.22$, $P < 0.05$). Also, the *si*III line survives more successfully to our time periods, although not significantly, with its original background than when introgressed with *si*I ($t = 1.45$, $P = 0.14$) or *si*II ($t = 0.70$, $P = 0.47$). Flies with *si*I and *si*III mtDNA have never been collected in sympatry and are the two most genetically divergent haplotypes (Ballard 2000).

DISCUSSION

We found significant differences among flies carrying distinct mitochondrial genotypes for both development time and survival. These mitochondrial effects were observed if we considered a simple model testing only the mitochondrial effects and a more complex model that also included the nuclear background, implying nucleo-mitochondrial interactions. Activity of *si*I males appears to be lower than that of *si*II or *si*III males; however, this result was not robust to a two-way ANOVA including the nuclear genome.

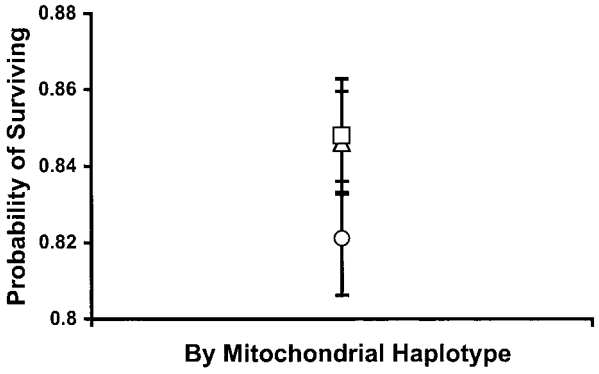

Figure 4.—Percentage of flies surviving to three age classes by haplotype (means and standard errors). Circles are *si*I fly lines, triangles are *si*II lines, and squares are *si*III lines. Each haplotype is represented by four introgressed lines and one control line.

It seems unlikely that Wolbachia infection can fully explain the geographic distribution of the mtDNA haplotypes. In contrast, it seems quite possible that the population subdivision observed in the mitochondrial genome of *D. simulans* is correlated with the Wolbachia strains that infect this species (Satta *et al.* 1988; James and Ballard 2000; M. D. Dean, K. J. Ballard, A. Glass and J. W. O. Ballard, unpublished results). We now consider how the results obtained in this study may reflect the geographic distribution of these flies.

Our results show that flies with the *si*I haplotype develop faster and live for a shorter period of time than those with the other two haplotypes. Flies with the *si*I mitochondrial haplotype have been collected on the islands of Hawaii, Tahiti, New Caledonia, and the Seychelles Islands (Merçot *et al.* 1995; Ballard 2000; James *et al.* 2002). They have not been collected in sympatry with flies harboring *si*II or *si*III mtDNA. It is possible that faster development gives these flies a selective advantage on islands where resources are particularly ephemeral and are vulnerable to deterioration. The breakup of coadapted nuclear-mitochondrial complexes in flies with *si*I mtDNA may inhibit the invasion of Indian/Pacific Islands by flies harboring *si*II mtDNA. The activity of males with *si*I mtDNA introgressed into MD106 flies (formally with *si*II mtDNA) is significantly reduced when compared to all other lines. Furthermore, flies from *si*I lines survive more successfully to each of our three experimental periods with their wild-type nuclear background than with the nuclear background of the *si*II or *si*III lines.

The *si*II haplotype is cosmopolitan, although it has not been collected in Hawaii, Tahiti, or New Caledonia. The *si*I and *si*II haplotypes have both been collected in the Seychelles, but not in sympatry. There is nothing exceptional in these data to help explain the wide geographic range of *si*II. One explanation is that flies with the *si*II haplotype are tolerant of temperature variations. As noted in materials and methods, the lines employed here were collected near the equator to control for clinal variation. This was necessary as the *si*I and *si*III lines have not been collected at sites with high latitudes. The transmission rates of Drosophila mtDNA haplotypes in flies made heteroplasmic by micro-injection can be temperature dependent across species (Tsujimoto *et al.* 1991; Matsuura *et al.* 1993, 1997). Perhaps the relative fitness of flies with different haplotypes is temperature dependent. To address this hypothesis it would be interesting to test a variety of traits at different temperatures. An alternate hypothesis that Wolbachia governs the distribution of the *si*II haplotype is unlikely. The *si*II haplotype is associated with two Wolbachia strains, one that consistently induces significant incompatibility (*w*Ri) and one that does not (*w*Au). If incompatibility were causing the spread of the *si*II

haplotype, it would be expected that all populations would be associated with the virulent strain. In fact, the two Wolbachia strains are equally geographically diverse. Thus, it appears that something about the mitochondrial genotype, and potentially nuclear genes interacting with the haplotype, influences the distribution of *si*II mtDNA in *D. simulans*.

The *si*III haplotype has been collected from Tanzania, Kenya, Madagascar, and Reunion Island, where it is sympatric with the *si*II type. At sites where *si*II and *si*III mtDNA have been collected in sympatry the frequency of the *si*III type is always ~40% (Satta *et al.* 1988; James and Ballard 2000; M. D. Dean, K. J. Ballard, A. Glass and J. W. O. Ballard, unpublished results). It is not clear why this is the case. When wild-type fly lines were compared, males with *si*III mtDNA were most active. Fly lines with *si*III mtDNA were slower developing and had a higher probability of surviving to our time periods than did *si*I lines, but did not differ in either regard from fly lines with *si*II mtDNA. Introgression of the *si*III haplotype with the *si*I line produced flies that were slow developing, but no haplotype-by-nuclear DNA interactions were attributed to the *si*III haplotype and the nuclear background of the *si*II line. Competing flies with the *si*II and *si*III haplotype flies in population cages may be an informative method to test the relative fitness of these mitochondrial genomes.

In natural populations that have both the *si*II and the *si*III haplotypes, there is a surprisingly high frequency of heteroplasmic individuals [5.8 and 11.8% reported by Matsuura *et al.* (1991) and Satta *et al.* (1988), respectively, on the island of Reunion; and 6.7% in Dar es Salaam, 1.7% in Malindi, Kenya, and 9.6% in Nairobi, Kenya observed by M. D. Dean, K. J. Ballard, A. Glass and J. W. O. Ballard (unpublished results)]. For heteroplasmy to exist at high frequency, "hybrid" flies should not have reduced fitness. Indeed, we found that the *si*II/*si*III lines with the wild-type nucleo-mitochondrial interactions were no different from each other or lines introgressed between the two. This contrasts with the reduced fitness of males with *si*I mtDNA in a nuclear background previously harboring *si*II mtDNA.

In this study, we limited our analyses of activity and longevity to males. As mitochondria are maternally inherited, it is theoretically predicted that negative selection may be greater in males than in females and a stable equilibrium can be maintained. Examples of mitochondria mutations that have a greater effect in males than in females are pointed out by Frank and Hurst (1996). However, it may be important to look at these components in females, particularly when considering the geographic distribution of mitochondrial haplotypes. Rand *et al.* (2001) found opposite fitness effects of cytoplasms when in males or females and tested in total fitness competition studies.

D. simulans is a perfect model system for distinguishing differential fitness of mitochondrial variation because it harbors three well-defined haplotypes. The *si*I haplotype differs from the *si*II and *si*III haplotypes by 39- and 40-amino-acid substitutions, respectively. One or more of these substitutions appears to cause detectable life-history trait differences that influence the geographic subdivision of the species. Further fine tuning of the specific differences that influence fitness will be interesting. Studying specific physiological variations in flies with different haplotypes or nucleo-mitochondrial interactions is of future interest. Products involved in the electron transport chain are potentially significant, as enzymes encoded by both nuclear and mitochondrial genes support this pathway. In particular, oxygen and hydrogen peroxide production (Ross 2000), oxidizing activity (Nagata and Matsuura 1991), and cytochrome c activity (Edmands and Burton 1998) are likely candidates.

We thank Wayne Johnson (Department of Physiology, University of Iowa) for lending us the "*Drosophila* Activity Monitoring System" (TriKinetics) and Matt Dean for general assistance. This research was supported by National Science Foundation CAREER grant DEB-9702824 to J. William O. Ballard.

LITERATURE CITED

Baba-Aïssa, F., M. Solignac, N. Dennebouy and J. R. David, 1988 Mitochondrial DNA variability in *Drosophila simulans*: quasi absence of polymorphism within each of the three cytoplasmic races. Heredity **61:** 419–426.

Ballard, J. W. O., 2000 Comparative genomics of mitochondrial DNA in *Drosophila simulans*. J. Mol. Evol. **51:** 64–75.

Ballard, J. W. O., B. Chernoff and A. C. James, 2002 Divergence of mitochondrial DNA is not corroborated by nuclear DNA, morphology, or behavior in *Drosophila simulans*. Evolution **56:** 527–545.

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

de Benedictis, G., G. Carrieri, O. Varcasia, M. Bonafe and C. Franceschi, 2000 Inherited variability of the mitochondrial genome and successful aging in humans. Ann. NY Acad. Sci. **908:** 208–218.

Dermitzakis, E. T., J. P. Masly, H. M. Waldrip and A. G. Clark, 2000 Non-Mendelian segregation of sex chromosomes in heterospecific Drosophila males. Genetics **154:** 687–694.

de Stordeur, E., 1997 Nonrandom partition of mitochondria in heteroplasmic *Drosophila*. Heredity **79:** 615–623.

Edmands, S., and R. Burton, 1998 Variation in cytochrome-c oxidase activity is not maternally inherited in the copeod *Tigriopus californicus*. Heredity **80:** 668–674.

Fos, M., M. A. Domínguez, 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.

García-Martínez, J., J. A. Castro, M. Ramón, A. Latorre and A. Moya, 1998 Mitochondrial DNA haplotype frequencies in natural and experimental populations of *Drosophila subobscura*. Genetics **149:** 1377–1382.

Hercus, M. J., and A. A. Hoffmann, 2000 Maternal and grandmaternal age influence offspring fitness in *Drosophila*. Proc. R. Soc. Lond. Ser. B Biol. Sci. **267:** 2105–2110.

Hiraizumi, Y., 1985 Genetics of factors affecting the life history of *Drosophila melanogaster*. I. Female productivity. Genetics **110:** 452–464.

Hoffmann, A. A., and M. Turelli, 1997 Cytoplasmic incompatibility

in insects, pp. 42–80 in *Influential Passengers: Inherited Microorganisms and Invertebrate Reproduction*, edited by S. L. O'Neill, A. A. Hoffmann and J. H. Werren. Oxford University Press, Oxford.

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.

James, A. C., and J. W. O. Ballard, 2000 Expression of cytoplasmic incompatibility in *Drosophila simulans* and its impact on infection frequencies and distribution of *Wolbachia pipientis*. Evolution **54:** 1661–1672.

James, A. C., M. D. Dean, M. E. McMahon and J. W. O. Ballard, 2002 Dynamics of double and single *Wolbachia* infections in *Drosophila simulans* from New Caledonia. Heredity **88:** 182–189.

Kaplan, N. L., R. R. Hudson and C. H. Langley, 1989 The "hitchhiking effect" revisited. Genetics **123:** 887–899.

Kilpatrick, S. T., 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.

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

Matsuura, E. T., H. Fukuda and S. I. Chigusa, 1991 Mitochondrial DNA heteroplasmy maintained in natural populations of *Drosophila simulans* in Reunion. Genet. Res. **57:** 123–126.

Matsuura, E. T., Y. Niki and S. I. Chigusa, 1993 Temperature-dependent selection in the transmission of mitochondrial DNA in *Drosophila*. Jpn. J. Genet. **68:** 127–135.

Matsuura, E. T., Y. T. Tanaka and N. Yamamoto, 1997 Effects of the nuclear genome on selective transmission of mitochondrial DNA in *Drosophila*. Genes Genet. Syst. **72:** 119–123.

Maynard Smith, J., and J. Haigh, 1974 The hitchhicking effect of a favourable gene. Genet. Res. **23:** 23–35.

Merçot, H., B. Llorente, M. Jacques, A. Atlan and C. Montchamp-Moreau, 1995 Variability with the Seychelles cytoplasmic incompatibility system in *Drosophila simulans*. Genetics **141:** 1015–1023.

Nagata, Y., and E. T. Matsuura, 1991 Temperature-dependency of electron-transport activity in mitochondria with exogenous mitochondrial DNA in *Drosophila*. Jpn. J. Genet. **66:** 255–261.

Nigro, L., 1994 Nuclear background affects frequency dynamics of mitochondrial DNA variants in *Drosophila simulans*. Heredity **72:** 582–586.

Rand, D. M., A. G. Clark and L. M. Kann, 2001 Sexually antagonistic cytonuclear fitness interactions in *Drosophila melanogaster*. Genetics **159:** 173–187.

Ross, R. E., 2000 Age-specific decrease in aerobic efficiency associated with increase in oxygen free radical production in *Drosophila melanogaster*. J. Insect Physiol. **46:** 1477–1480.

Satta, Y., H. Ishiwa and S. I. Chigusa, 1987 Analysis of nucleotide substitutions of mitochondrial DNAs in *Drosophila melanogaster* and its sibling species. Mol. Biol. Evol. **4:** 638–650.

Satta, Y., N. Toyohara, C. Ohtaka, Y. Tatsuno, K. Watanabe *et al.*, 1988 Dubious maternal inheritance of mitochondrial DNA in *D. simulans* and evolution of *D. mauritiana*. Mol. Gen. Genet. **52:** 1–6.

Scheffler, I. E., 2001 A century of mitochondrial research: achievements and perspectives. Mitochondrion **1:** 3–31.

Solignac, M., and M. Monnerot, 1986 Race formation and introgression within *Drosophila simulans*, *D. mauritiana* and *D. sechellia* inferred from mitochondrial DNA analysis. Evolution **40:** 531–539.

Tsujimoto, Y., Y. Niki and E. T. Matsuura, 1991 Further study on selective transmission of mitochondrial DNA in heteroplasmic lines of *Drosophila melanogaster*. Jpn. J. Genet. **66:** 609–616.

Communicating editor: S. W. Schaeffer