Cytonuclear Interactions and the Economics of Mating in Seed Beetles

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abstract: Recent studies have uncovered an abundance of nonneutral cytoplasmic genetic variation within species, which suggests that we should no longer consider the cytoplasm an idle intermediary of evolutionary change. Nonneutrality of cytoplasmic genomes is particularly intriguing, given that these genomes are maternally transmitted. This means that the fate of any given cytoplasmic genetic mutation is directly tied to its performance when expressed in females. For this reason, it has been hypothesized that cytoplasmic genes will coevolve via a sexually antagonistic arms race with the biparentally transmitted nuclear genes with which they interact. We assess this prediction, examining the intergenomic contributions to the costs and benefits of mating in *Callosobruchus maculatus* females subjected to a mating treatment with three classes (kept virgin, mated once, or forced to cohabit with a male). We find no evidence that the economics of mating are determined by interactions between cytoplasmic genes expressed in females and nuclear genes expressed in males and, therefore, no support for a sexually antagonistic intergenomic arms race. The cost of mating to females was, however, shaped by an interaction between the cytoplasmic and nuclear genes expressed within females. Thus, cytonuclear interactions are embroiled in the economics of mating.

Keywords: mitochondrial DNA, evolution, cytoplasm, cytonuclear, cost of mating, sexual conflict.

Introduction

In eukaryotes, genetic elements that reside permanently within the cytoplasm, but outside of the cell nucleus, are likely to play a role in driving adaptive evolution (Charlat et al. 2003; Ballard and Whitlock 2004; Rand et al. 2004; Dowling et al. 2008; Werren et al. 2008). Such genetic elements include the mitochondrial genome, the chloroplast genome in plants, a range of persistent bacteria such as *Wolbachia*, and certain viruses that lack routine horizontal transmission. A defining feature of these cytoplasmic genomes is that they typically exhibit strict maternal transmission, in contrast to the biparental transmission of the nuclear genome. Recently, Zeh (2004) and Zeh and Zeh (2005) proposed that the asymmetry in transmission of the cytoplasmic and nuclear genomes will promote sexual antagonism between these genomes.

Maternal inheritance of cytoplasmic genetic elements sets the stage for male-harming alleles to accumulate within the cytoplasmic genomes (Frank and Hurst 1996). Cytoplasmic genes reach a dead end in males and can therefore not respond to selection on male phenotypes (Zeh 2004), at least in the absence of inbreeding and kin selection (Wade and Brandvain 2009). This poses no problem for traits in which the phenotypic optimum is equivalent across sexes, because selection on females will result in optimal cytoplasmic genetic functioning in both females and males. However, for traits exhibiting sexually dimorphic expression, cytoplasmic genomes are expected to evolve for optimal functioning in females, with males suffering a genetic load attributable to the lack of response to selection on male expression–specific cytoplasmic alleles. In principle, cytoplasmic alleles that suppress male fitness can increase in frequency within populations, via drift if neutral when expressed in females (Frank and Hurst 1996) or by selection if beneficial when expressed in females. The buildup of cytoplasmic alleles that are detrimental to males should, however, result in selection on the nuclear genome to mitigate their harmful effects via the evolution of compensatory counteradaptations (Rand et al. 2004; Zeh and Zeh 2005; Dowling et al. 2008). Conversely, alleles may arise within the nuclear genome that promote relative fitness in males at the expense of female fitness (Arnqvist and Rowe 2005). Given that such sexually antagonistic alleles come at a cost to female fitness, counteradaptations should be favored within the cytoplasm to alleviate costs to females (Zeh 2004; Zeh and Zeh 2005).

The idea of a sexually antagonistic arms race between the cytoplasmic and nuclear genomes is intriguing and is based on at least four general requirements. The first re-

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quirement is that genetic variation within the cytoplasm leads to phenotypic differences. Leaving aside the well-documented phenotypic effects that result from *Wolbachia* infections (Charlat et al. 2003; Werren et al. 2008), genetic polymorphisms located outside of the nucleus have traditionally been considered to be of little phenotypic and evolutionary significance. However, an alternative view is emerging, suggesting that the existence of genetic variation within the cytoplasm and, in particular, the mitochondrial genome will often be instrumental in driving evolutionary change (Ballard and Whitlock 2004; Dowling et al. 2008). In theory, several genetic processes exist that will facilitate the accumulation of nonneutral mitochondrial genetic variation within populations, including mutation accumulation under drift (Lynch 1997) and balancing selection on the joint cytoplasmic-nuclear genotype (Gregorius and Ross 1984; Rand et al. 2001). Empirically, nonneutral cytoplasmic and mitochondrial genetic variation has been documented across (Ballard and Kreitman 1995; Blier et al. 2001; Rand 2001; Ballard and Whitlock 2004; Ballard and Rand 2005; Dowling et al. 2008) and within (Rand et al. 2001; Dowling et al. 2007*c*) populations of several species.

The second requirement is that there are epistatic interactions between cytoplasmic and nuclear genes. Again, such intergenomic epistasis has been documented for life-history traits in several taxa (Rand et al. 2001; James and Ballard 2003; Zeyl et al. 2005; Rand et al. 2006; Dowling et al. 2007*c*, 2007*d*; Clancy 2008). The third condition is that cytoplasmic genetic variation affects traits that either are sexually dimorphic or in which the optimal expression differs across the sexes. Indeed, empirical studies have now documented cytoplasmic and/or intergenomic contributions to fitness-related traits that are sexually dimorphic, such as gamete quality (Dowling et al. 2007*b*), larval development rates (Dowling et al. 2007*d*), and life span (James and Ballard 2003; Maklakov et al. 2006; Rand et al. 2006), as well as traits that are likely to be involved in sexual conflict, such as female mating rate (Dowling et al. 2007*a*).

The fourth requirement is the existence of cytoplasmic alleles with sex-specific effects on fitness. Although it is clear that sex-specific and sexually antagonistic alleles are common within the nuclear genome (Arnqvist and Rowe 2005; Ellegren and Parsch 2007; Ayroles et al. 2009; Innocenti and Morrow 2009), it is less clear how prevalent such alleles are within the cytoplasm. However, recent studies in *Drosophila melanogaster* suggest that mitochondrial genomes harbor alleles that exert sex-specific effects (Rand et al. 2001; Dowling et al. 2007*c*). For instance, Rand et al. (2001) demonstrated that mitochondrial haplotypes that perform well in juvenile females perform poorly in juvenile males and vice versa. In sum, all conditions required to promote an intergenomic arms race between the sexes are, at least sometimes, realized.

Although it has long been known that reproduction is costly (Reznick 1985), the act of mating alone and the sexual interactions that go with it (i.e., leaving aside the costs of egg laying and parental care) also come at a cost to females (Fowler and Partridge 1989; Chapman et al. 1995; Yanagi and Miyatake 2003; Kemp and Rutowski 2004; Rönn et al. 2006). Such costs are manifested, for example, in reductions to female life span that result from mating. In a number of species, the costs to females of mating are caused by a variety of male persistence adaptations (e.g., male harassment [Sakurai and Kasuya 2008; Gay et al. 2009], genital spines [Crudgington and Siva-Jothy 2000; Hotzy and Arnqvist 2009], or seminal proteins that manipulate female physiology [Chapman et al. 1995; Fiumera et al. 2006]), which are beneficial to males in terms of male-male competition (Arnqvist and Rowe 2005). Such male adaptations, however, impose strong selection for counteradaptations in females (e.g., mechanisms to thwart male mating attempts [Arnqvist and Rowe 1995] or adaptations that minimize harm to females from mating [Rönn et al. 2007]).

In this study, we test the potential for male-female cytoplasmic-nuclear coevolution (Zeh 2004; Zeh and Zeh 2005) in the seed beetle *Callosobruchus maculatus*. We take a between-population approach, in which we make use of novel combinations of distinct cytoplasmic and nuclear genomes extracted from three different populations to test for effects of the cytoplasmic genomes (purged of any *Wolbachia* infections) when expressed in females, the nuclear genomes when expressed in both females and males, and all possible genomic interactions, on the life span of females subjected to one of three different mating regimes (retained as virgins, mated once, or forced to cohabit with males). In this model system, the act of copulation is generally costly in terms of both reduced life span, when controlling for egg production (Tatar et al. 1993; Rönn et al. 2006), and reduced lifetime offspring production (Arnqvist et al. 2005). Furthermore, male harassment negatively affects female life span even in the absence of any copulations (i.e., simply cohabitation with males; Gay et al. 2009). Thus, female life span when measured across these mating regimes should provide an informative gauge of the costs associated with escalating sexual interactions (including matings and harassment) in this species. Our experimental design enables us to determine whether there are intergenomic contributions to female life span and, if so, whether the outcomes of these interactions are contingent on the level of sexual interaction a female experiences. More specifically, it enables us to experimentally address the ideas of Zeh (2004) and Zeh and Zeh (2005) explicitly for the first time, by testing whether the cost of

increased sexual interaction to females is affected by intergenomic interactions involving cytoplasmic genomes expressed in females and nuclear genomes expressed in males.

The between-population approach that we take here enables us to assess whether cytoplasmic genes that are expressed in females interact with nuclear genes that are expressed in males to affect the expression of a key life-history trait (female life span) and, as such, represents a test of the potential for sexually antagonistic cytonuclear coevolutionary interactions. Choosing to screen for this interaction at the between-population level increases our inferential power, because it is clear that more cytoplasmic genetic variation is found when sampled across rather than within populations (Dowling et al. 2008). Indeed, in the presence of a sexually antagonistic cytonuclear arms race within populations, we assume that we might actually find very low levels of polymorphism in the interacting cytonuclear genes within populations, given that new mutations conferring adaptations and counteradaptations are likely to be fixed or purged. This process should, however, generate divergence in the interacting cytonuclear gene complexes across populations, given that the coevolutionary trajectories of each population are likely to differ (Arnqvist and Rowe 2005). We note, however, that although our comparative approach is critical to determine the potential for sexually antagonistic coevolutionary interactions of the type suggested by Zeh (2004) and Zeh and Zeh (2005), it is very difficult to distinguish between alternative underlying coevolutionary processes by using this approach (Rowe et al. 2003; Arnqvist and Rowe 2005).

Methods

Construction of the Cytonuclear Lines

The construction of these lines has been described in detail previously (Dowling et al. 2007*a*, 2007*b*, 2007*d*). In short, outbred stocks of five *Callosobruchus maculatus* populations were used to construct 25 combinations of cytoplasmic and nuclear lineages (Brazil [BR], California [CA], Yemen [YE], Lossa [LO], and Oyo [OY]). A single virgin female from each of the five stocks was first mated to a male from the same stock. These five females were essentially "mitochondrial Eves." Groups of full-sib virgin daughters were then placed with males from one of the five stock populations, in each of the 25 possible combinations. In each subsequent generation, virgin females from each of the 25 lines were backcrossed to outbred males from the same stock population as their fathers. This repeated backcrossing was used to disassociate each of the sampled cytoplasmic genomes 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). In theory, after 15 generations of backcrossing, more than 99.9% of the original nuclear genome of each line had been replaced, resulting in each of the cytoplasmic genomes expressed in five distinct and controlled nuclear backgrounds.

Following generation 15, cytonuclear lines were maintained as separate populations on black-eyed beans *Vigna unguiculata* at large population sizes (>100 individuals) at 30°C, 50% relative humidity, without food or water, on a 12L : 12D cycle and a 26- to 28-day discrete generation cycle. To avoid the possibility of line-specific adaptation, backcrossing to the outbred stock populations was again conducted in generations 18, 29, and 39–42. The experimental assay described below took place in generations 43 and 44.

Line-specific cytonuclear coadaptation might, in theory, have affected our interpretation of the interactive female effects in the experiments outlined below. Specifically, there could be two modes of cytonuclear coadaptation. First, the incoming nuclear genomes of the parental strain may coadapt to their new cytoplasmic haplotypes alongside which they are expressed. However, the four generations of backcrossing that immediately preceded this experiment would have greatly mitigated this possibility, since this alone will have resulted in the replacement of about 94% of the nuclear background of each line. We note that all lines were highly fecund throughout the backcrossing protocol, suggesting that there was no strong selection against the formation of new cytonuclear combinations. Second, the cytoplasmic haplotype could coadapt to its new nuclear background. However, the knowledge that an earlier study demonstrated a main effect of cytotype across introgression lines on sperm viability and sperm length (Dowling et al. 2007*b*) suggests that this form of coadaptation did not drive significant levels of cytoplasmic genetic divergence across the cytonuclear lines.

Antibiotic Treatment of Lines to Eliminate Potential Cytoplasmic Bacteria

The primary goal of our experiment was to explore the evolutionary significance of genetic variation within the mitochondrial genome. In this regard, infection with cytoplasmically transmitted bacteria such as *Wolbachia* could confound our interpretations if not controlled (Dowling et al. 2008). We note that previous screens have failed to detect *Wolbachia* in *C. maculatus* (Tuda et al. 2006). Nonetheless, we treated all lines with tetracycline hydrochloride at generation 9 to eliminate any maternally inherited bacterial infections that might have been present (Dowling et al. 2007*a*, 2007*b*, 2007*d*). Despite this precaution, it is possible that any cytoplasmic genetic effects we identify

might still reflect variation in elements other than the mitochondrial genome, such as infection with cytoplasmic and maternally transmitted viruses (López-Ferber et al. 1989; Juchault et al. 1991; Ferber et al. 1997; Tsai et al. 2008). Although such a scenario would not detract from our conclusions regarding the evolutionary significance of the cytoplasm, given that such viruses are essentially cytoplasmic genetic elements, we believe that any cytoplasmic effects found here will more likely reflect underlying mitochondrial genetic variation for two reasons. First, we know that genetic variation exists within protein-coding regions of the mitochondrial genome among the cytoplasmic lineages used in this study (Dowling et al. 2007*d*). Second, although little is known about the fitness effects of cytoplasmic-transmitted viruses, it seems that such effects are relatively benign (Shabalina et al. 1997) and, further, that such viruses seemingly exhibit much higher levels of paternal leakage (Ferber et al. 1997; Tsai et al. 2008) than expected for mitochondrial genomes (Lansman et al. 1983; Kondo et al. 1990). Therefore, if nontransient cytoplasmic viruses exist among populations of *C. maculatus*, the backcrossing protocol that we outlined above would have presumably resulted in the cross-infection of all of the lines (i.e., no variation in infection status among lines), and viral infections therefore seem unlikely to cause any effects observed across genetic lineages.

Experimental Design

All females used in the experiment were sampled from a subset (nine lines) of the 25 cytonuclear lines. This reduced the number of required crosses in the experiment described below from 125 to 27. These nine female lines comprised all possible cytonuclear combinations of the OY, YE, and LO populations. All males used in the experiment possessed either OY, YE, or LO nuclear genotypes expressed within a standardized CA cytoplasm. Hence, the number of crosses assayed was nine female genotypes × three male genotypes.

The experiment was conducted in two blocks that were separated in time by one generation. In each block, 54 virgin females were collected from each of the nine cytonuclear lines over a 2-day period (27 females per line per day). Each of these females was then randomly assigned to one of three mating treatments. On each day, nine of the 27 females per line were kept individually in small petri dishes as virgins (remained unmated throughout their life; hereafter referred to as the virgin class). Nine females were placed in a small dish and then mated once (and once only) to a virgin male possessing one of three possible nuclear genotypes (OY, LO, or YE; hereafter referred to as the once-mated class). Nine females were placed in a dish with a male from one of the three nuclear genotypes and cohabited with this male for 6 days (in block 1) or 5 days (in block 2; hereafter referred to as the cohabit class). Female *C. maculatus* are polyandrous, and when presented with males at time intervals separated by 48 h, about 50% (although this value varies across strains) of females will remate (Dowling et al. 2007*a*). When paired in a small dish, males continually attempt copulations, chasing and mounting the female (Gay et al. 2009). However, female remating propensity is presumably much higher, and female ability to resist remating lower, than 50% when exposed to males continually rather than at discrete time points. Thus, females in the cohabit treatment experienced male harassment and were assumed to mate multiply. For all females, life span was determined by spot checks every 24 h.

All females were kept in dishes without oviposition substrate (i.e., no beans) to mitigate the significant costs associated with egg production in this species (Rönn et al. 2006). Although denying females access to beans is largely effective in removing the cost of egg production (Tatar et al. 1993), mated females will sometimes deposit a few eggs onto the surface of the dish. We therefore counted these eggs for all females and included this as a covariate in our analyses (Rönn et al. 2006). On the day of death, the size of each female was measured as the length of the elytra, using a digitizing tablet placed under a side-mounted camera lucida attached to a dissecting microscope.

Statistical Analysis

We analyzed the data in two separate mixed-model ANOVAs, using Type III sum of squares and Satterthwaite's approximation of denominator synthesis. The first model incorporated female life span data across all three mating treatment classes. In this model, female cytotype, female nuclear genotype, and block were treated as random effects factors, while mating treatment, elytra length, and number of eggs laid were modeled as fixed effects (the two latter as continuous covariates). The second model was similar to the first but also included the male nuclear genotype as a random effect. Because one-third of the focal females were assigned to a virgin treatment and remained unexposed to males during the experiment, life span data for virgin females were omitted from this second model. Our final inferential models represent subsets of the full models, in which the block factor was excluded (it did not significantly improve model fit in any of our models) and three- and four-way interaction effects were included only when statistically significant. We note here that our data were fully balanced and that assessments of the residuals of our inferential models confirmed that the assumptions of the models were upheld (i.e., normality of errors and homoscedasticity).

Table 1: Mixed-model ANOVA of the effects of female genotype and mating treatment on female life span

Source	Sum of squares	Numerator		Denominator		*F*	*P*
		df	Mean square	df	Mean square		
Female nuclear	651.9	2	325.9	3.5	482.7	.675	.564
Female cytotype	94.2	2	47.1	.1	10.7	4.414	.824
Treatment	6,332.9	2	3,166.5	3.7	527.1	6.007	.069
Female nuclear × female cytotype	130.9	4	32.7	8.0	68.1	.481	.749
Female nuclear × treatment	2,348.1	4	587.1	8.1	67.2	8.732	.005
Female cytotype × treatment	184.3	4	46.1	7.9	68.2	.676	.627
Female nuclear × female cytotype × treatment	545.4	8	68.2	1,105	24	2.841	.004
No. eggs laid	211.7	1	211.7	1,105	24	8.823	.003
Elytra length	197.5	1	197.5	1,105	24	8.228	.004
Error	26,518.8	1,105	23.9				

Results

The cost of mating treatment affected the life span of females, although the precise pattern of the effect of mating regime differed across female genotypes (table 1). Virgin females generally outlived those that had mated once, while females forced to cohabit with a male had markedly shorter life spans than each of the other classes (fig. 1).

Female Genetic Contributions to Female Life Span (Model 1)

Female life span was affected by nuclear genotype, but this effect was contingent on the mating class to which the females were subjected (table 1; nuclear genotype × treatment, $P = .005$). In our case, this interaction was generated by the OY genotype, which was associated with the longest life span in virgin and once-mated females but the shortest life span in females subjected to male cohabitation (fig. 1).

However, genetic contributions to female life span were more complex than this, involving an interaction between the cytotype × nuclear genotype × treatment (table 1). That is, the cost of mating to females was in part determined by the specific combination of cytoplasmic-nuclear genotype harbored within each female. Further exploration of this interaction (fig. 2) offers three additional insights. First, the coevolved cytonuclear combinations (e.g., YE cytoplasm in YE nuclear background) did not outperform the novel cytonuclear combinations created via introgressive backcrossing (e.g., YE cytoplasm in LO background), and the performance of the coevolved cytonuclear combinations also varied across the mating classes (fig. 2). Second, although most cytonuclear combinations showed gradual reductions in mean female life span with increasing levels of sexual interaction (i.e., from virgin to once-mated to cohabitation), two combinations showed marginally increased mean life span in the once-mated relative to the virgin treatment on the basis of visual inspection of the life span means in figure 2. That is to say, mating once does not always entail a cost to life span in this species (Rönn et al. 2006). Third, the changes to life span between the cohabit and other mating classes were predominantly driven by the nuclear genotypes (figs. 1, 2).

Male Genetic Contributions to Female Life Span (Model 2)

Our second model confirmed that the cost of sexual interactions to females was contingent on female genotype to a large extent, as demonstrated by the interaction be-

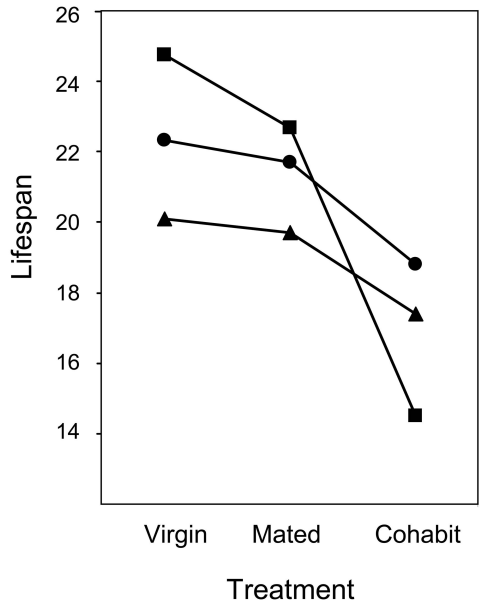

Figure 1: Mean (least squares mean) female life span of the three nuclear female genotypes across the three mating treatments (*squares*, Oyo; *circles*, Lossa; *triangles*, Yemen).

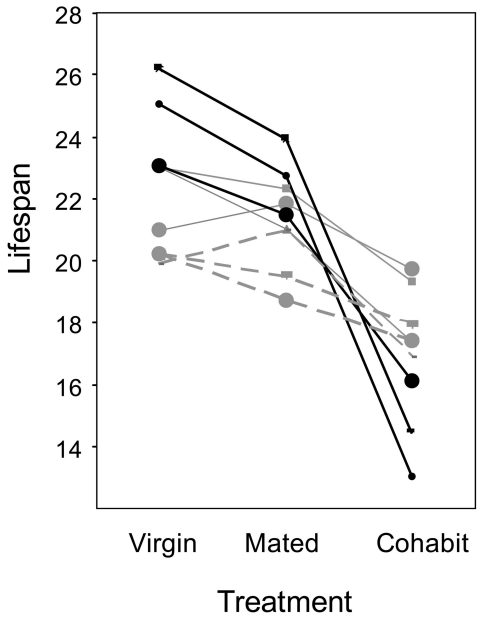

Figure 2: Mean (least squares mean) female life span of each combination of cytotype × nuclear genotype across mating treatments, illustrating the three-way interaction between cytotype, nuclear type, and mating treatment. Coevolved cytonuclear combinations are denoted by enlarged circles, while introgressed combinations are denoted by lines connected by smaller shapes. Black solid lines denote cytonuclear combinations inclusive of an Oyo nuclear genotype, gray solid lines denote combinations inclusive of a Lossa nuclear type, and gray dashed lines denote combinations inclusive of a Yemen nuclear type.

tween the mating treatment and female cytotype and female nuclear genotype (table 2).

Although the effect of male nuclear genotype on female life span differed in the two mating treatments (table 2; fig. 3), we did not detect any genetic interactions between male and female genotypes for the cost of sexual interactions to females. Notably, the interaction between female cytotype and male nuclear genotype was weak and nonsignificant (table 2). This is not congruent with predictions based on sexually antagonistic cytoplasmic-nuclear conflict (Zeh 2004; Zeh and Zeh 2005). Similarly, none of the more complex interactions between female-expressed and male-expressed genomes and mating treatment were significant.

Discussion

Our findings provide two insights into our understanding of cytonuclear genetics. First, the fact that variation in cytoplasmic factors affected female life span substantiates previously supposed links between the cytoplasmic genome and the process of aging in *Drosophila melanogaster* (James and Ballard 2003; Maklakov et al. 2006; Rand et al. 2006; Dowling et al. 2009) and lends support to the mitochondrial theory of aging (Harman 1956, 1972). However, our results suggest that cytoplasmic genetic effects on aging are more complicated than previously appreciated. In *Callosobruchus maculatus*, not only is female life span shaped by interactions between the cytoplasmic and nuclear genomes, but also the outcomes of these intergenomic interactions are contingent on the mating status of the females. The cytonuclear combinations that encoded long life in virgin females were not by default the same combinations that encoded long life in females that mated once or in females forced to cohabit with males. Thus, cytonuclear interactions are linked to the economics of mating in this model system, when gauged by the reduction to female life span that results from increases in sexual interaction.

Second, our results indicate that the cytonuclear interactions for female life span across mating classes were mediated by genetic effects within focal females. Although nuclear genes expressed by the interacting males affected female life span, these effects were independent of the female genotype. Most importantly, we found no evidence of intersexual genetic interactions for the economics of mating either between the nuclear genomes expressed by each sex (Fricke and Arnqvist 2004) or between cytoplasmic genomes expressed in females and nuclear genomes expressed in males. Thus, although intergenomic interactions play a role in mediating the cost and benefits of mating to females in *C. maculatus*, we failed to uncover any signs of intergenomic sexual antagonism over the outcomes of these sexual interactions, as predicted by Zeh (2004) and Zeh and Zeh (2005). Nonetheless, ideas of intergenomic sexual antagonism are intriguing and deserve further empirical attention before we can fully assess their biological relevance. This is reinforced by the observation that the general requirements for such antagonism between genomes seem to, at least sometimes, be in place (see "Introduction").

Recent empirical studies have provided support for the emerging view that cytonuclear interactions commonly affect life-history traits and fitness (see Dowling et al. 2008). These studies substantiate theory, which suggests that cytonuclear interactions can contribute to maintaining nonneutral genetic variation in the mitochondrial genome within populations (Gregorius and Ross 1984; Rand et al. 2001). Further, by highlighting a ubiquity of nonneutral cytoplasmic or mitochondrial genetic variation (Dowling et al. 2008), particularly within populations (Rand et al. 2001; Dowling et al. 2007*c*), these studies suggest that the cytoplasm may play a nontrivial role in driving life-history evolution (Dowling et al. 2008). In light of the fact that the mitochondrial genome is haploid and nonrecombining, a key question is how nonneutral mitochondrial genetic var-

Table 2: Mixed-model ANOVA of the effects of female genotype, male genotype, and mating treatment on female life span

Source	Sum of squares	Numerator df	Numerator MS	Denominator df	Denominator MS	*F*	*P*
Female nuclear	459.4	2	229.7	1.5	478.8	.479	.689
Female cytotype	226.4	2	113.2	.1	4.7	24.293	.679
Male nuclear	504.8	2	252.4	1.8	205.8	1.226	.461
Treatment	3,738.5	1	3,738.5	2.4	741.8	5.039	.131
Female nuclear × treatment	1,317.9	2	658.9	4.0	89.2	7.387	.045
Male nuclear × treatment	428.2	2	214.1	724	27.9	7.660	<.001
Female cytotype × treatment	17.7	2	8.8	4	89.7	.098	.908
Female cytotype × female nuclear	33.2	4	8.3	4	89.4	.093	.979
Male nuclear × female nuclear	89.67	4	22.4	724	27.9	.802	.524
Male nuclear × female cytotype	97.5	4	24.4	724	27.9	.872	.480
Female cytotype × female nuclear × treatment	358.9	4	89.7	724	27.9	3.210	.012
No. eggs laid	310.5	1	310.5	724	27.9	11.108	<.001
Elytra length	270.3	1	270.3	724	27.9	9.669	.002
Error	20,237.3	724	27.9				

iation can be maintained within populations (Clark 1984, 1985; Gregorius and Ross 1984; Clark and Lyckegaard 1988; Lynch 1997; Rand et al. 2001). Theory suggests that cytonuclear interactions might uphold a stable mtDNA polymorphism only under the certain conditions, namely negatively frequency-dependent (Gregorius and Ross 1984) or sexually antagonistic (Rand et al. 2001) selection. In addition, recent empirical research suggests that cytonuclear dynamics are likely to be more complex than previously broached by theory, because the outcomes of cytonuclear interactions may depend on the thermal environment (Matsuura et al. 1997; Rawson and Burton 2002; Willett and Burton 2003; Dowling et al. 2007*d*). It is conceivable that the existence of environment × cytoplasmic × nuclear interactions will broaden the conditions under which cytonuclear interactions might uphold stable mtDNA polymorphisms. Our results represent yet another example of an environment × cytoplasmic × nuclear interaction for a life-history trait but are unique because environmental variation in our study (mating regime) reflects a social rather than an abiotic aspect of the environment. Thus, when combined with earlier findings, it appears that the outcomes of cytonuclear interactions may commonly be highly context dependent, varying across both abiotic (e.g., temperature) and biotic (e.g., social setting) environmental factors.

Innocenti and Morrow (2009) recently reported genome-wide changes in the nuclear expression profiles of *D. melanogaster* females subjected to a similar mating treatment (virgin, once mated, or twice mated) as the *C. maculatus* females in this study. They revealed striking differences in the expression of genes related to metabolism and innate immune response (Innocenti and Morrow 2009). The results of our study are consistent with their findings. Mitochondrial-nuclear interactions lie at the heart of cellular metabolism (Blier et al. 2001; Dowling et al. 2008), and emerging evidence shows that metabolic phenotypes are linked to cytonuclear genotypes (G. Arnqvist, D. K. Dowling, P. Eady, L. Gay, T. Tregenza, M. Tuda, and D. Hosken, unpublished data). Therefore, it is likely that the intergenomic effects we found on the economics of mating in this species are, at least partly, caused by underlying variation in metabolic phenotypes across the cytonuclear genotypes. Furthermore, it is well known that

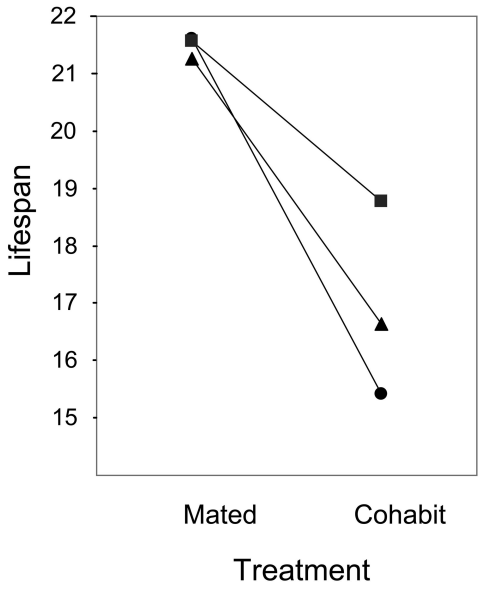

Figure 3: Mean (least squares mean) female life span when mating once or cohabiting with males harboring one of three distinct nuclear genotypes (*squares*, Oyo; *circles*, Lossa; *triangles*, Yemen).

variance in female immune function may affect the cost of mating in insects (Rolff and Joop 2002; Innocenti and Morrow 2009), and there are clear links between metabolism and immune function (e.g., Dowling and Simmons 2009). In *C. maculatus*, males possess sclerotized spines on their genitalia that cause melanized scars within the female reproductive tract (Crudgington and Siva-Jothy 2000; Rönn et al. 2007; Hotzy and Arnqvist 2009). Melanization is the end product of a fundamental innate immune response in insects (Cerenius and Söderhäll 2004), and the genetic effects on the economics of mating documented here may therefore have been mediated in part by variance in immune function across females.

We found a strong effect of the male nuclear genotype on the life span of cohabiting, but not once-mated, females (fig. 3). This is consistent with earlier studies in this model system, which have found nuclear genetic variation for several male traits that affect the costs and benefits of mating to females, including the harmfulness of male genitalia (Hotzy and Arnqvist 2009), male ejaculate weight (Savalli and Fox 1998), and male effects on female remating rate (Fricke et al. 2006; Harano and Miyatake 2007).

In sum, we have shown that the economics of mating to females depend on the genotypes of both focal females and the interacting males. Notably, the genetic determinants of these economics include complex interactions between the cytoplasmic and nuclear genomes. These intergenomic interactions, however, were not played out between the sexes (i.e., cytoplasmic genomes expressed in females did not interact with nuclear genomes expressed in males); therefore, our genetic dissection provides no support for the idea that the two genomes are entwined in a coevolutionary arms race between the sexes.

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Callosobruchus maculatus beetles mating. Photograph by Fleur Champion de Crespigny.