doi:10.1111/j.1558-5646.2007.00016.x

TEMPERATURE-SPECIFIC OUTCOMES OF CYTOPLASMIC-NUCLEAR INTERACTIONS ON EGG-TO-ADULT DEVELOPMENT TIME IN SEED BEETLES

Damian K. Dowling,[1,2] Katia Chávez Abiega,[1] and Göran Arnqvist[1]

[1]*Animal Ecology/Department of Ecology and Evolution, Evolutionary Biology Centre Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden*

[2]*E-mail: damian.dowling@ebc.uu.se*

Received March 23, 2006
Accepted October 1, 2006

The integration of the mitochondrial and nuclear genomes coordinates cellular energy production and is fundamental to life among eukaryotes. Therefore, there is potential for strong selection to shape the interactions between the two genomes. Several studies have now demonstrated that epistatic interactions between cytoplasmic and nuclear genes for fitness can occur both at a "within" and "across" population level. Genotype-by-environment interactions are common for traits that are encoded by nuclear genes, but the effects of environmental heterogeneity on traits that are partly encoded by cytoplasmic genes have received little attention despite the fact that there are reasons to believe that phenotypic effects of cytoplasmic genetic variation may often be environment specific. Consequently, the importance of environmental heterogeneity to the outcomes of cyto-nuclear fitness interactions and to the maintenance of mitochondrial polymorphism is unclear. Here, we assess the influence of temperature on cyto-nuclear effects on egg-to-adult development time in seed beetles (*Callosobruchus maculatus*). We employed an "across-population" design, sourcing beetles from five distinct populations and using backcrossing to create orthogonal combinations of distinct introgression lines, fixed for their cytoplasmic and nuclear lineages. We then assayed development times at two different temperatures and found sizeable cyto-nuclear effects in general, as well as temperature- and block-specific cyto-nuclear effects. These results demonstrate that environmental factors such as temperature do exert selection on cytoplasmic genes by favoring specific cyto-nuclear genetic combinations, and are consistent with the suggestion that complex genotype-by-environment interactions may promote the maintenance of polymorphism in mitochondrial genes.

KEY WORDS: ***Callosobruchus maculatus*, coadaptation, coevolution, cytonuclear, epistasis, genotype by environment, mitochondria, mtDNA, thermal.**

Energy production in eukaryotes requires the integration of both the mitochondrial and nuclear genomes, and these intergenomic interactions are thus fundamental to life. Although genetic variation in mitochondrial genes has traditionally been considered to be selectively neutral, recent research indicates that selection has played a role in shaping this variation and several reviews have thus highlighted the need for further research in this area (see Rand 2001; Ballard and Whitlock 2004; Rand et al. 2004; Ballard and Rand 2005). For instance, recent theoretical and empirical advances have demonstrated that cytoplasmic genes can interact with nuclear genes to affect fitness, both at a "within population" (Rand et al. 2001; Dowling et al. 2007a) and "across population" level (Clark 1985; Clark and Lyckegaard 1988; Willett and Burton 2001; James and Ballard 2003; Zeyl et al. 2005; Ellison and Burton 2006). In other words, cytoplasms may have differential fitness depending on the nuclear background in which they are

expressed. Current theory also suggests that such cyto-nuclear fitness interactions within populations may be important in maintaining sequence polymorphism in the mitochondrial genome (Rand et al. 2001).

It has been suggested that variation in mitochondrial genes among populations may be associated with local climatic conditions and that this genetic variation may represent local adaptation via natural selection to such conditions (Somero 2002; Ballard and Whitlock 2004). Although it is theoretically established that spatial environmental heterogeneity can promote polymorphism in nuclear genes when migration between demes is limited (Hedrick 1986), no such theoretical investigation has examined whether this may also be true for mitochondrial genes. Yet, empirical research exists that is consistent with such a possibility. For instance, several studies have found cyto-nuclear effects on fitness when cytoplasms from a given population were expressed in nuclear backgrounds derived from distinct, reproductively isolated populations (Clark 1985; Clark and Lyckegaard 1988; Rand et al. 2001; James and Ballard 2003; Zeyl et al. 2005). Notably, some studies have reported that the disruption of the coevolved cyto-nuclear gene complexes specific to particular populations results in a reduction in fitness (Nagao et al. 1998; Edmands and Burton 1999; Rawson and Burton 2002; Sackton et al. 2003; Ellison and Burton 2006). These findings are consistent with the suggestion that the cyto-nuclear genotypes of particular populations have been shaped through natural selection and coadaptation to the local environment. Disruption of these coadapted genotypes may thus lead to functional incompatibilities and reductions in fitness.

Thus, empirical support exists for coadaptation of cyto-nuclear gene complexes within populations. However, the importance of intrinsic versus extrinsic (i.e., environmental) factors in this adaptation is unclear (Willett and Burton 2003). Results of recent studies on plants suggest that environmental influences may affect the outcome of cyto-nuclear interactions (Galloway and Fenster 1999; Campbell and Wasser 2001). A process that was recently proposed is that mitochondria may adapt to the prevailing thermal environment (Blier and Lemieux 2001; Somero 2002; Ballard and Whitlock 2004). There are a number of mechanisms by which such adaptation could occur. For instance, given the essential role of mitochondrial genes in metabolism, we might expect that adaptation to a novel thermal environment may result in selection for mtDNA gene products with different thermal properties. This may be particularly pertinent to poikilotherms, because the mitochondria of such organisms experience the external temperature (Ballard and Whitlock 2004). Moreover, it is clear that both mitochondrial and nuclear gene products are essential for a functional oxidative phosphorylation (OXPHOS) system (Ruiz-Pesini et al. 2000). Two functions of the OXPHOS system in endotherms are ATP production and heat generation (Brand 2000). In such organisms, adaptation to different temperatures may require differential allocations toward these two primary functions of the OXPHOS system, and may thus involve adaptation in mitochondrial genes.

To date, the results of several studies have provided evidence that differential thermal regimes may exert selection on mtDNA or support for the idea that mtDNA may adapt to the thermal environment. First, exposure to different temperature regimes in the marine copepod (*Tigriopus californicus*) appears to affect the enzymatic activity of particular cytochrome *c* oxidase variants (encoded in part by mtDNA genes) when expressed with particular cytochrome *c* variants (encoded by nuclear genes) (Rawson and Burton 2002). Second, temperature-dependent transmission of heteroplasmic mtDNA has been demonstrated experimentally in *Drosophila melanogaster* (Matsuura et al. 1991, 1997; Doi et al. 1999). In these studies, heteroplasmy of mtDNA was induced by germ plasm transplantation from *D. mauritiana*, and then heteroplasmic lines were maintained at two different temperatures. The transmission of each mtDNA variant was biased according to the specific temperature treatment. Moreover, this transmission depended on the nuclear genome used, indicating the importance of cyto-nuclear interactions (Matsuura et al. 1997; Doi et al. 1999). In contrast, Kann et al. (1998) did not find temperature-dependent transmission of mtDNA variants in lines of *D. melanogaster* exhibiting naturally occurring heteroplasmy. Third, support for the idea of thermal adaptation of mtDNA has been provided by two studies reporting associations between mutations in human mtDNA and climatic regions (Mishmar et al. 2003; Ruiz-Pesini et al. 2004). Combined, these studies support the hypothesis that certain ancient mtDNA variants enabled humans to adapt to colder climates. However, Kivisild et al. (2006) questioned the above findings after demonstrating that an excess of non-silent mutations in mtDNA is not only a characteristic of Arctic populations, but is a general feature of the young branches of the phylogenetic tree.

Thus, there is some evidence suggesting that certain mtDNA variants may be selected in one climatic/temperature condition but not in another. However, no study has directly addressed whether the relative fitness of individuals carrying distinct cytoplasmic/mitochondrial lineages varies according to the temperature that they are reared in. Here, we test the effects of temperature on egg-to-adult development time in lines of seed beetles (*Callosobruchus maculatus*) fixed for orthogonal combinations of distinct cytoplasmic and nuclear lineages. Development time is generally considered a key fitness component (Roff 1992). This should be particularly true in species such as *C. maculatus* that develop on ephemeral resources vulnerable to deterioration, attack by predators/parasites, or destruction by humans. Accordingly, previous studies have demonstrated that egg-to-adult development time is a fitness-related trait in *C. maculatus* (e.g., Fox 1993; Fox and Dingle 1994; Fox et al. 2003).

Materials and Methods

STUDY SPECIES AND STOCK POPULATIONS

Callosobruchus maculatus is a pest of stored legumes. Females lay their eggs on the surface of seeds. Larvae hatch from these eggs and burrow directly into the seed. The entire larval and pupal development phases are completed within the seeds and the emerging adults are immediately ready to reproduce.

Outbred stocks of five distinct *C. maculatus* wild-type populations were used in this experiment. Each was originally collected from a distinct geographic area, and each has been maintained under controlled, laboratory conditions for at least 60 generations. These stocks are Brazil (BR), California, USA (CA), Yemen (YE), and two Nigerian stocks: Lossa (LO) and Oyo (OY). The BR was obtained from Robert Smith at the University of Leicester and all other stocks from Peter Credland at the University of London. Since acquiring replicated populations of these strains in April 2002, each was cultured in large (1 L) glass jars at 30°C, 50% R.H. on a 12hL:12hD light cycle and a 25- to 28-day discrete generation cycle for 23 generations. For each strain, each generation was propagated by approximately 300 mated individuals on 120 g of black-eyed beans, *Vigna unguiculata*.

CONSTRUCTION OF CYTO-NUCLEAR INTROGRESSION LINES

In February 2004, a single virgin female from each of the five stocks was mated to a male from the same stock and then placed with him on 100 g of beans in a glass jar. These five females were effectively mitochondrial "Eve's." Twenty to thirty full-sib virgin daughters were collected from each of these matings and separated into five groups of four to six daughters. Each of these groups was then placed with 6 to 10 males from one of the five stock populations in each of the 25 possible combinations. For each combination of matings, the resulting offspring had inherited 100% of their cytoplasmic genes from their mother, 50% of their nuclear genes from their mother, and 50% from their father. These offspring were used to found 25 corresponding cyto-nuclear introgression lines. In each subsequent generation, 10 virgin daughters, from each of the 25 lines, were collected and backcrossed to 6 to 10 outbred males from the same stock population as their fathers (in jars with 120 g beans). In this way, 15 successive generations of backcrossing were used to disassociate each of the sampled cytoplasmic genomes (each genome derived from one of the five stocks) from the nuclear genome with which it was originally associated, replacing it with a new complement of nuclear genes (derived from one of the five stocks) (Table 1). In theory, after 15 generations of such backcrossing, 99.99% of the original nuclear genome of each cyto-nuclear introgression line had been replaced, resulting in each of the cytoplasmic genomes expressed in five distinct nuclear backgrounds. Inbreeding was avoided because females were backcrossed to outbred males each generation. This backcrossing protocol also reduced the opportunity for any of the cyto-nuclear genotypes/populations to evolve along independent trajectories because half of the nuclear genes contributing to each generation were sourced directly from the stock populations. For this reason, we did not replicate the cyto-nuclear lines. In the 15th generation, the number of individuals used in the backcrosses was increased (20 virgin females and 30 males) to ensure that sampling error would not create differences in nuclear DNA among the cyto-nuclear introgression lines. From generation 15, the lines were kept as separate populations. From this time, the experimental assay described below was conducted in two blocks that were separated in time (using offspring of generation 15 and 16). To maintain similar densities of eggs per bean in both blocks, in generation 16, 35–40 non-virgin females were placed with 40 males from each line. This increase in sampled females was necessary because the females here were non-virgin and had thus already oviposited a substantial proportion of their eggs before being transferred to fresh jars to propagate the next generation.

Many insects are infected by a range of maternally inherited, cytoplasmic bacteria such as *Wolbachia*. The presence of such bacteria can potentially confound the results of experiments designed to explore mitochondrial effects on fitness-related traits. Despite efforts to identify *Wolbachia* spp. in *C. maculatus* (Tuda et al. 2006), there are currently no records of *Wolbachia* in this species. Infections have, however, been recorded in some congeners, such as *C. chinensis* (Kondo et al. 1999), where it is established that treatment of the ovipositioning substrate with the antibiotic, tetracycline hydrochloride, is an effective method to eliminate *Wolbachia* (Kondo et al. 2002) and assumingly other

Table 1. "Cyto-nuclear introgression" lines—five cytoplasmic genomes (c) introgressed into five nuclear (n) backgrounds. Cytoplasmic genes are inherited maternally and introgressed with nuclear DNA from males of the stock populations.

	c BR	c CA	c LO	c OY	c YE
n BR	n BR×c BR	n BR×c CA	n BR×c LO	n BR×c OY	n BR×c YE
n CA	n CA×c BR	n CA×c CA	n CA×c LO	n CA×c OY	n CA×c YE
n LO	n LO×c BR	n LO×c CA	n LO×c LO	n LO×c OY	n LO×c YE
n OY	n OY×c BR	n OY×c CA	n OY×c LO	n OY×c OY	n OY×c YE
n YE	n YE×c BR	n YE×c CA	n YE×c LO	n YE×c OY	n YE×c YE

such bacteria. We employed a similar protocol here. In the ninth generation, all beans used in the backcrossing process were thoroughly soaked in a 3% aqueous solution of tetracycline and then air-dried prior to their use as oviposition substrates. All beetles laid their eggs on these tetracycline-treated beans in this generation and the subsequent larvae developed inside and fed on these beans.

EXPERIMENTAL DESIGN

In each block, we isolated 50 beans (infested with larvae) per cyto-nuclear introgression line in "virgin chambers" at day 17 of the life cycle. These chambers separate individual beans, which can then be checked regularly for emerging, virgin adults. Adults began emerging on day 20. At this point, 20 virgin females and 30 males were collected from each line over a two-day period (days 21 and 22 of the life cycle) and each sex was stored in separate Petri dishes. The 20 females and 30 males collected per line were divided into two groups (10 females and 15 males per group). On day 23, individuals of each group were exposed to a corresponding group of the opposite sex from the same line, and females were allowed 2 h to mate. During this time, most females will mate only once (Edvardsson 2005). The males were then discarded and each group of females placed in separate dishes containing 150 beans and allowed to oviposit for exactly 2 h, and then discarded. This ratio of females:beans results in an egg density of 1–2 eggs per bean, thus minimizing any density-dependent effects.

Following the above protocol, there were two groups of beans (with eggs) per cyto-nuclear introgression line. One of these groups was placed in a 30°C, 50% R.H. incubator, and the other a 25°C, 50% R.H. incubator. Both incubators were set at identical 12hL:12hD light cycles. The following day, 50 beans were then selected from each group, attempting to select only those beans carrying one egg. When beans were selected with two eggs, the surplus egg was scraped off with a razor. Despite these efforts to restrict egg density per bean to a single egg, we nonetheless observed some instances during the subsequent experiment in which more than one beetle emerged from a given bean. We thus subsequently controlled for potential effects of larval density statistically.

Seventeen days after oviposition, the 50 beans from each line in the 30°C temperature treatment were transferred to virgin chambers. Nineteen days after oviposition, we commenced hourly spot-checks of the virgin chambers of each line in the 30°C treatment. These checks were conducted on the hour, 24 h per day, for five consecutive days to detect emergence of offspring. As offspring emerged, we recorded the time of emergence for each individual, sex, the number of beetles developing in the bean the individual emerged from (termed "density" from hereon), and the date and time of maternal oviposition of the egg the individual hatched from. The first 10 males and 10 females that hatched from beans within which only one beetle was developing were collected and kept in individual vials with 99% ethanol. The size of these individuals was subsequently measured as the length of elytra, using a digitizing tablet placed under a side-mounted camera lucida attached to a dissecting microscope.

Twenty-eight days after ovipositioning, beans from the 25°C temperature treatment were transferred into virgin chambers. The following day, we commenced hourly spot-checks of these beans that continued for nine consecutive days. These spot-checks followed the same protocol as for the 30°C treatment.

Egg-to-adult development time per individual was calculated as the number of hours elapsing from the mid-point of the ovipositioning period (of the relevant group/line) to the hour of emergence. Given that the original ovipositioning period was 2 h and that we monitored offspring hatching using hourly spot-checks, we were thus able to estimate the egg-to-adult development time of every hatching individual to within 2 h of its true time. This value was then divided by 24 to convert the measure into days. The experiment was conducted in two blocks that were separated in time (two sequential generations). As mentioned above, the size of elytra was only measured for a sub-sample of 500 individuals per temperature treatment per block. In total, development times were recorded for 4416 individuals, 2000 for which the elytra size was also measured.

STATISTICAL ANALYSES

Data were analyzed using restricted maximum likelihood estimation (Proc Mixed statement) in SAS v. 9.1 (SAS Institute, Inc., Cary NC). Egg-to-adult development times were $\log_{10}$ transformed prior to analysis. Examination of the residuals of the subsequent models confirmed that they did not deviate significantly from a normal distribution. Cytoplasmic lineage, nuclear lineage, and block were analyzed as random effects and temperature, density, and sex were fitted as fixed effects. All third-order interactions were initially included in a full model, and non-significant interactions dropped sequentially. The model was checked by adding each non-significant term back into the model, one at a time. Our data were fitted to two parallel models: the first incorporated the whole dataset whereas the second model was constrained to use only the subset of our data that contained individuals for which the size of elytra was available as a fixed covariate. This second model showed that the size of elytra had a significant effect, both as a main effect ($F_{1,1918} = 4.11$, $P = 0.043$) and in interactions with sex ($F_{1,1924} = 10.93$, $P = 0.001$) and temperature ($F_{1,1890} = 7.39$, $P = 0.0066$). We note that density was not included in the second model because individuals included in this analysis hatched exclusively from beans in which they were the sole occupant. All results of the second model were qualitatively identical, in terms of our ability or inability to reject null hypotheses, and quantitatively very similar to the first model based on our entire data set. We thus present only the results of the first model below.

Table 2. Variance component estimates for cyto-nuclear effects on egg-to-adult development times. Fixed effects model is denoted below the variance components table.

Source	Estimate ($\times 10^{-6}$)	Standard error ($\times 10^{-6}$)	Z	P	Scaled estimate total variance
Cytoplasmic lineage	5.211	18.00	0.29	0.3868	1.18
Nuclear lineage	58.00	57.00	1.03	0.1510	13.12
Block	11.00	19.00	0.59	0.2790	2.49
Cytoplasmic × nuclear	57.00	30.00	1.93	0.0268	12.90
Cytoplasmic × temperature	4.59	5.74	0.80	0.2119	1.04
Nuclear × temperature	11.00	10.00	1.07	0.1423	2.49
Cytoplasmic × block	2.274	5.025	0.45	0.3255	0.51
Nuclear × block	4.889	6.68	0.73	0.2322	1.11
Temperature × block	0.00	–	–	–	0.00
Cytoplasmic×nuclear × temp	14.00	5.888	2.33	0.0098	3.17
Cytoplasmic×nuclear × block	16.00	8.091	1.93	0.0270	3.62
Residual	258.00	5.565	46.42	<0.0010	58.37
Fixed effects	Numerator df	Denominator df	F	P	
Temperature	1	5.27	3105.7	<0.001	
Density	2	4353	3.90	0.0204	
Sex	1	4333	167.96	<0.001	

Results

There were no additive effects of cytoplasmic or nuclear lineage on egg-to-adult development times, but a significant cyto-nuclear interaction that explained about 13% (95% C.I.: 5.9–50.7%) of the variance in development time (Table 2). Some cytoplasmic lineages developed faster when expressed in certain nuclear backgrounds and slower when expressed in other backgrounds. In four out of five cases, the matching of coevolved cytoplasms and nuclear backgrounds resulted in relatively slow development times, compared to the introgressed combinations (Fig. 1). Moreover, the three-way interaction between cytoplasmic and nuclear lineages and temperature treatment explained about 3% (95% C.I.: 1.6–9%) of the variance in development times (Table 2). In other words, the development time of a given, joint cyto-nuclear genotype was, in part, contingent upon the temperature the genotype was reared under. Some cyto-nuclear genotypes had relatively faster developments when reared at 25°C, whereas some had relatively faster developments at 30°C (Fig. 2). Finally, we found an interaction between cytoplasmic and nuclear lineages and block that affected development times (Table 2). This explained about 3.5% (95% C.I.: 1.6–13.8%) of the variance in development time and indicates that the development time of a given cyto-nuclear genotype was additionally affected by the block in which the assay was conducted.

Discussion

Our results demonstrate that epistatic interactions between cytoplasmic and nuclear genes affect egg-to-adult development time in seed beetles, but we failed to document any additive effects of either genome. Notably, the outcomes of these cyto-nuclear interactions on development time were partly contingent upon the environmental conditions in which the beetles develop. First, the rate of development of a joint cyto-nuclear genotype was affected by the temperature treatment, demonstrating thermal selection on

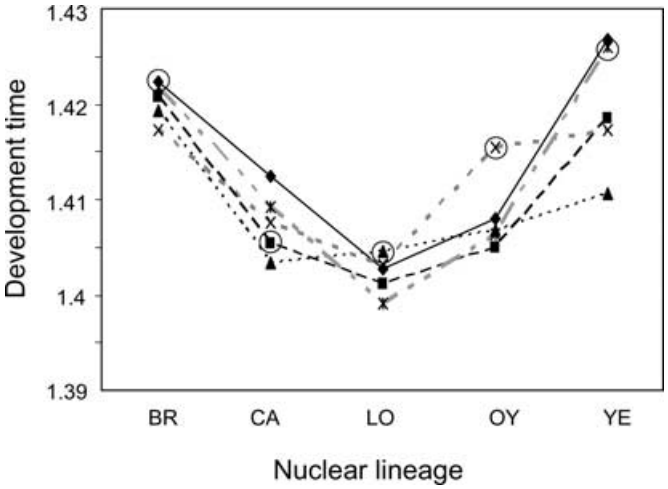

Figure 1. Interaction plot of cytoplasmic × nuclear lineage on development time (least square means generated from GM using the model in Table 2). The five nuclear lineages are denoted along the horizontal axis and each of the five lines on the plot represents a distinct cytoplasmic lineage: BR, —◆— CA, —■— LO, - - -▲- - - OY, -×- and YE —✳— . Coevolved cytoplasm-nuclear combinations (e.g., BR × BR) are circled.

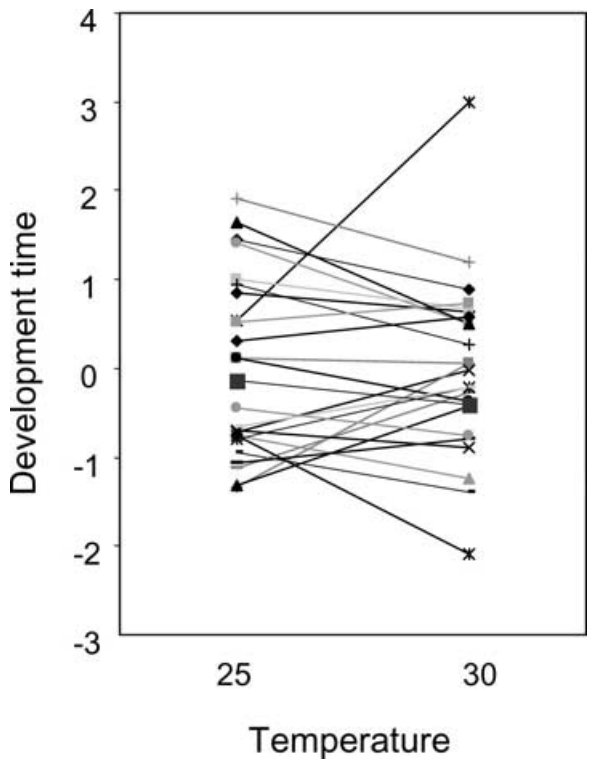

Figure 2. **Interaction plot of cytoplasmic × nuclear lineage × temperature treatment on development times. To facilitate visual comparison, the plot shows least square means (generated from GLM using the model in Table 2) standardized (to a mean of zero and unit variance) within each temperature treatment. Temperature treatment is represented along the horizontal axis and each of the 25 lines on the plot represents a specific joint cyto-nuclear combination.**

this genotype. Although this result does not provide evidence that cytoplasmic genes have adapted to the thermal environment, it is nonetheless consistent with the suggestion that natural selection may promote adaptation of mtDNA to the thermal environment (Blier and Lemieux 2001; Somero 2002; Ballard and Whitlock 2004). Second, the phenotypic effects of cyto-nuclear combinations were also influenced by the block in which the assay was conducted. Because the experimental protocol and environmental conditions were carefully controlled during the experiments, we suggest that this block effect reflects minor random environmental fluctuations between blocks (e.g., in relative humidity).

Our finding of a significant cyto-nuclear effect on egg-to-adult development is consistent with research on *Drosophila*. James and Ballard (2003) found evidence for cyto-nuclear effects on development in *D. simulans* with known sequence differences in the mitochondrial genome, having controlled for the nuclear background through backcrossing. Further, Christie et al. (2004) presented evidence for differential development times in *D. subobscura* dependent on two distinct mitochondrial haplotypes. Notably, the cyto-nuclear effects that we revealed in our study were sizeable, accounting for at least 6% (lower 95% C.I. of the scaled variance estimate) of the variation in egg-to-adult development time. This result highlights the potential importance of cytoplasmic genes in shaping organismal fitness, and is consistent with the suggestion that mtDNA may evolve adaptively (Ballard and Whitlock 2004; Rand et al. 2004; Ballard and Rand 2005).

The cyto-nuclear interactions that we document suggest that there is between-population polymorphism in both coding nuclear and mitochondrial genes in *C. maculatus*. Indeed, previous studies have revealed substantial evidence of functionally significant nuclear genetic variation between populations in this species (Bieri and Kawecki 2003; Fox et al. 2004) and Dowling et al. (2007b) have provided evidence of genetic variation for sperm length between the five nuclear lineages used in this study. Furthermore, there are good reasons to assume that the cytoplasmic effects seen in our experiment reflect variation in mitochondrial genes. First, cytoplasmic elements such as *Wolbachia* have not been found in our study species (Tuda et al. 2006). Second, any infections with such maternally inherited bacteria, should they have been present, should have been eliminated by our tetracycline treatment (see Methods). Third, previous molecular work in our laboratory has revealed large amounts of genetic variation in mtDNA across *C. maculatus* populations (G. Arnqvist and J. Rönn, unpub. data). For example, sequences of 553 bp of the COII region across nine different populations (two of which were used in the current study) revealed a very high proportion of variable sites (approximately 15%) and showed that all nine populations carried distinct mtDNA sequences for this fragment. Based on these data, we can confidently assume that each of the five populations used in this study harbors a distinct mtDNA haplotype.

The relative fitness of a given cyto-nuclear genotype was partly contingent upon the temperature treatment in which the beetles were reared. That is, some cyto-nuclear genotypes resulted in faster relative development in the 25°C treatment and slower relative development in the 30°C treatment, and vice versa. This direct demonstration of temperature-specific outcomes of cyto-nuclear interactions on a fitness-related trait is congruent with other recent findings (see Introduction), and suggests that variation in the thermal climate may indeed exert selection on cyto-nuclear genotypes. However, the percentage of variance explained in development time by the cytoplasmic × nuclear × temperature interaction (about 3%) was relatively small in comparison to the cytoplasmic × nuclear interaction (about 13%) and about equal in magnitude to the cytoplasmic × nuclear × block interaction. Thus, although the potential exists for coadaptation of cytoplasmic and nuclear genomes to the thermal environment, the magnitude of variation in selection was rather small and perhaps no greater than variation in selection caused by other environmental factors (such as the random environmental effects accounted for in the block effect).

The development time of a given cyto-nuclear genotype was also affected by the block in which the experiment was conducted. As mentioned above, we consider this "block" effect to reflect the random environmental heterogeneity that occurs between blocks, in factors such as relative humidity, and that this environmental heterogeneity was sufficient to generate differences in the relative fitness ranks of the cyto-nuclear genotypes studied here. An alternative possibility is that the "block" effect reflects an environmental maternal effect. It is possible that small but consistent differences in larval density during the parental generation between blocks (see Methods) resulted in mothers of differing condition. If so, then the cyto-nuclear × block effect observed may have been a cyto-nuclear × maternal effect, with block-specific differences in maternal condition generating differences in the fitness ranks of the cyto-nuclear genotypes. However, we note that mean development times were not significantly different across the two blocks, and this suggests that there were no block-specific maternal effects on development. Although the precise environmental factors underlying the cyto-nuclear × block interaction are unclear, this interaction nonetheless substantiates the importance of environmental factors in affecting the outcome of cyto-nuclear fitness interactions.

Several recent studies have demonstrated that the disruption of coevolved cyto-nuclear gene complexes specific to particular populations may result in a breakdown of fitness (Nagao et al. 1998; Edmands and Burton 1999; Rawson and Burton 2002; Sackton et al. 2003; Ellison and Burton 2006). This is in line with the idea that cyto-nuclear gene complexes of particular populations have been shaped through natural selection and coadaptation to the local environment. In our study, visual examination of mean development time for each cyto-nuclear combination suggested that the coevolved cyto-nuclear genotypes did not show particularly rapid development. The disruption of these genotypes did not seem to lead to functional incompatibilities, at least in terms of development times, as might have been expected. One possible explanation for why the introgressed cyto-nuclear combinations appeared to have done at least as well as the coevolved combinations is that the original cyto-nuclear genotypes may have adapted in the wild to conditions that are distinct from the laboratory conditions used here. Other examples exist in which the disruption of cyto-nuclear gene complexes did not lead to reductions in fitness (Hutter and Rand 1995; Kilpatrick and Rand 1995). For instance, when Hutter and Rand (1995) introgressed the mtDNA of *D. pseudoobscura* into the nuclear background of its sibling species *D. persimilis*, and vice versa, they found no evidence that the mtDNA from *D. persimilis* performed better in its own nuclear background than did the introgressed *D. pseudoobscura* mtDNA.

In conclusion, we detected sizable, epistatic interactions between cytoplasmic and nuclear genes on egg-to-adult development times in *C. maculatus*. The outcomes of these interactions were partly dependent upon the temperature and block in which the beetles were reared. These results suggest that mitochondrial genes, as well as epistatic interactions between mitochondrial and nuclear genes, may be shaped by natural selection to the local environment.

ACKNOWLEDGMENTS

We thank Agnetha Säfsten for her assistance in conducting the experimental assay, as well as Candice Slevinsky, Alexei Maklakov, Claudia Fricke, and Johanna Rönn for laboratory assistance during the experiment. We thank Urban Friberg for advice regarding the construction of the cytoplasmic introgression lines, Magdalena Nystrand for statistical assistance, the referees for their valuable comments on the manuscript, and the sub-department of Animal Ecology, Uppsala University, for supporting this work. DKD was supported by a Wenner-Gren Foundations postdoctoral scholarship, Swedish Institute guest scholarship, and research grants from Stiftelsen för zoologisk forskning, Uppsala University, and The Royal Swedish Academy of Sciences (Stiftelsen Th. Nordström). GA was supported by the Swedish Research Council.

LITERATURE CITED

Ballard, J. W. O., and D. M. Rand. 2005. The population biology of mitochondrial DNA and its phylogenetic implications. Annu. Rev. Ecol. Evol. Syst. 36: 621–642.

Ballard, J. W. O., and M. C. Whitlock. 2004. The incomplete natural history of mitochondria. Mol. Ecol. Evol. 13:729–744.

Bieri, J., and T. J. Kawecki. 2003. Genetic architecture of differences between populations of cowpea weevil (*Callosobruchus maculatus*) evolved in the same environment. Evolution 57:274–287.

Blier, P. U., and H. Lemieux. 2001. The impact of the thermal sensitivity of cytochrome c oxidase on the respiration rate of Arctic charr red muscle mitochondria. J. Comp. Physiol. B 171:247–253.

Brand, M. D., 2000. Uncoupling to survive? The role of mitochondrial inefficiency in ageing. Exp. Gerontol. 35:811–820.

Campbell, D. R., and N. M. Waser. 2001. Genotype-by-environment interaction and the fitness of plant hybrids in the wild. Evolution 55:669–676.

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 mtDNA in *Drosophila melanogaster*. Genetics 118:471–481.

Christie, J. S., J. A. Castro, P. Oliver, A. Picornell, M. M. Ramon, and A. Moya. 2004. Fitness and life-history traits of the two major mitochondrial DNA haplotypes of *Drosophila subobscura*. Heredity 93:371–378.

Doi, A., H. Suzuki, and E. T. Matsuura. 1999. Genetic analysis of temperature-dependent transmission of mitochondrial DNA in *Drosophila*. Heredity 82:555–560.

Dowling, D. K., U. Friberg, F. Huiler, and G. Arnquist. 2007a. Intergenomic epistasis for fitness: within-population interactions between cytoplasmic and nuclear genes in *Drosophila melanogaster*. Genetics 175.

Dowling, D. K., A. Larkeson Nowostawski, and G. Arnquist. 2007b. Effects of cytoplasmic genes on sperm viability and sperm morphology in a seed beetle: implications for sperm competition theory?. J. Evol. Biol. 20: 358–368.

Edmands, S., and R. S. Burton. 1999. Cytochrome *c* oxidase activity in interpopulation hybrids of a marine copepod: a test for nuclear-nuclear or nuclear-cytoplasmic coadaptation. Evolution 53:1972–1978.

Edvardsson, M. 2005. Cryptic female choice and male mating behaviour: sexual interactions in beetles. Ph.D. diss., Uppsala University, Uppsala, Sweden.

Ellison, C. K., and R. S. Burton. 2006. Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. Evolution 60:1382–1391.

Fox, C. W. 1993. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Oecologia 96:139–146.

Fox, C. W., and H. Dingle. 1994. Dietary mediation of maternal age effects on offspring performance in a seed beetle (Coleoptera: Bruchidae). Funct. Ecol. 8:600–606.

Fox, C. W., M. L. Bush, and W. G. Wallin. 2003. Maternal age affects offspring lifespan of the seed beetle, *Callosobruchus maculatus*. Funct. Ecol. 17:811–820.

Fox, C. W., M. E. Czesak, and W. G. Wallin. 2004. Complex genetic architecture of population differences in adult lifespan of a beetle: nonadditive inheritance, gender differences, body size and a large maternal effect. J. Evol. Biol. 17:1007–1017.

Galloway, L. F., and C. B. Fenster. 1999. The effect of nuclear and cytoplasmic genes on fitness and local adaptation in an annual legume, *Chamacristae fasciculata*. Evolution 53:1734–1743.

Hedrick, P. W. 1986. Genetic polymorphism in heterogeneous environments: a decade later. Ann. Rev. Ecol. Syst. 17:535–66.

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. 2003. Mitochondrial genotype affects fitness in *Drosophila simulans*. Genetics 164:187–194.

Kann, L. M., E. B. Rosenblum, and D. M. Rand. 1998. Aging, mating and the evolution of mtDNA heteroplasmy in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 95:2372–2377.

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.

Kivisild, T., P. Shen, D. P. Wall, B. Do, R. Sung, K. Davis, G. Passarino, P. A. Underhill, C. Scharfe, A. Torroni, R. Scozzari, D. Modiano, A. Coppa, P. de Knijff, M. Feldman, L. L. Cavalli-Sforza, and P. J. Oefner. 2006. The role of selection in the evolution of human mitochondrial genomes. Genetics 172:373–387.

Kondo, N., M. Shimada, and T. Fukatsu. 1999. High prevalence of *Wolbachia* in the azuki bean beetle *Callosobruchus chinensis* (Coleoptera, Bruchidae). Zool. Sci. 16:955–962.

Kondo, N., N. Nikoh, N. Ijichi, M. Shimada, and T. Fukatsu. 2002. Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. Proc. Nat. Acad. Sci. 99:14280–14285.

Matsuura, E. T., S. I. Chigusa, and Y. Niki. 1991. Selective transmission of mitochondrial DNA in heteroplasmic lines for intra- and interspecific combinations in *Drosophila melanogaster*. Jap. J. Genet. 65:87–93.

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

Mishmar, D., E. Ruiz-Pesini, P. Golik, V. Macaulay, A. G. Clarke, S. Hosseini, M. Brandon, K. Easley, E. Cheng, M. D. Brown, R. I. Sukernik, A. Olckers, and D. C. Wallace. 2003. Natural selection shaped regional mtDNA variation in humans. Proc. Natl. Acad. Sci. USA 100:171–176.

Nagao, Y., Y. Totsuka, Y. Atomi, H. Kaneda, K. F. Lindahl, H. Imai, and H. Yonekawa. 1998. Decreased physical performance of congenic mice with mismatch between the nuclear and the mitochondrial genome. Genes Genet. Syst, 73:21–27.

Rand, D. M. 2001. The units of selection on mitochondrial DNA. Annu. Rev. Ecol. Syst. 32:415–448.

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

Rand, D. M., R. A. Haney and A. J. Fry. 2004. Cytonuclear coevolution: the genomics of cooperation. Trends Ecol. Evol. 19:645–653.

Rawson, P. D., and R. S. Burton. 2002. Functional coadaptation between cytochrome *c* and cytochrome *c* oxidase within allopatric populations of a marine copepod. Proc. Natl. Acad. Sci. USA 99:12955–12958.

Roff, D. A. 1992. The evolution of life histories: theory and analysis. Chapman & Hall, New York.

Ruiz-Pesini, E., A.-C. Lapeña, C. Díez-Sánchez, A. Péres-Martos, J. Montoya, E. Alvarez, M. Díaz, A. Urriés, L. Montoro, M. J. López-Péres, and J. A. Enríquez. 2000. Human mtDNA haplotypes associated with high or reduced spermatozoa motility. Am. J. Hum. Genet. 67:682–696.

Ruiz-Pesini, E., D. Mishmar, M. Brandon, V. Procaccio, and D. C. Wallace. 2004. Effects of purifying and adaptive selection on regional variation in human mtDNA. Science 303:223–226.

Sackton, T. B., R. A. Haney, and D. M. Rand. 2003. Cytonuclear coadaptation in Drosophila: disruption of cytochrome *c* oxidase activity in backcross genotypes. Evolution 57:2315–2325.

Somero, G. N. 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integr. Comp. Biol. 42:780–789.

Tuda, M., J. Rönn, S. Buranapanichpan, N. Wasano, and G. Arnqvist. 2006. Evolutionary diversification of the bean beetle genus *Callosobruchus* (Coleoptera: Bruchidae): traits associated with stored-product pest status. Mol. Ecol. 15: 3541–3551.

Willett, C. S., and R. S. Burton. 2001. Viability of cytochrome *c* genotypes depends on cytoplasmic backgrounds in *Tigriopus californicus*. Evolution 55:1592–1599.

Willett, C. S., and R. S. Burton. 2003. Environmental influences on epistatic interactions: viabilities of cytochrome *c* genotypes in interpopulation crosses. Evolution 57:2286–2292.

Zeyl, C., B. Andreson, and E. Weninck. 2005. Nuclear-mitochondrial epistasis for fitness in *Saccharomyces cerevisiae*. Evolution 59:910–914.

Associate Editor: C. Goodnight