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Energy demand and the context-dependent effects of genetic interactions

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ABSTRACT

Genetic effects are often context dependent, with the same genotype differentially affecting phenotypes across environments, life stages, and sexes. We used an environmental manipulation designed to increase energy demand during development to investigate energy demand as a general physiological explanation for context-dependent effects of mutations, particularly for those mutations that affect metabolism. We found that increasing the photoperiod during which *Drosophila* larvae are active during development phenocopies a temperature-dependent developmental delay in a mitochondrial-nuclear genotype with disrupted metabolism. This result indicates that the context-dependent fitness effects of this genotype are not specific to the effects of temperature and may generally result from variation in energy demand. The effects of this genotype also differ across life stages and between the sexes. The mitochondrial-nuclear genetic interaction disrupts metabolic rate in growing larvae, but not in adults, and compromises female, but not male, reproductive fitness. These patterns are consistent with a model where context-dependent genotype-phenotype relationships may generally arise from differences in energy demand experienced by individuals across environments, life stages, and sexes.

IMPACT SUMMARY

Genetic effects on traits are often context dependent, such that a genotype that improves fitness

20   under one context may have no effect or even a deleterious effect in another context. The

external environment is a common context that affects the degree to which a genotype

determines a phenotype, but the internal environment of an organism (e.g., its genetic

background, sex or life stage) also provides an important context that may modify the phenotypic

expression of a genotype. Here we combine new data on the phenotypic effects of a well-

25   characterized genetic interaction between the mitochondrial and nuclear genomes of the fruit fly

Drosophila with prior observations to support a model of energy demand as a general

explanation for context-dependent genetic effects, particularly for mutations that affect

metabolism. We show that the magnitude of fitness effects of this genetic interaction correlates

positively with the degree of energy demand among developmental treatments that accelerate

30   growth rate, across developmental stages that differ in the cost of growth, and between sexes

with potentially different costs of reproduction. These internal and external contexts create

variable demands on energy metabolism that will impact the efficacy of natural selection acting

on metabolic mutations in populations.

35 **INTRODUCTION**

Environment, development and physiological state can all modify the phenotypic expression of genetic variation (e.g. Hartman *et al.* 2001; Raj *et al.* 2010). Because natural selection acts upon the subset of expressed genetic variation that affects fitness, the fate of new mutations may depend on the landscape of genetic backgrounds and environments experienced by a population

40 (e.g. Remold and Lenski 2004, Chandler *et al.* 2013, Lachance *et al.* 2013, Wang *et al.* 2013, Kammenga *et al.* 2017). Routine variation in the internal – genetic, developmental, physiological – or external environment can challenge the capacity of individuals to maintain homeostasis, and this can magnify deleterious mutational effects (e.g. Kondrashov and Houle 1994, Hoekstra *et al.* 2013). Conversely, favorable environments can mask potential genotype-phenotype relationships

45 (Harshman and Zera 2007, Agrawal *et al.* 2010, Hoekstra *et al.* 2013). If the relationship between genotype and fitness is generally conditional on internal or external environmental factors (i.e., is context dependent), then elucidating general principles underlying genotype-phenotype-environment interactions is critical for understanding evolutionary processes such as the maintenance of genetic variation for life-history traits (Roff and Fairbairn 2007, Van Dyken

50 and Wade 2010, Mackay 2013).

If context-dependent genetic effects mediate phenotypic tradeoffs – often manifest as negative phenotypic trait correlations – then dissecting the underlying physiology can provide mechanistic explanations of phenotypic correlations that better enable predictions regarding the performance of particular genotypes in particular environments (Harshman and Zera 2007, Flatt

55 and Heyland 2011). Many phenotypic tradeoffs likely result from the differential allocation of finite resources to growth, survival, and reproduction (Van Noordwijk and Dejong 1986, Roff 2002). Traits such as growth rate and gamete production demand sufficient energy production

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supplied by metabolic processes, but the rate of metabolism itself is subject to homeostatic regulation that can influence energy allocation and obscure trait correlations (Clarke and Fraser

60   2004, Harshmann and Zera 2007, Leopold and Perrimon 2007). While many good examples of the importance of environmental context for tradeoffs exist (reviewed in Asplen *et al.* 2012), understanding the genetic architecture underlying tradeoffs and the physiological mechanisms mediating them lags behind (Roff and Fairbairn 2007).

To begin to fill this gap in our understanding, we combined well-characterized

65   mitochondrial-nuclear (hereinafter mito-nuclear) genotypes that affect metabolism, physiology and fitness in Drosophilid flies(Hoekstra *et al.* 2013, Meiklejohn *et al.* 2013, Holmbeck *et al.* 2015, Zhang *et al.* 2017) with an environmental perturbation designed to manipulate energy demand. Phenotypic effects of variation in the mitochondrial genome (mtDNA) often depend upon variation in the nuclear genome due to the functional interactions between gene products

70   from these two genomes (reviewed in Burton and Barreto 2012). In ectotherms, the phenotypic effects of these mito-nuclear genetic interactions frequently depend upon temperature (Dowling *et al.* 2007a; Arnqvist *et al.* 2010; Hoekstra *et al.* 2013; Paliwal *et al.* 2014). For example, cool development temperatures masked the deleterious effects of a mito-nuclear incompatibility in *Drosophila*, while warmer temperatures generated inefficiencies in larval metabolism that

75   magnified the deleterious effects of the incompatibility on development rate (Hoekstra *et al.* 2013). We proposed that this was due to the accelerating effect of temperature in speeding up development and increasing demand on energetic processes during more rapid larval growth. Here we show that manipulating the developmental photoperiod to accelerate growth and putatively increase energy demand (Kyriacou *et al.* 1990, Paranjpe *et al.* 2005) generates similar

80   context-dependent effects of this genotype on development that are independent of temperature.

This suggests that energy demand may be a general physiological explanation for why some environments expose, while others mask, genetic effects.

85　　Energy demand may also provide a general explanation for why genetic effects vary across life stages and between sexes. The substantial metabolic cost of growth (Parry 1983, Glazier 2005) may cause the energy budget of developing organisms to be more constrained than that of adults. This may be particularly true for holometabolous insects that experience exponential growth during development before reaching a relatively static adult size (Church and Robertson, 1966). After the cessation of growth, adults of different sexes may partition energy in different ways due to the differential costs of reproduction (Bateman 1984; Hayward and

90　Gillooly 2011), potentially generating sex-specific effects of mutations. Here we present patterns of context-dependent effects of a mito-nuclear incompatibility that are consistent with a model where internal and external environments that cause energy demand to exceed supply may generally expose mutational effects on phenotypes. This has important consequences for the efficacy of natural selection acting on mutations in populations, and particularly for those

95　mutations that impact metabolism.

METHODS

Drosophila *genotypes*

We used mito-nuclear genotypes that precisely pair mtDNAs from *Drosophila melanogaster* and

100　*D. simulans* with nuclear genomes from wild-type *D. melanogaster* (Montooth *et al.* 2010). Pairing the *D. simulans* mtDNA from the *simw*^{501} strain with two *D. melanogaster* nuclear genomes reveals a strong mito-nuclear epistatic interaction for fitness. The *D. simulans simw*^{501} mtDNA is phenotypically wild type when combined with the *D. melanogaster AutW132* nuclear

105 genome (hereinafter *Aut*), but is incompatible with the *D. melanogaster Oregon-R* (hereinafter *OreR*) nuclear genome, resulting in a significant increase in development time and decrease in fitness of the (*simw^{501}*);*OreR* (mtDNA);nuclear genotype (Montooth *et al.* 2010, Meiklejohn *et al.* 2013). The molecular genetic basis of this interaction is an incompatible pairing between a single nucleotide polymorphism (SNP) in the *simw^{501}* mitochondrial-encoded tRNA^{Tyr} and a naturally segregating amino acid polymorphism present in the *OreR* nuclear-encoded

110 mitochondrial tyrosyl-tRNA synthetase gene, *Aatm*. These mutations act epistatically to decrease OXPHOS activity, as predicted by the critical role that these genes play in mitochondrial protein translation (Meiklejohn *et al.* 2013). The four genotypes that we use here – (*ore*);*Aut*, (*ore*);*OreR*, (*simw^{501}*);*Aut*, and (*simw^{501}*);*OreR* – provide a well-characterized model of epistasis between naturally occurring polymorphisms that affects energy metabolism and fitness, allowing

115 us to test how internal and external environment influences the phenotypic expression of genetic interactions (Table 1). Fly cultures were maintained on Bloomington *Drosophila* Stock Center media with a 12:12h light:dark cycle, unless otherwise indicated.

Manipulating developmental photoperiod

120 We tested the specific prediction that an arrhythmic photoperiod (24:0h L:D) would accelerate growth rate in wild-type genotypes, but that the increased energy demand of accelerated growth would induce a developmental delay of the incompatible (*simw^{501}*);*OreR* genotype at 16°C – a temperature where this genotype has a wild-type development time (Hoekstra et al. 2013). We also tested whether arrhythmic photoperiods at 22°C would phenocopy the developmental delay

125 caused by (*simw^{501}*);*OreR* at higher developmental temperatures (Hoekstra *et al.* 2013). We quantified the effect of extended day length on development time using four different

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combinations of temperature and light:dark cycle (16°C, 12:12h; 16°C, 24:0h; 22°C, 12:12h; 22°C, 24:0h). For each genotype, replicate pools of fifty 0-12 hour old eggs were collected into fresh food vials and randomly assigned to one of the four developmental treatments. We scored

130   the number of new pupae and new adults to eclose once per day at 16°C and twice per day at 22°C for approximately 20 vials of each genotype under each developmental treatment. The fixed effects of genotype and photoperiod on development time within each temperature were tested using mixed-model analysis of variance (ANOVA) models that were fit using restricted maximum likelihood and included rearing vial as a random factor. All analyses were performed

135   in the R statistical package (R Core Team 2013).

Adult mass and metabolic rate

Incompatible (*simw^{501}*);*OreR* larvae have inefficient larval metabolism, manifest as higher metabolic rates and longer development times at 25°C, but develop and respire at a normal pace

140   at 16°C (Hoekstra *et al.* 2013). To test whether this mito-nuclear incompatibility also affects adult metabolism, we reared all four genotypes from egg to adult with controlled densities at 16°C or 25°C and measured mass and metabolic rate of 3-6 day old adults. At 48 hrs. post-pupal eclosion, adult flies were lightly anaesthetized with CO_2, sexed, and sorted into groups of ten flies. The wet mass of each group of ten flies was recorded to the nearest μg and adults were

145   allowed to recover in fresh, yeasted food vials for at least 24hrs. Mass was log-transformed to improve normality and genetic effects on mass were tested using ANOVA and Tukey's post-hoc contrasts corrected for the number of multiple tests.

We used flow-through respirometry to estimate routine metabolic rate (hereinafter, metabolic rate) as the volume of CO_2 (VCO_2) produced by groups of ten female or male flies of

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150 the same genotype that were confined to a small, dark space to minimize activity. VCO_2 is a good proxy for metabolic rate in insects like *D. melanogaster* that largely use carbohydrates for respiration and have a respiratory quotient of approximately one (Chadwick 1947). We measured at least ten biological replicates of each combination of genotype, sex, development temperature (T_{DEV} = 16°C and 25°C), and measurement temperature ($T_{MEASURE}$ = 16°C and 25°C) using

155 offspring collected from multiple cultures and multiple parental generations in order to average across micro-environmental effects. Metabolic rates were measured between 11:00 am and 7:00 pm, and genotypes were distributed across this timeframe and across respirometry chambers using a random, balanced design. All measurements were made in a Peltier-controlled thermal cabinet (Tritech Research, Inc.), and measurement temperature was monitored using a

160 thermocouple meter wired into an empty respirometry chamber.

 For flow-through measurement of adult VCO_2 we pushed CO_2-free air through glass respirometry chambers containing flies at a rate of 100 ml min^{-1}. Air that leaves the chamber carries CO_2 produced by the flies, as well as water. The water vapor was removed from the airstream using magnesium perchlorate, and the CO_2 in the airstream was measured using a

165 Licor 7000 infrared CO_2 detector (Licor, Lincoln, NE, USA). We used the RM8 Intelligent Multiplexer to switch the airstream sequentially through five respiratory chambers (Sable Systems International, Las Vegas, NV, USA), one of which serves as an empty baseline chamber. Each experimental run measured the VCO_2 of four pools of ten adult flies, each sampled twice for ten minutes during a 100-minute period. There is no death from this treatment.

170 Baseline CO_2 values were recorded before and after each sample and used to drift-correct CO_2-tracings using the two-endpoint automatic method in Expedata, version 1.1.15 (Sable Systems International, Las Vegas NV). Raw CO_2 values were converted from parts per million to

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µL hr^{-1} (VCO$_2$) and then log-transformed to improve normality and homoscedasticity. To allow

metabolic rate to acclimate in response to temperature shifts (e.g., T$_{DEV}$ = 16°C and T$_{MEASURE}$ =

175   25°C), we used the second recording of each respirometry run to estimate metabolic rate such

that flies were acclimated for 50-80min.

Because there is measurement error in adult body mass, we estimated the scaling

relationship between mass and VCO$_2$ using Type II Model regression implemented with smatR,

version 3.4.3 (Warton *et al.* 2006). When justified by a homogeneity of slopes test for the log-log

180   relationship between mass and VCO$_2$, we fit a common slope to all genotypes and tested for

shifts along the common x axis (i.e., differences in mass) and for shifts in elevation (i.e.,

differences in mass-specific metabolic rate) among genotypes. Across genotypes within each of

the four T$_{MEASURE}$ x Sex combinations, we were able to fit a common slope and test for genotype

differences in mass and in mass-specific metabolic rate. We could then correct for the effect of

185   mass on metabolic rate by taking the residuals of each of these regressions and adding back the

grand mean of all fitted values to provide meaningful scale. We refer to these values as mass-

corrected metabolic rates (MCMR). We tested for the fixed effects of T$_{DEV}$, mtDNA, nuclear

genotype, and all possible interactions on MCMR using analysis of variance (ANOVA).

Respirometry chambers were housed inside infrared activity detectors (AD-2, Sable

190   Systems International, Las Vegas NV), providing a simultaneous measurement of activity. We

summarized the activity data by taking the median absolute difference of activity across each

seven-minute metabolic rate measurement. This measure of activity neither significantly affected

metabolic rate nor interacted with any genetic effects and was not included in our final statistical

models.

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Adult reproductive traits

Incompatible ($simw^{501}$);*OreR* females have significantly reduced fecundity, measured as the number of eggs laid over 10 days (Meiklejohn *et al.* 2013). To test whether males of this genotype also suffer a decrease in reproductive fitness, we measured one aspect of male fertility

200   – the number of offspring sired by an individual male mated to virgin females of a control wild-type genotype, *Canton-S*. Thirty males were assayed for fertility across two experimental blocks that spanned multiple parental generations and used slightly different female genotypes. Both female genotypes were *Canton-S*, but in the first block the strain carried the *cn,bw* eye mutation. Block was included as a fixed factor in the analysis. However, rank orders of genotypes for the

205   number of females fertilized and the number of offspring sired were similar between blocks.

Males were given 48-hours to mate with three virgin females. After 48-hours, we placed each female into a separate vial to lay fertilized eggs for an additional week. Progeny emerging from each vial were counted every other day until all progeny were counted. Males that sired fewer than 25 offspring were removed from the analysis, which resulted in a sample size of 29

210   males per genotype, except for ($simw^{501}$);*Aut* for which n = 28 males. By placing females in separate vials after they were housed with the focal male, we could infer how many females were mated by each male, with the caveat that some females may have laid eggs in the first vial, but not in their individual vials. The median number of females that produced offspring in their vial per male was 3 (mean = 2.64) and this was not affected by genotype ($P > 0.15$ for genotype and

215   all interactions). We estimated fertility as the total progeny sired by each male divided by the number of females with whom that male produced progeny. We tested for fixed effects of mtDNA, nuclear genotype, experimental block and the interaction between these factors using ANOVA.

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220   **RESULTS**

An arrhythmic photoperiod phenocopies the temperature-dependent developmental delay of a mito-nuclear incompatibility

The developmental delay of incompatible *(simw^{501});OreR* larvae is strongly mediated by temperature, with warmer temperatures exacerbating and cooler temperatures masking the delay

225   (Hoekstra *et al.* 2013). *Drosophila* development rate depends on variation in the rhythmicity of the photoperiod (e.g. Kyriacou *et al.* 1990, Paranjpe *et al.* 2005). We tested whether an arrhythmic, continuous-light photoperiod (24:0h L:D) that typically accelerates larval-to-adult development (Paranjpe *et al.* 2005) could phenocopy this developmental delay in a manner similar to the accelerating effect of increased temperature. The accelerating effect of arrhythmic

230   photoperiod influenced the severity of the *(simw^{501});OreR* developmental delay in a pattern remarkably similar to the effect of increased development temperature (Figure 1, Supplemental Tables S1 & S2). The incompatible *(simw^{501});OreR* genotype developed at the same pace as other genotypes when reared at 16°C with a fluctuating photoperiod (12:12h L:D) (mtDNA x Nuclear: $F_{1, 80} = 0.38$, $P = 0.5413$). However, the incompatible *(simw^{501});OreR* genotype

235   experienced a significant developmental delay relative to other genotypes when developed at 16°C with constant light (24:0h L:D) (mtDNA x Nuclear: $F_{1, 79} = 30.13$, $P < 0.0001$). At 22°C, where the incompatible genotype normally experiences a significant developmental delay with a fluctuating photoperiod, the constant light photoperiod magnified the developmental delay (Photoperiod x mtDNA x Nuclear: $F_{1, 153} = 299.07$, $P < 0.0001$). This was a particularly striking

240   effect; constant light accelerated development of compatible mito-nuclear genotypes by ~ 2 days at 22°C, which is comparable to development time at 25°C. In contrast, constant light at 22°C

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significantly slowed development of the incompatible *(simw^{501});OreR* genotype, resulting in an ~

4-day developmental delay between *(simw^{501});OreR* and compatible genotypes (Figure 1).

Within each developmental temperature, the magnitude of the effect of the mito-nuclear genetic

245 interaction on development time was conditional on photoperiod (G x G x E, Table 1; *P*<0.0001

for both developmental temperatures, Supplemental Table S1). However, there was no evidence

that the highest-order interaction between development temperature, photoperiod, mtDNA and

nuclear genotype affected development time (G x G x E x E, Table 1; $F_{1,312} = 0.41, P = 0.5244$). In

other words, the effects of development temperature and photoperiod to delay development

250 specifically in the incompatible genotype were independent.

Adults with a mito-nuclear incompatibility achieve similar masses as compatible genotypes

Gene-environment (G x E) effects on adult mass were dominated by interactions between

nuclear genotype and the known effects of both development temperature and sex on mass

255 (Supplemental Table S3). Relative to these large effects, there was a small, but statistically

significant effect of the mito-nuclear interaction on mass (mtDNA x nuclear: $F_{1,317} = 6.078, P =$

0.0142). Rather than eclosing as smaller adults, *(simw^{501});OreR* adults were slightly larger than

(ore);OreR adults ($P_{Tukey} = 0.04$). However, the magnitude of the effect was small (+ 0.04 mg/10

flies) and the mtDNA x nuclear interaction was only statistically significant for females raised at

260 16°C (Supplemental Table S4). This small but consistent effect may be caused by smaller

(simw^{501});OreR larvae not surviving development as well as smaller individuals of other

genotypes, as this genotype has reduced egg-to-pupal survival (Hoekstra *et al*. 2013). In

summary, adults with a mito-nuclear incompatibility that survive development achieve body

masses that are similar to or greater than compatible genotypes, potentially as a consequence of

265   their extended time spent as feeding larvae.

In contrast to larvae, adult metabolic rate is more robust to mito-nuclear interactions

The scaling of metabolic rate as a function of mass can be characterized by the slope of the

relationship between *ln*(metabolic rate) and *ln*(mass) (i.e., the mass-scaling exponent). The mass-

270   scaling exponent differed slightly, but significantly between sexes and between measurement

temperatures for adults in this study ($P < 0.0001$ for both Sex x Mass and $T_{MEASURE}$ x Mass

interactions). However, within each combination of sex and $T_{MEASURE}$, there was no evidence

that genotype or development temperature affected the mass-scaling exponent ($P > 0.05$ for

both). Thus, we fit common slopes to the metabolic rate data for all genotypes within sex-

275   $T_{MEASURE}$ combination to test for effects of mito-nuclear genotype (Supplemental Figure S1 and

Table S5). Within each sex-$T_{MEASURE}$ combination, the range of masses for all genotypes were

largely overlapping and there was no evidence for differences in mass among genotypes

(Supplemental Figure S1 and Table S5).

In contrast to the larval life stage where *(simw^{501});OreR* larvae have significantly elevated

280   25°C metabolic rates (Hoekstra et al. 2013), there was no evidence of elevated metabolic rate in

the incompatible *(simw^{501});OreR* adults measured at any temperature. The only significant effect

of the mito-nuclear genotype on adult metabolic rate was a lower *(simw^{501});OreR* metabolic rate

relative to compatible genotypes at 16ºC. However, once again, this effect was small and only in

females (Figure 2A, Supplemental Figure S1 and Table S5). Male metabolic rate was unaffected

285   by mito-nuclear genotype (Supplemental Figure S1 and Table S5). Significant effects of the

nuclear genome at 25°C (Supplemental Table S5) and in our prior work (Hoekstra & Montooth

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2013, Hoekstra et al. 2013, Greenlee *et al.* 2014) demonstrate the sensitivity of this method to detect both genetic and environmental effects on metabolic rate.

290   Mass-corrected metabolic rates allow for comparisons of genotypes across development and measurement temperatures (i.e., metabolic plasticity) (Figure 2B-D, Supplemental Figure S2, Supplemental Tables S6 & S7). This analysis revealed that the lower 16°C metabolic rates in (*simw^{501}*);*OreR* females were largely the consequence of lower metabolic rates of females developed at 25°C and measured at 16°C. However, this difference was not statistically significant and supports generally weak effects of this genetic interaction on adult, relative to

295   larval, metabolic rates. There was no evidence that the mito-nuclear interaction affected adult male MCMR within any combination of development or measurement temperatures (Supplemental Fig S2 and Table S6). In contrast to larvae, where (*simw^{501}*);*OreR* have compromised thermal plasticity of metabolic rate (i.e., the Q_{10} for metabolic rate), we found no evidence that adults of this genotype differ in their Q_{10} for metabolic rate ($T_{MEASURE}$ x mtDNA x

300   nuclear, *P* > 0.65 for both sexes) and this was independent of development temperature ($T_{MEASURE}$ x T_{DEV} x mtDNA x nuclear, *P* > 0.50 for both sexes)(Supplemental Table S7). Thus, relative to larvae, both adult metabolic rate and metabolic plasticity are more robust to the effects of this mito-nuclear genetic incompatibility.

305   *Mito-nuclear effects on reproductive fitness are stronger in females*

Patterns of male fertility and female fecundity provide further evidence that the effects of the mito-nuclear incompatibility are sex specific. The mito-nuclear interaction did not compromise the number of offspring sired by males (mtDNA x nuclear: $F_{1,\ 107}$ = 0.185, *P* = 0.668)(Figure 3A, Supplemental Table S8). This is in contrast to strong mito-nuclear effects on female fecundity,

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310   with females of the *(simw^{501});OreR* genotype producing ~50% fewer eggs than *(ore);OreR*

females (Figure 3B, Supplemental Table S8)(Meiklejohn *et al.* 2013). Thus, while both sexes

generally maintain metabolic rate independent of mito-nuclear genotype, *(simw^{501});OreR* females

appear to do so at the cost of egg production.

315   **DISCUSSION**

Previously, we found that a synergistic, epistatic interaction between polymorphisms in the mt-

tRNA^{Tyr} and the nuclear-encoded, mitochondrially targeted tRNA-synthetase for this mt-tRNA

decreases OXPHOS activity and negatively impacts a number of life-history traits in a

temperature-dependent manner in *Drosophila* (Montooth *et al.* 2010, Meiklejohn *et al.* 2013,

320   Hoekstra *et al.* 2013, Zhang *et al.* 2017). Here we show that the phenotypic expression of this

genetic interaction is generally environment-dependent and depends on intrinsic factors such as

life stage and sex in a manner consistent with greater energy demands revealing fitness effects of

mutations that compromise metabolism.

325   *The physiological basis of environment-dependent, mito-nuclear effects*

Many organismal traits, particularly in ectotherms, are temperature dependent, including the

universal, but mechanistically not well understood, unimodal thermal performance curve for

metabolic rate (Angiletta 2009, Schulte 2015, DeLong *et al.* 2017). Given this relationship

between temperature and metabolic processes, the thermal environment is likely an ecologically

330   relevant and critical determinant of the relationship between genotype and phenotype for traits

that depend on metabolic function. Phenotypic effects of cytonuclear interactions, including the

mito-nuclear incompatibility described here, can be temperature sensitive (Arnquvist *et al.* 2010,

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Dowling *et al.* 2007a, Hoekstra *et al.* 2013). The temperature dependence of these mito-nuclear genetic effects could result from direct thermodynamic effects on the physical interaction

335   between the mutations, e.g., between mutations in the mt-tRNA^{Tyr} and its tRNA synthetase. Alternatively, the temperature dependence may arise indirectly, as metabolic rate and development rate increase with temperature and place greater demand on the energetic products of mito-nuclear interactions. This latter, energy-dependent explanation is also consistent with the observations that mitochondrial genetic effects can be temperature sensitive (Pichaud *et al.* 2013)

340   and both mitochondrial and mito-nuclear effects can be diet sensitive (Zhu *et al.* 2014, Ballard and Youngson 2015, Mossman *et al.* 2016). Here we found that manipulating the photoperiod to accelerate growth rate independent of temperature produced patterns of context-dependent mito-nuclear genetic effects strikingly similar to those revealed by varying development temperature. While this does not exclude the possibility that there are direct thermal effects on the physical

345   interaction between mt-tRNA and tRNA synthetase, it demonstrates that temperature is not required to expose this genetic interaction and suggests a more general physiological explanation – external and internal contexts that place greater demand on energy metabolism expose deleterious effects of genetic interactions that compromise energy supply.

In some insects, including *Drosophila*, pupal eclosion behavior is under control of the

350   circadian clock (Kyriacou *et al.* 1990, Paranjpe *et al.* 2005). Under cyclic or rhythmic photoperiodic regimes, the circadian clock entrains to the light cycle and eclosion behavior is gated such that pupae will delay the initiation of eclosion behavior until lights off in order to synchronize eclosion with dawn. In arrhythmic photoperiodic environments, however, eclosion behavior is unregulated and pupae initiate eclosion behavior as quickly as possible. While

355   compatible mito-nuclear genotypes developed under arrhythmic photoperiods experienced an

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unregulated acceleration of development, incompatible mito-nuclear genotypes do not appear to have the energetic capacity to similarly accelerate growth. ($simw^{501}$);$OreR$ larvae also have significantly reduced thermal plasticity for metabolic rate (i.e., they have a very low Q_{10}) (Hoekstra et $al.$ 2013). Thus under two independent contexts that normally accelerate growth

360   (arrhythmic photoperiod and warm temperatures), this mito-nuclear incompatibility appears to limit larval growth. Mito-nuclear incompatibilities likely limit the scope for growth, potentially because incompatibilities compromise ATP production and result in energy supplies that are very close to demand.

365   *Ontogeny of the energy budget*

Even at temperatures that most exacerbate mito-nuclear effects on larval metabolic rate and survivorship (Hoekstra et $al.$ 2013), we found that the effects of this mito-nuclear incompatibility on adult metabolic rate are minimal. This suggests that the deleterious effects of mito-nuclear incompatibility on metabolic rate and survivorship may be alleviated by the cessation of growth.

370   Larval growth in *D. melanogaster* proceeds extremely quickly and challenges metabolic processes (Church and Robertson 1966, Tennessen et $al.$ 2011). Furthermore, metabolism during growth is estimated to be 40% to 79% above that of fully developed conspecifics (Parry 1983), and cessation of growth in holometabolous insects is correlated with an ontogenetic decrease in metabolic rate per unit mass (Glazier 2005, Callier and Nijhout 2012, Greenlee et $al.$ 2014,

375   Maino and Kearney 2014). Thus, the cessation of growth in *Drosophila* likely results in the excess metabolic capacity needed to compensate adult metabolic rate of incompatible, mito-nuclear genotypes. Hoekstra et $al.$ (2013) also observed that for those individuals that survive to pupation, there is no further effect of the mito-nuclear incompatibility on survival through

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metamorphosis, when metabolic rates decrease to a minimum (Dobzhansky & Poulson 1935,

380 Merkey *et al.* 2011) and those individuals that have committed to pupation appear to have the

energy stores needed for successful metamorphosis. Consistent with this, we observed that adult

(*simw^{501}*);*OreR* attain similar mass at eclosion as do adults with compatible mitochondrial and

nuclear genomes. Changes in the energy budget of holometabolous insects across development

(e.g., Merkey *et al.* 2011) likely generate important ontogenetic contingency for the fitness

385 effects of mutations in populations. The fate of conditionally neutral alleles, such as those

underlying this mito-nuclear incompatibility, will then depend not only on what environments

are experienced, but when those environments are experienced in an organism's lifespan (*sensu*

Diggle, 1994).

390 *Sex-specific costs of reproduction*

The small effect that this mito-nuclear interaction did have on adult metabolic rate was sex

specific. (*simw^{501}*);*OreR* females had significantly depressed metabolic rates when measured at

cool temperatures. There was some indication that this was driven by disruption of metabolic

plasticity, similar to what we have observed in larvae of this genotype, although of much weaker

395 effect; (*simw^{501}*);*OreR* females developed at one temperature and measured at the other

temperature had the lowest mass-corrected metabolic rates relative to all other compatible

genotypes.

If energy stores in mito-nuclear incompatible individuals are limited, then the

maintenance of metabolic rate using an inefficient OXPHOS system might support adult, but at a

400 cost to more energetically demanding function, such as reproduction. We observed this pattern in

females, but not in males. Females with incompatible mito-nuclear genomes laid far fewer eggs,

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while the fertility of males with the same mito-nuclear combination was unaffected. The fecundity defects in (*simw^501*);*OreR* females are also strongly temperature dependent and involve defects in the development and maintenance of the ovary (Zhang *et al.* 2017). Furthermore,

405   mothers of this genotype developed at 28°C produce defective eggs that have a lower probability of being fertilized and, when fertilized, die during embryogenesis presumably due to insufficient maternal provisioning or the inheritance of sub-functional mitochondria (Zhang *et al.* 2017).

       Although we measured only one aspect of reproductive fitness for each sex, this pattern is consistent with a higher cost of reproduction in females (Bateman 1948) and with empirical

410   estimates of the costs of gamete production that suggest that high costs of egg production may specifically constrain female gamete production (Hayward and Gillooly 2011). Oogenesis in *Drosophila* is regulated in response to nutrient availability (Drummond-Barbosa & Spradling 2001), but nutrient checkpoints for spermatogenesis are less well studied (but see e.g., McLeod *et al.* 2010, Yang & Yamashita 2015). Further experiments are warranted to determine whether

415   there are more subtle fertility defects in (*simw^501*);*OreR* males, as cytoplasmic effects on sperm morphology and viability have been measured in seed beetles (Dowling *et al.* 2007b). However, these viability differences do not contribute to cytoplasmic effects on sperm competition in seed beetles or in *D. melanogaster* (Dowling *et al.* 2007c, Friberg & Dowling 2008). Our findings suggest that homeostasis for metabolic rate (or ATP production) combined with potentially

420   differential costs of gametogenesis, may generate sex-specific allocation tradeoffs between maintenance and reproduction as a consequence of genetic variation in metabolic processes.

Conclusion

       Our observation that the degree of expression of a mito-nuclear incompatibility correlates

425   with energetic demand – among developmental treatments that accelerate growth rate, across

        developmental stages with substantial differences in the cost of growth, and between sexes with

        putatively different costs of reproduction – suggests that the phenotypic effects of genetic

        interactions that impact metabolism will depend broadly on the context of energy use and the

        metabolic cost of producing focal traits. These energetic contexts may change as a function of

430   the external (e.g. temperature or photoperiod) or internal (e.g., life stage, sex or tissue)

        environment in which a trait is expressed. The context-dependent genotype-phenotype

        relationships that we describe exemplify how energy allocation principles and design constraints

        can generate complicated environment-dependence, potentially confounding attempts to define

        fitness in energetic terms (e.g., Bruning *et al.* 2013). Yet, many components of fitness are

435   expected to depend on the pathways of metabolism, and the variable phenotypic expression of

        mutations in these pathways presents a dynamic and perhaps challenging context for both

        purifying and adaptive selection, as context-dependent mutational effects only experience

        selection in a fraction of possible environments (Van Dyken and Wade 2010). This weakening of

        selection may be an important contribution to the maintenance of genetic variation for

440   metabolism and life-history traits in populations.

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AUTHOR CONTRIBUTIONS

LAH and KLM conceived and designed the study, LAH, CRJ, KMM and KLM collected and

450   analyzed the data, LAH and KLM drafted the initial version of the manuscript, and all authors

contributed to later versions of the manuscript.

DATA ACCESSIBILITY

All data will be available in Dryad upon publication of the manuscript.

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Table 1. Biological interpretation of context-dependent genetic effects in this study system

Interaction[1]	Tested in this system[2]	Biological interpretation
G x G	mtDNA x Nuclear	Phenotypic effects of mtDNA variation depend upon nuclear genomic variation (i.e., epistasis)
G x E	mtDNA | Nuclear x Photoperiod | T_{DEV} | $T_{MEASURE}$	Phenotypic effects of variation in either genome depend upon the environment, which can also be interpreted as genetic variation for phenotypic plasticity
G x G x E	mtDNA x Nuclear x Photoperiod | T_{DEV} | $T_{MEASURE}$	Phenotypic effects of the mitochondrial-nuclear interaction are conditional on environment (i.e., context-dependent epistasis)
G x E x E	mtDNA | Nuclear x Photoperiod x T_{DEV}	The differential effect of photoperiod on the development time of particular genotypes depends upon development temperature
	mtDNA | Nuclear x $T_{MEASURE}$ x T_{DEV}	Phenotypic effects of variation in either genome on thermal plasticity of metabolic rate (the Q_{10}) depend upon development temperature
G x G x E x E	mtDNA x Nuclear x Photoperiod x T_{DEV}	Phenotypic effects of the mitochondrial-nuclear interaction on development time in response to photoperiod are conditional upon development temperature
	mtDNA x Nuclear x $T_{MEASURE}$ x T_{DEV}	Phenotypic effects of the mitochondrial-nuclear interaction on thermal plasticity of metabolic rate (the Q_{10}) depend upon development temperature (as in Hoekstra *et al*. (2013))

605   [1] Phenotypic effects of any of these interactions may also differ between males and females (i.e., sex-specific effects).
[2] " | " denotes one or the other factor.

Figure legends

Figure 1. An arrhythmic photoperiod phenocopies the effects of increasing temperature on a

610   mito-nuclear incompatibility that extends development. An arrhythmic, constant light

photoperiod (L:L) accelerates development in control genotypes relative to a rhythmic

photoperiod (L:D), but delays development in the mito-nuclear incompatible genotype

(*simw^{501}*);*OreR* (Supplemental Table S1). For comparison, the developmental delay of

(*simw^{501}*);*OreR* larvae relative to control genotypes under constant light at 22°C is greater than

615   the delay observed at 25°C under fluctuating light (L:D) (25°C data from Hoekstra *et al.* (2013)).

Asterisks denote a significant effect of the mtDNA x nuclear genetic interaction within each

temperature-photoperiod combination at the level of $P < 0.0001$ (Supplemental Table S2), with

the associated mean days delayed of (*simw^{501}*);*OreR* relative to (*simw^{501}*);*Aut* and the percent

increase in development time in parenthesis.

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Figure 2. Female adult metabolic rate is robust to mito-nuclear genetic effects. A. The only

mito-nuclear genetic effect was a small, but significant decrease in 16°C routine metabolic rate

(RMR) in incompatible (*simw^{501}*);*OreR* females ($P < 0.05$, Supplemental Table S5). B.

Developmental reaction norms show robust thermal acclimation of female mass-corrected RMR

625   at both measurement temperatures. C & D. Thermal reaction norms show that the Q_{10} for female

mass-corrected RMR is similar under both developmental temperatures, and that incompatible

(*simw^{501}*);*OreR* females have similar metabolic plasticity as their nuclear genotypic control

(*ore*);*OreR*. Patterns were similar for adult male metabolic rate (Supplemental Figure S2). The

mtDNA x nuclear interaction did not affect mass-corrected RMR of males or females at either

630   measurement temperature ($P > 0.28$; Supplemental Table S6). Error bars are +/- 1 SEM.

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Figure 3. Mito-nuclear effects on reproductive phenotypes are stronger in females. A. Male fertility was not significantly affected by the mito-nuclear incompatibility (mtDNA x nuclear: $F_{1, 107}$ = 0.185, P = 0.668). B. This was in contrast to strong effects of mito-nuclear genotype on

635   female fecundity (mtDNA x nuclear: $F_{1, 31}$ = 16.976, P = 0.0003), with females of the incompatible *(simw^{501});OreR* genotype laying on average 48% the number of eggs produced by *(ore);OreR* females. Female fecundity data from Meiklejohn *et al.* (2013) are total eggs produced over 10 days by individual females for n = 7-10 females per genotype. Whiskers on boxplots represent interquartile ranges. Green denotes the mitochondrial-nuclear incompatible

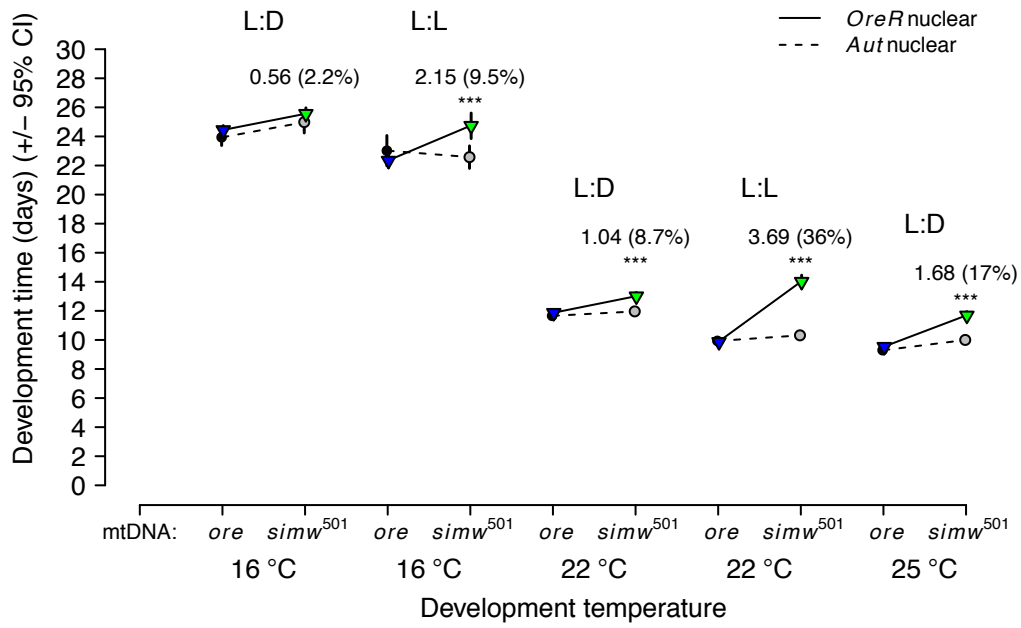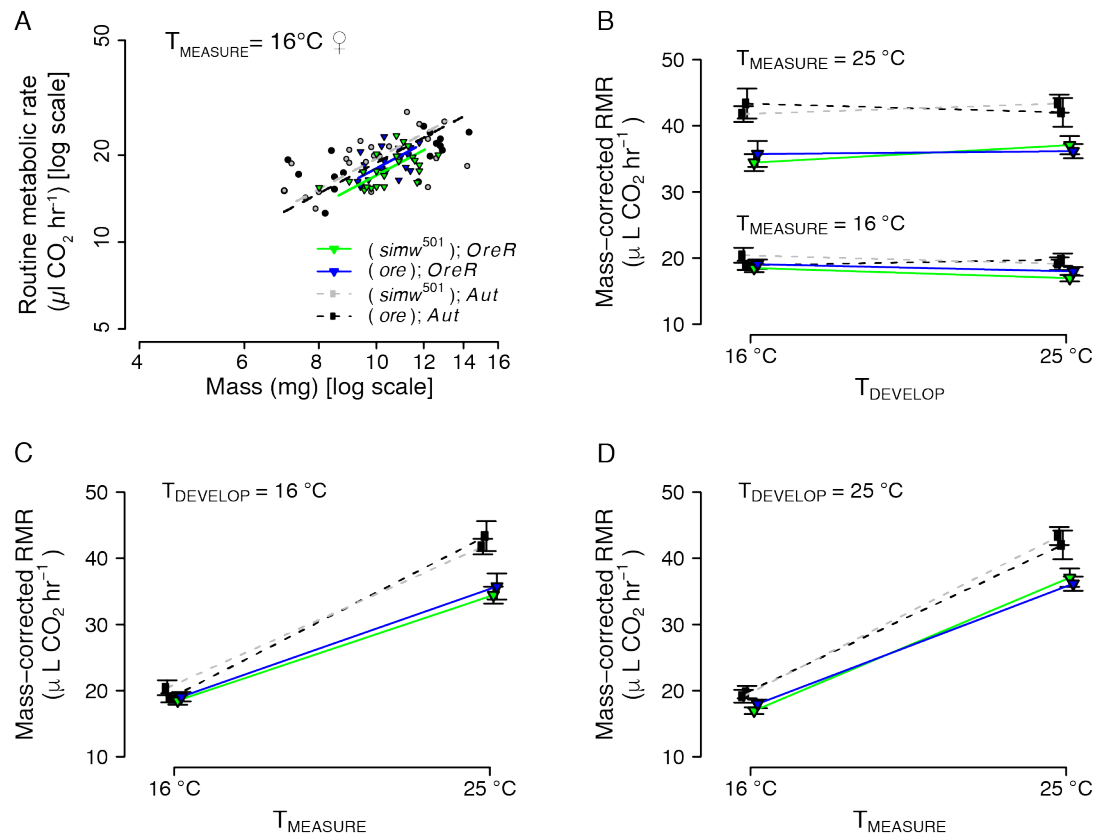640   genotype, as in other figures.

Figure 1

Figure 2

645

Figure 3

A. Male fertility

B. Female fecundity