Mitochondrial background can explain variable costs of immune deployment

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Abstract

Organisinal health and survival depend on the ability to mount an effective immune response against infection. Yet immune defence may be energy-demanding, resulting in fitness costs if investment in immune function deprives other physiological processes of resources. While evidence of costly immunity resulting in reduced longevity and reproduction is common, the role of energy-consuming mitochondria on the magnitude of these costs is unknown. Here we employed Drosophila melanogaster cybrid lines, where several mitochondrial genotypes (mitotypes) were introgressed onto a single nuclear genetic background, to explicitly test the role of mitochondrial variation on the costs of immune stimulation. We exposed female flies carrying one of nine distinct mitotypes to either a benign, heat-killed bacterial pathogen (stimulating immune deployment) or a sterile control and measured lifespan, fecundity, and locomotor activity. We observed mitotype-specific costs of immune stimulation and identified a positive genetic correlation between life span and the proportion of time cybrids spent moving while alive. Our results suggest that costs of immunity are highly variable depending on the mitochondrial genome, adding to a growing body of work highlighting the important role of mitochondrial variation in host–pathogen interactions.

Keywords: mitochondria, costs of immunity, trade-offs, host–parasite interaction, insects, life history evolution

Introduction

Life history theory predicts that upregulation of the immune system will come at a physiological cost (McKean et al., 2008; Moret & Schmid-Hempel, 2000; Stearns, 1992). In line with this prediction, the requirement to reallocate finite energy stores to maintain and activate immune function has been shown to give rise to an array of physiological costs across metazoan taxa (McKean & Lazzaro, 2011; McKean et al., 2008; Nystrand & Dowling, 2020). For instance, there is a strong negative relationship between immune function and reproductive success across species (Schwenke et al., 2016), while in many species, there is evidence that immune deployment can be costly in terms of reduced longevity (Eraud et al., 2009; Hanssen et al., 2004; Jacot et al., 2004; Moret & Schmid-Hempel, 2000). Additionally, immune activation can trigger significant behavioural changes, such as in locomotor activity, that are intimately intertwined with host energy intake and reallocation (Lopes et al., 2021; Siva-Jothy & Vale, 2019; Vincent et al., 2022). Even prokaryotic organisms, which lack what is usually considered to be a conventional immune system, have shown that those with strong pathogen defence mechanisms may experience reduced growth rates (Vale et al., 2015; Westra et al., 2015). Therefore, while the type and form of immunity may vary, its associated fitness costs are likely to be common (Nystrand & Dowling, 2020).

While the occurrence of life history costs associated with immune deployment is well known, the physiological and genetic basis of these trade-offs is rarely understood (Gupta et al., 2022). Mitochondria, the powerhouse of the cell, generate energy in the form of adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS). OXPHOS requires the coordinated action of protein subunits encoded by the nuclear (nDNA) and mitochondrial genomes (mtDNA). Variation in the function of the mitochondria may therefore arise through mutations in nDNA or mtDNA, which can affect signalling between the mitochondrial and nuclear genomes, transcription and translation of mitochondrial proteins, and through the interaction between OXPHOS components of both the nDNA and mtDNA (Dordević et al., 2017; Rand & Mossman, 2019; Salminen & Vale, 2020). In the absence of infection or immune deployment, variation in mitochondrial function has been associated with decreased life span, changes to locomotor activity and reproductive success, as well as developmental time and weight (Anderson et al., 2022; Camus & Dowling, 2018; Clancy, 2008; Salminen et al., 2017; Samstag et al., 2018). In addition to a potential effect on immune deployment via energy production, it is also possible that mitochondrial signalling or by-products of mitochondrial metabolism intervene directly in response to pathogens by controlling apoptosis, regulating various signalling pathways, and producing reactive oxygen species (ROS) (Angajala et al., 2018; Mills et al., 2017; Salminen & Vale, 2020; Shekhova, 2020). Thus, we may predict variation in mtDNA to generate, or at least contribute to, heterogeneity in the immune response (Buchanan et al., 2018; Salminen & Vale, 2020; Vesala et al., 2024). Following this reasoning, polymorphisms that lead to changes in the efficiency of ATP production may also impact the cost of immune deployment.
in the form of reduced fecundity, life span, or other aspects of host physiology. However, the role of mitochondrial variation on the costs associated with immune stimulation remains poorly understood (Buchanan et al., 2018).

Here we asked if variation in the mitochondrial genomes in nine cytoplasmic hybrid lines (cybrids) impacted the expression of two life history traits, survival, and fecundity, as well as locomotor activity in immune-stimulated female fruit flies (Drosophila melanogaster) exposed to heat-killed Pseudomonas entomophila, a natural fly pathogen (Dieppois et al., 2015; Vodovar et al., 2005). In response to P. entomophila infection, D. melanogaster responds through a combination signalling cascade that may involve the production of ROS (especially during gut infection) and the detection of bacterial peptidoglycan (PGN) that trigger signalling cascades such as the Immune Deficiency (IMD) pathways leading to the downstream expression of IMD-responsive antimicrobial peptides (AMPs) (Myllymäki et al., 2014; Vodovar et al., 2005). mtDNA is inherited maternally, so donor mtDNA can be introgressed (i.e., repeatedly backcrossed) into a specific nuclear background over sixteen generations, resulting in flies with less than 0.01% paternal nDNA, 99.99% paternal nDNA, and 100% maternal donor mtDNA (Salminen & Vale, 2020; Salminen et al., 2017). We used these cybrid lines to focus on naturally occurring variation in the mitochondrial genome, while introgressing each mitotype onto a single nuclear background helped to ensure that any observed effects were not influenced by epistasis between mitochondrial and nuclear genomes, which has been shown to strongly affect a number of life history traits (Camus et al., 2020; Đorđević et al., 2017; Rand & Mossman, 2019). We chose to use heat-killed bacteria to disentangle the effects of immune stimulation and pathology on our life history readouts: longevity, locomotor activity, and long-term fecundity.

Materials and methods

Generation of cybrid fly lines and rearing conditions

The lines used herein were generated using donor mtDNA from previously described cybrid lines in a wild-type Oregon-R nuclear background (Anderson et al., 2022; Salminen et al., 2017). The donor mtDNA in each of the eight lines was sampled from naturally occurring geographic variants (Salminen et al., 2017). To obtain cybrid strains with distinct mitochondrial genomes on the same nuclear background, eight wild-type cybrid strain females (ORT, KSA2, WTS5A, BS1, BV1, M2, BOG1, and PVM) were backcrossed for 16 generations with males in a W1118 nuclear background (Vienna Drosophila Resource Centre). Theoretically, this procedure yields flies with over 99.9% paternal nDNA and 100% maternal donor mtDNA, although this percentage may be lower due to processes such as genetic linkage (Salminen & Vale, 2020). The mtDNA of all strains (except BV1 and w1118) had been previously sequenced, and the known non-synonymous polymorphisms in OXPHOS complex coding genes are summarized in Supplementary Table S1. All flies were maintained at 25 ± 1 °C on a 12 hr: 12 hr light:dark cycle at 60% humidity on a modified Lewis cornmeal-yeast-sugar diet (Lewis, 2014) (14% protein; 1:6 protein:carbohydrate, see Hudson et al., 2020; Savola et al., 2021) for the complete recipe. Experimental flies were raised at constant density for one generation: ten females and five males were placed in a vial for two days at 25 °C, after which the adults were discarded, and the offspring were left to close, which ensures relatively constant egg densities in each vial (Gupta et al., 2017; Monteith, 2018; Savola et al., 2021). Mated female offspring were collected at 2–3 days post-eclosion for both experiments.

Heat-killed bacteria preparation and confirmation of immune deployment

To stimulate the immune response without a pathogenic infection, we pricked flies with a heat-killed bacterial culture. This procedure is commonly employed in Drosophila and other invertebrates to achieve an upregulation of antimicrobial peptide expression in the absence of a viable, replicating pathogen (Kutzer et al., 2019; Pham et al., 2007; Wen et al., 2019). One hundred microliter aliquots of Pseudomonas entomophila, a commonly used fly pathogen (Dieppois et al., 2015; Prakash et al., 2022; Prakash, Monteith, et al., 2023), were grown overnight in 10 ml of LB broth in an orbital shaker at 140 rpm at 30 °C until the culture reached exponential growth (i.e., OD 0.6–0.8). The next morning, cultures were spun down at 2,500 rpm for 15 min at 4 °C, and the pellet was resuspended in PBS. The OD was then adjusted to 0.1 and further diluted to 0.01 (~2,000 live cells/fly when pricked). Although our treatment only included heat-killed bacteria, controlling for the concentration of bacteria in the inoculum ensured that all flies were exposed to a comparable quality of bacterial peptidoglycan (PNG). We placed 500 μl aliquots of the dilution into a heat block set to 70 °C for 30 min and then froze the aliquots for later use. We confirmed that the bacteria had been inactivated by streaking the aliquot out onto an LB agar plate and growing it overnight at 30 °C after each experimental block. No viable colony-forming units were detected. Further, all tested fly lines exposed to the heat-killed culture showed an upregulation of the AMP Dipterin of between 5 and 50-fold compared to those pricked with sterile PBS (Supplementary Figure S1), confirming that our heat-killed treatment successfully induced immune deployment.

Locomotor activity and life span

We lightly anesthetized 2–3 day old female flies (20–31 flies per mitotype per treatment for a total of 472 flies) and pricked them in the thorax with a 5 mm needle dipped in an aliquot of heat-killed P. entomophila or in sterile PBS to control for the effect of pricking. The flies were placed in new vials to recover until being placed in the Drosophila activity monitors (DAM5M, hereafter referred to as DAM), as described previously (Anderson et al., 2022; Pfeifferberger et al., 2010; Siva-Jothy & Vale, 2019; Vale & Jardine, 2015). Eight DAM monitors were set up to measure activity and sleep levels in D. melanogaster. We performed the experiment over two separate days or experimental blocks because of constraints in the capacity of the DAM. Prior to starting the experiment, we prepared DAM tubes by filling each with 2 cm of a 5% sucrose-agar solution. When loading the DAM monitors, flies were anaesthetized using CO₂ and then placed individually into the tubes of each 32-tube activity monitor at random. The DAM monitors were kept in an incubator (25 °C on a 12 hr: 12 hr light:dark cycle, 60% relative humidity) placed to minimize possible disturbance and disruption, and each monitor contained either one empty tube or no tube as negative controls. Each monitor was connected to a laptop placed in the incubator running the DAMSystem3 data collection.
software. Activity counts and life span were recorded in 1-min bins (Pfeiffenberger et al., 2010) for 13 days, after which we checked individual survival in each tube.

**Fecundity**
A total of 234 flies (13 replicate flies per mitotype/treatment) were pricked with PBS or heat-killed *P. entomophila* as described above and then placed individually into vials to monitor egg laying. In contrast to the survival experiment, females retained ad libitum access to food throughout the fecundity experiment. We transferred the females to fresh food every 24 hr and then counted the eggs laid during the previous day. We counted the total eggs laid per day per female for 14 days.

**Statistics**
All data are available at https://doi.org/10.5281/zenodo.8411236. Statistical analyses were performed in R version 4.2.2 and RStudio 2022.07.01. We tested for significant interactions and main effects using type 3 Wald $\chi^2$ tests or likelihood ratio tests (Bolker et al., 2009). We evaluated model fits using model selection criteria (Brooks et al., 2019) and the simulateResiduals/testResiduals functions in the DHARMa package or the check_model function in the performance package. The models are described below and in Table 1.

Survival over the experimental period (Model 1) was analyzed using a Cox mixed effects survival model using the coxme function in the coxme package (Therneau, 2022) with the day of death as the response variable and DAM tube nested within the monitor and experimental block as random effects. We tested the model without random effects to check whether it fulfilled the assumptions of proportional hazards over time using the cox.zph function. The model explaining significance variance in survival was:

**Model1**: Survival $\sim$ Line + Treatment + Line $\times$ Treatment + (1|Block/Monitor/Tube)

We tested for differences in activity levels, measured as the proportion of time spent active (Model 2a) or movements per minute (Model 2b), using linear mixed-effects models in the glmmTMB package (Brooks ME et al., 2017) and tube nested in monitor and experimental block as random effects. We observed that activity levels were largely driven by whether an individual survived, so we included censor/survived (yes/no) as a factor in our models.

**Model2a**: Proportion time active $\sim$ Line + Treatment + Survived + (Line $\times$ Treatment) + (Line $\times$ Survived) + (Treatment $\times$ Survived) + (Line $\times$ Treatment $\times$ Survived) + (1|Block/Monitor/Tube)

**Model2b**: Movements per minute $\sim$ Line + Treatment + Survived + (Line $\times$ Treatment) + (Line $\times$ Survived) + (Treatment $\times$ Survived) + (Line $\times$ Treatment $\times$ Survived) + (1|Block/Monitor/Tube)

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$p$-values in bold indicate significance ($p < 0.05$).
We used a generalized linear mixed model with a negative binomial error structure and a quadratic parameterization to evaluate Model 3 using the glmmTMB function (Brooks ME et al., 2017). We used fecundity, measured as eggs laid per day, as our response variable, time (day) as a covariate, and included FlyID as a random intercept to account for pseudoreplication in the time series.

\[ \text{Model3} : \text{Fecundity} \sim \text{Time} + \text{Line} + \text{Treatment} + (\text{Time} \times \text{Line}) + (\text{Time} \times \text{Treatment}) + (\text{Line} \times \text{Treatment}) + (\text{Time} \times \text{Line} \times \text{Treatment}) + (1)\text{FlyID} \]

**Results**

**Exposure to heat-killed bacteria induces mitotype-specific mortality rates**

We found that individuals pricked with heat-killed *P. entomophila* showed increased expression of the AMP Diptericin, confirming several previous studies that this treatment stimulates the immune response despite not containing any viable bacterial cells (see Materials and methods section for details and Supplementary Figure S1) (Pham et al., 2007; Wen et al., 2019). Flies pricked with heat-killed *P. entomophila* also experienced higher mortality compared to sham-treated flies (0.82 ± 0.13; Figure 1; Model 1), indicating there is a survival cost associated with mounting an immune response. However, the magnitude of the survival cost varied among mitotypes (Figure 1A). Mitochondrial genome differentially affected survival following immune activation when compared to the w^{1118} control (Model 1b, Line × Treatment: \( p = 0.0018 \)). This effect was largely driven by mtPVM where survival of the sham control was significantly greater than in w^{1118} and by mtBS1, where survival was virtually identical in the sham and heat-killed treatments (Figure 1A).

**Fly activity patterns are strong predictors of future survival**

Given that different mitotypes are known to impact the extent of fly locomotor activity (Anderson et al., 2022), we tested if costs associated with immune deployment were also apparent in the form of reduced activity and if the magnitude of these varied across mitochondrial lines. We observed that a fly’s survival during the experiment seemed to significantly influence the proportion of time it spent being active while alive (Figure 2A and C). Specifically, we found that individuals who ultimately died tended to spend proportionally less of their lifetime moving while alive, with the magnitude of this decrease depending on mitotype and treatment (Figure 2A; Table 1, Model 2a: Line × Treatment × Survival: \( p = 0.0437 \)). Additionally, we found a significant and strong positive correlation between the average life span of each mitotype and the proportion of time that mtDNA remained active while alive (\( R^2 = 0.85, p = 0.0045 \), Figure 2E). Surprisingly, flies that died displayed a comparable activity rate (e.g., movements per minute) to flies that survived, regardless of treatment (Figure 2D, Model 2b).

**Egg-laying rate reveals a mitotype-dependent cost of immune stimulation**

Costs of immunity are often associated with decreased fecundity in females, so we carried out a separate experiment to test how immune stimulation with heat-inactivated bacteria affected fecundity in the cybrid lines over fourteen days. In general, the egg-laying rate in flies treated with heat-killed bacteria remained lower than controls from days 1 to 3, stabilizing at day 4 (Figure 3B). However, this time-dependent effect varied according to mtDNA background. The egg-laying rate changed over time based on exposure treatment and mitotype (Table 1, Model 3a, Time × Line × Treatment: \( p = 0.017 \)). All lines exhibited decreased egg laying in the heat-killed treatments 1-day post-exposure, but the magnitude of this effect varied according to mitotype, and the initial cost of immune activation tended to decrease over time. This three-way interaction was driven by BS1, BV1, and WT5A. BS1 and BV1 both recovered fecundity after immune activation compared to w^{1118}, whereas fecundity in WT5A remained low in the heat-killed treatment over the 2-week experimental period. This suggests that mitochondrial background affects immune activation, manifested as reproductive trade-offs.

![Figure 1](https://academic.oup.com/jeb/article/37/4/442/7624384) (A) Kaplan–Meier survival curves showing the mortality of flies pricked with either a heat-killed bacterial culture (red) or a sterile PBS control (blue). Each panel shows a cybrid line with the mitotype indicated above. All lines were introgressed onto a w^{1118} nuclear background for 16 generations. \( N = 20–31 \) flies per line per treatment. (B) the overall effect of exposure to heat-killed *P. entomophila* on the survival of all flies, averaged across all cybrid backgrounds.
Discussion

Overall, we found evidence for mitochondrial effects on the cost of immune stimulation in all measured traits. Further, we identified an intriguing positive and strong association between the life span of a given cybrid line and the proportion of time it spent moving while alive.
Mitotype-specific effects on survival
We found cybrid-specific effects on life span after immune activation (Figure 1; Table 1, Model 1). Costs of immunity are often associated with reduced longevity in a range of species. For example, immune stimulation in male field crickets decreased life span compared to the controls (Jacot et al., 2004), and a similar result was observed in female eiders (Hanssen et al., 2004) and Eurasian collared dove nestlings (Eraud et al., 2009). A meta-analysis examining trade-offs between immune activation and life history traits revealed that survival tended to be negatively affected in a range of organisms after immune stimulation by heat-killed bacteria or other antigenic substances (Nystrand & Dowling, 2020). Although much work exists examining the effects of previous exposure to a heat-killed pathogen on survival, many of these studies examine immune stimulation under ad libitum laboratory conditions, which may mask life history costs. Costs are often revealed in resource-limited or otherwise challenging environments (Boots, 2011; Boots & Begon, 1993; McKea et al., 2008), and we therefore measured the activity and survival of individual flies within DAM tubes, which is both a resource-limited and stressful environment due to constrained space and reduced access to food. For instance, resource-limited bumblebee workers (Bombus terrestris) challenged with lipopolysaccharide (LPS), an antigen derived from E. coli, or micro-latex beads had significantly reduced life spans compared to workers with ad libitum access to resources, indicating that life history costs are sometimes masked by compensatory resource intake (Moret & Schmid-Hempel, 2000). In an Indian meal moth, experimental selection for resistance to a granulosis virus under low or high levels of food resources resulted in reduced resistance in the moths selected on lower resources, suggesting also that immune deployment is costly, and its evolution is resource-dependent (Boots, 2011).

Mitotype-specific effects on fecundity
We measured the mean reproductive output of each cybrid line as the daily number of eggs laid by each fly over a 14-day period following a single period of mating. We found a significant 3-way interaction between treatment, line, and time (Model 3, Table 1), revealing a complex effect of immune stimulation on fecundity. Some cybrid lines showed a significant reduction in the number of eggs following stimulation with a heat-killed pathogen, but this effect changed over time and was especially visible within the first 3–5 days of egg-laying (Figure 3). Given that female flies were only mated at the start of the assay, this initial period of egg-laying is, therefore, the biggest contributor to fitness, as egg-laying rates would be highest during this period and then decrease.

Female fecundity is often—though not always (Hudson et al., 2020; Kutzer & Armitage, 2016; Labbé et al., 2010)—found to be reduced following pathogen exposure, and this reduction is frequently associated with immune-derived trade-offs. In birds, stimulation of the immune response by novel antigen injection in female house martins was accompanied by a decrease in reproductive success compared to non-injected individuals (Marzal et al., 2007). Studies using female Drosophila have also found that mounting an immune response is followed by a decrease in fecundity (McKea et al., 2008; Schwenke et al., 2016), and similarly, a post-mating decline in immune function has been observed, presumably due to a reallocation of resources to reproduction (Winterhalter & Fedorka, 2009).

Immune deployment does not result in changes to activity
In addition to the commonly observed survival and fecundity-based trade-offs, we also measured locomotor activity, a behavioural trait that is likely to be highly energy dependent (Anderson et al., 2022). We hypothesized that immune activation would trigger significant behavioural changes because of the relationship between host energy intake and energy reserves. However, activity levels were associated with survival rather than immune status in our study. Conflicting results in other studies suggest that activity levels after immune activation tend to be context dependent. For example, immune stimulation by LPS resulted in a marked decrease in locomotor activity in mice up to 36 hr post-inoculation (Meneses et al., 2018), and Eurasian collared dove nestlings injected with LPS had reduced life spans as a result of predation, which was likely due to reduced activity levels (Eraud et al., 2009).

In contrast, injection with heat-killed Micrococcus luteus, a Gram-positive bacterium, induced an increase in activity levels in D. melanogaster (Vincent et al., 2022). Thus, immune activation sometimes comes with significant costs, manifested as behavioural changes, but these tend to be context dependent (Lopes, 2014; Lopes et al., 2012), as was evident in the current study.

Flies that move a lot, live a lot
One intriguing result was the observation of a strong positive genetic correlation between life span and the proportion of time flies spent moving (Figure 2B). While we did not set out to test any hypothesis relating to the two traits, it became clear during our analysis of locomotor activity that the data generally clustered in two distinct groups within each of the control and heat-killed treatments. Stratifying the data by whether flies had died or remained alive during the experiment showed clearly that these clusters were driven by the much lower proportion of active movement in flies that eventually died (Figure 2). No difference between flies that lived or died was observed in our other measure of activity, movements per minute (Figure 2C and F). The proportion of time spent actively should, in principle, be independent of an individual’s life span, so this positive correlation is likely to reflect a true biological effect rather than any potential statistical artefact of how we measured activity and survival. It is noteworthy that this effect occurs in both heat-killed treated and sham-treated flies, so it is not clearly a consequence of immune deployment or other immunopathology. Across all tested lines, even within a group of otherwise healthy control flies, a low proportion of time spent active was associated with death later in the experiment (Figure 2A). One potential interpretation is that cybrid lines that are more likely to die are those lines that are already in severe metabolic deficit and, therefore, spend a greater proportion of their time inactive. Further work is clearly needed to disentangle the physiological basis of this genetic association. It also suggests that the proportion of time spent actively while alive offers a potentially useful early warning signal for death (Deb et al., 2022; Scheffer et al., 2009; Trefois et al., 2015).

Understanding the mechanisms of mitochondrial effects on life history costs
We have shown evidence that flies carrying distinct mtDNA also experience different levels of costs following immune stimulation, which we measured as reductions in survival, fecundity, or activity. It remains difficult to understand,
however, which aspect of mitochondrial metabolism contributes to these differences. Mitochondrial function can impact the deployment of immunity in at least three distinct ways (Salminen & Vale, 2020): (1) mitochondria play a direct role in immune signalling via intermediates of the mitochondrial tricarboxylic acid (TCA) cycle; (2) mitochondrial metabolism may have a direct antimicrobial action by producing ROS; and (3) mitochondria generate energy by producing ATP during OXPHOS. The non-pathogenic nature of the exposure we employed allows us to disregard the first two sources of variation: All cybrid lines maintained the ability to signal via TCA cycle, and variation in the production of antimicrobial ROS is unlikely to affect the performance of each cybrid line during a benign heat-killed exposure.

This leaves variation in energy production, which we initially predicted would lead to variable costs of immunity. Most cybrid lines we used are known to harbour non-synonymous mutations in several OXPHOS complexes (Supplementary Table S1; Salminen et al., 2017). Mutations in any mitochondrial encoded OXPHOS could affect the total electron transfer chain, potentially causing a decrease in the total production of ATP, with detrimental effects on the expression of life history traits (Salminen et al., 2017, 2019). Changes or loss of mitochondrial function often cause issues with locomotion or flight in D. melanogaster. For instance, flies that are mutant for clueless and parakin, genes associated with altered mitochondrial function, have degenerated flight muscles, display uncoordinated movement, and have shorter life spans compared to wild-type flies (Cox & Spradling, 2009).

Recent work on some of the cybrids used in the present work has also found that mitochondrial variation can affect immune deployment directly by affecting the production of ROS and the proliferation and differentiation of haemocytes during parasitoid wasp infection, particularly in lines with low mitochondrial copy number and non-synonymous substitutions in cytochrome b (OXPHOS complex II) and cytochrome c oxidase subunit 3 (COIII), such as mitotype mtKSA2 (Salminen et al., 2019; Vesala et al., 2024). In addition, there is some evidence that trade-offs between immunity and fecundity are associated with disruptions in mitochondrial function (Buchanan et al., 2018). Female D. melanogaster lacking normal energy metabolism that survived a Providencia rettgeri infection had reduced fecundity compared to control genotypes with normal energy metabolism (Buchanan et al., 2018). These findings highlight the importance of mitochondrial function in shaping the life history costs of immunity.

In summary, we found evidence for costs of immune stimulation measured as fecundity, survival, and activity. The extent of these costs varied according to the mitochondrial genome of each line. Further, we identified an intriguing positive and strong association between the life span of a given cybrid line and the proportion of time it spent moving while alive. Our work adds to a growing body of literature highlighting the significant role of mitochondrial variation on the expression of life history traits (Camus & Dowling, 2018; Camus et al., 2020; Nystrand & Dowling, 2020; Rand et al., 2006). By impacting the costs of immune deployment, mitochondrial variation may, therefore, also play a role in the maintenance of variation in immunity, with a possibly underappreciated role in host–pathogen evolutionary dynamics (Downey et al., 2022; Salminen & Vale, 2020).

### Supplementary material

Supplementary material is available at Journal of Evolutionary Biology online.

### Data availability

All data and code are available at https://doi.org/10.5281/zenodo.8411236 (Kutzer et al., 2023).

### Author contributions

Megan Kutzer (Data curation [lead], Formal analysis [lead], Software [equal], Validation [lead], Visualization [lead], Writing—original draft [lead], Writing—review & editing [equal]), Beth Cornish (Investigation [equal], Methodology [equal], Writing—review & editing [equal]), Michael Jamieson (Investigation [equal], Methodology [equal], Writing—review & editing [equal]), Olga Zawistowska (Investigation [equal], Methodology [equal], Writing—review & editing [equal]), Katy Monteith (Investigation [equal], Methodology [equal], Supervision [equal], Writing—review & editing [equal]), and Pedro Vale (Conceptualization [lead], Funding acquisition [lead], Project administration [lead], Resources [lead], Supervision [lead], Writing—original draft [equal], Writing—review & editing [equal])

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### Conflicts of interest

The authors declare no conflict of interest.

### References


