Thermal performance curves: from mitochondrial physiology to population growth rate in *Drosophila*

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ABSTRACT

Biological processes at the mitochondrial level could play a key role in setting thermal sensitivity of organisms and delineating the thermal range of ectotherms. With the current effects of climate change, many ectotherms will experience greater energetic stress, warranting investigation into the role of mitochondria as an important component of how they will cope with climate change. To determine whether changes in mitochondrial properties could underlie trade-offs and constraints in thermal limits and adaptation, we examined the thermal performance of mitochondrial function in outbred population of *Drosophila melanogaster*. Our approach is to connect thermal performance at different levels of biological organization in order to mechanistically understand how temperature-dependent changes in mitochondrial function scale up to impact population dynamics.

Here we present data on thermal performance curves for mitochondrial function and the emergent physiological components and life-history traits including metabolic rate, survivorship, development rate, fecundity and population growth rate in an outbred population of *Drosophila melanogaster*. These data provide the baseline for an experimental evolution experiment to ask how different levels of biological organization together respond adaptively to temperature. We discuss a predictive framework for forecasting the dynamic responses to environmental change from thermal metabolic responses through a series of currently unknown nested functions up to population level responses. This is important for comprehensive and quantitative understanding of the driving forces, tradeoffs and constraints in thermal adaptation.

HYPOTHESIS

- Adaptive metabolic responses to temperature will scale up through levels of organization from subcellular to population growth rate.
- The prediction of organismal thermal performance at any level requires understanding how the functional at lower levels is transformed to the above level.

EXPERIMENTAL DESIGN

12 strains derived from wild-caught female flies from Lincoln, NE

Rearing conditions: 22 C, 60% RH, and 12:12 h L:D

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Thermal performance assays at different level of organization

Cellular level

Mitochondrial respiratory function (Matoo *et al*., 2019)
- State 3: oxygen-coupled ATP production.
- State 4: leakiness of membrane
- Maximal respiration capacity

Organismal level

- Metabolic rate using flow-through respirometry (Hoekstra et al. 2013).
- Development rate & survivorship
- Adult longevity and survival analysis (Kleinbaum & Klein, 2012)
- Fecundity, fertility, and offspring survivorship

Population level

Population parameters (Southwood & Henderson, 2000)

R0: the net reproductive rate per generation (∑LxMx)

G: cohort generation time (∑lx mx x) / R0

λ: The finite rate of population change (lambda = R0/G)

r: the intrinsic rate of natural increase (r = ln(R0)/G = ln(lambda)

ex: the life expectancy (ex)

Mathematical modeling (Luhring and DeLong 2017)

Statistical analysis using R software version 3.5.1 (2018-07-02) in R-studio Version 1.1.456 – © 2009-2018 RStudio, Inc.

RESULTS

Mitochondrial Physiology TPCs

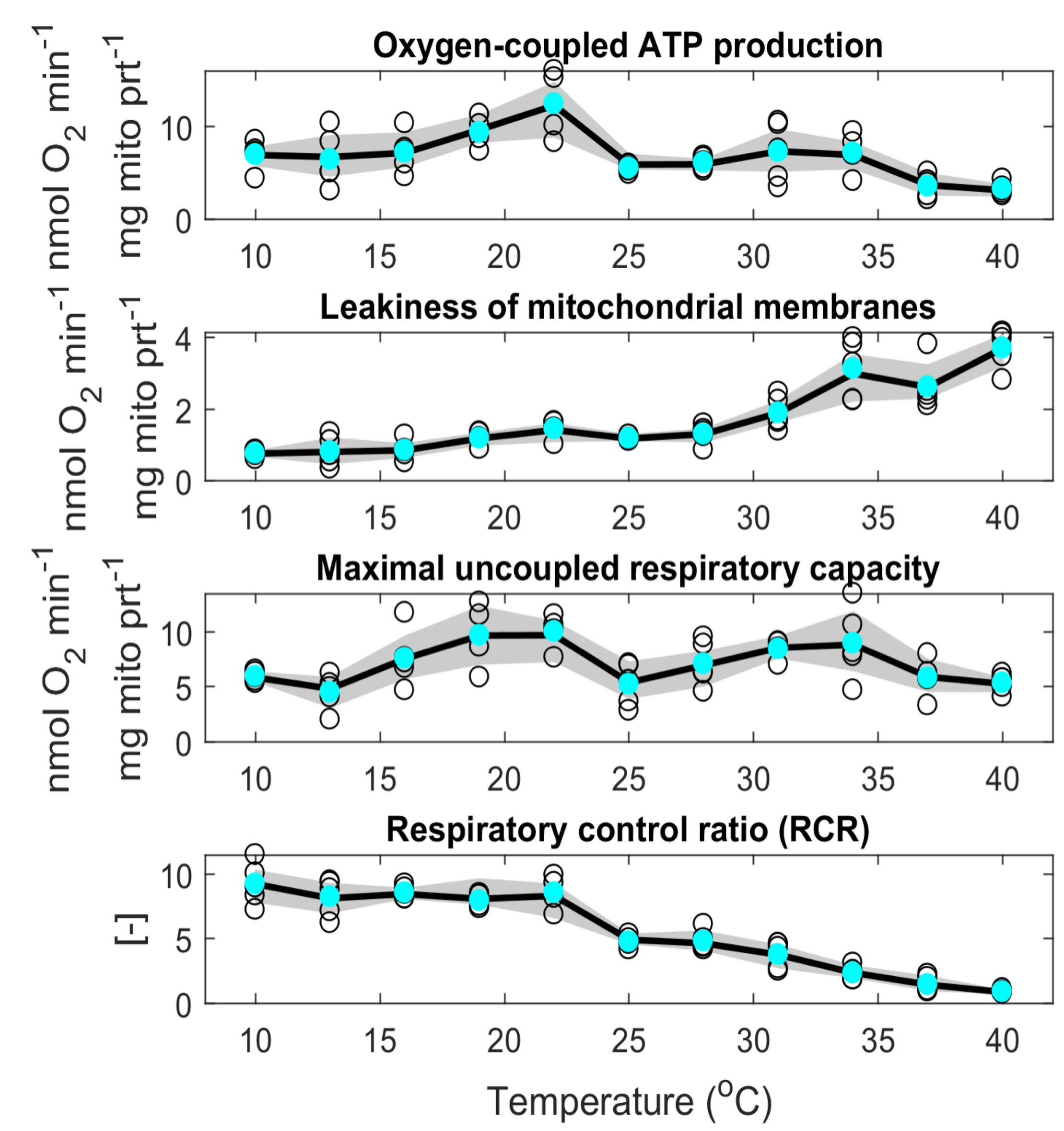

Fig. 1: TPCs for female mitochondrial function : O_2-coupled ATP production is maintained over temps, but mitochondrial integrity declines at higher temperatures

Whole-organism Metabolic Rate (TPCs)

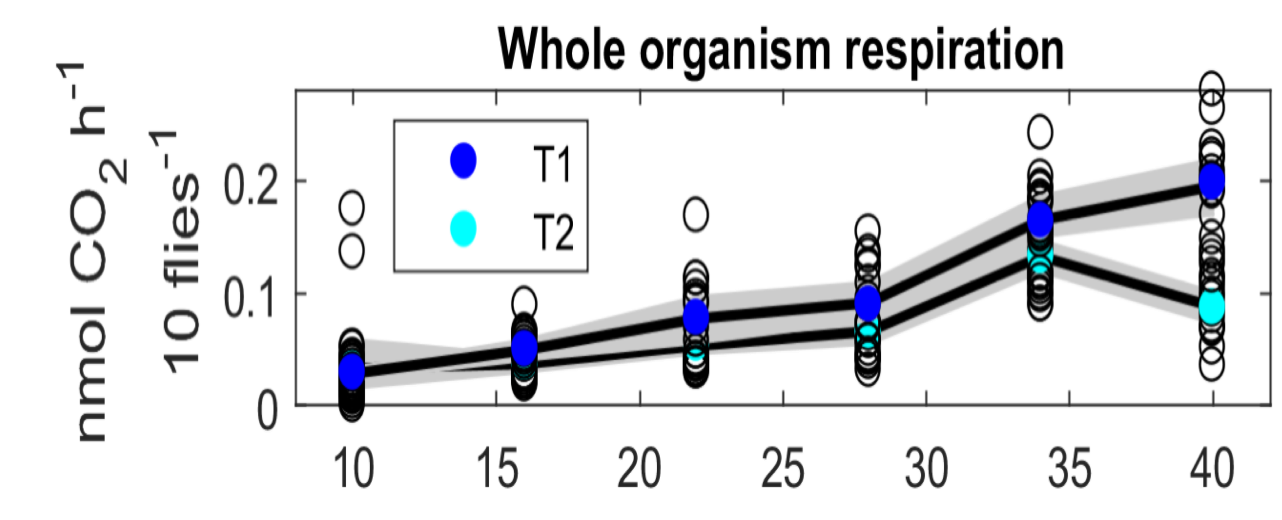

Fig. 2: TPCs for female metabolic rate: Metabolic rate decreases upon exposure to high temp. Time point T2 is a second measure of metabolic rate for the same biological replicate of female flies after one hours from the first measure (T1) at the exposed temp.

Metabolic rate (VCO_2) differed significantly among temperatures (df=5, F=126.23, $P < 2x10^{-16}$) and time points (df=1, F= 92.47, $P < 2x10^{-16}$) with a significant interaction between temp and time points (df=5, F= 14.28, $P < 1.1x10^{-12}$) .

The whole organism metabolic rate reflects mitochondrial respiration. The leakiness of mitochondrial membranes (State 4) increased at higher temperatures, resulting in a a decrease in the respiratory control ratio (RCR) despite the maintenance of O_2-consumption linked to ATP production (State 3) across temperatures. This may contribute to the dampening of organismal metabolic rate after a prolonged exposure to high temperatures.

Development Rate TPCs

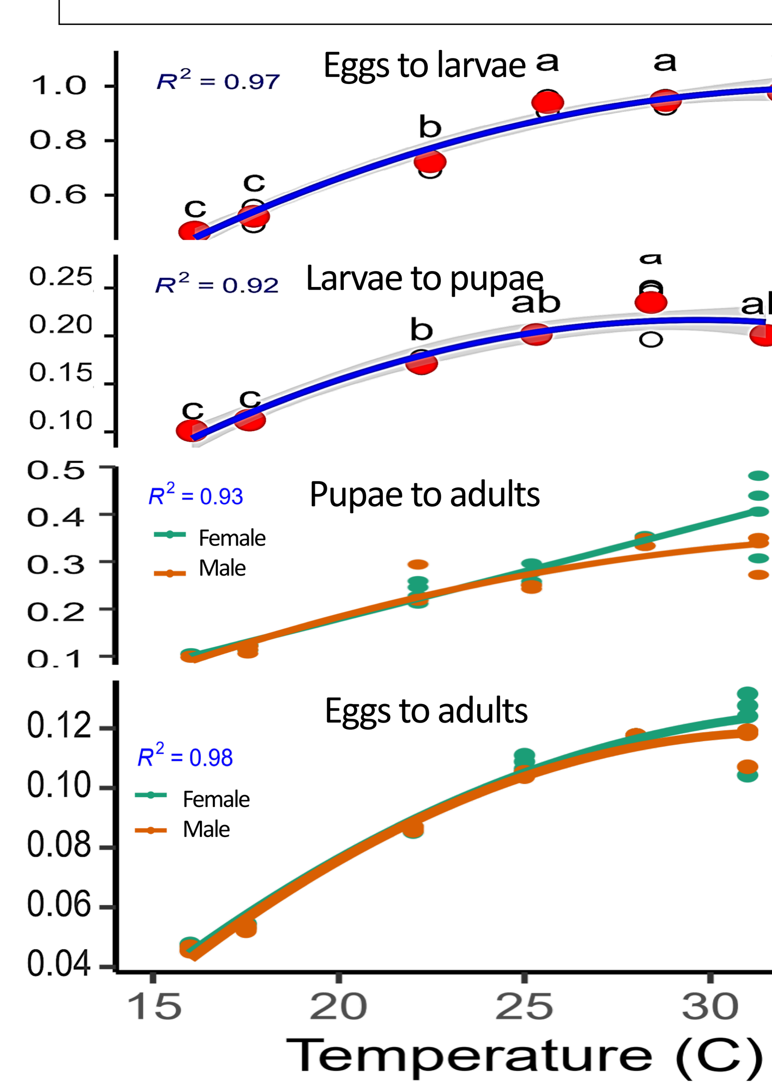

Fig. 3: TPCs for development rate: The development rates of egg, larval and pupal stages were positively correlated with temperature (Quadratic model). However, larval development rate decreased when temperature increased above 28 C.

Population Parameters TPCs

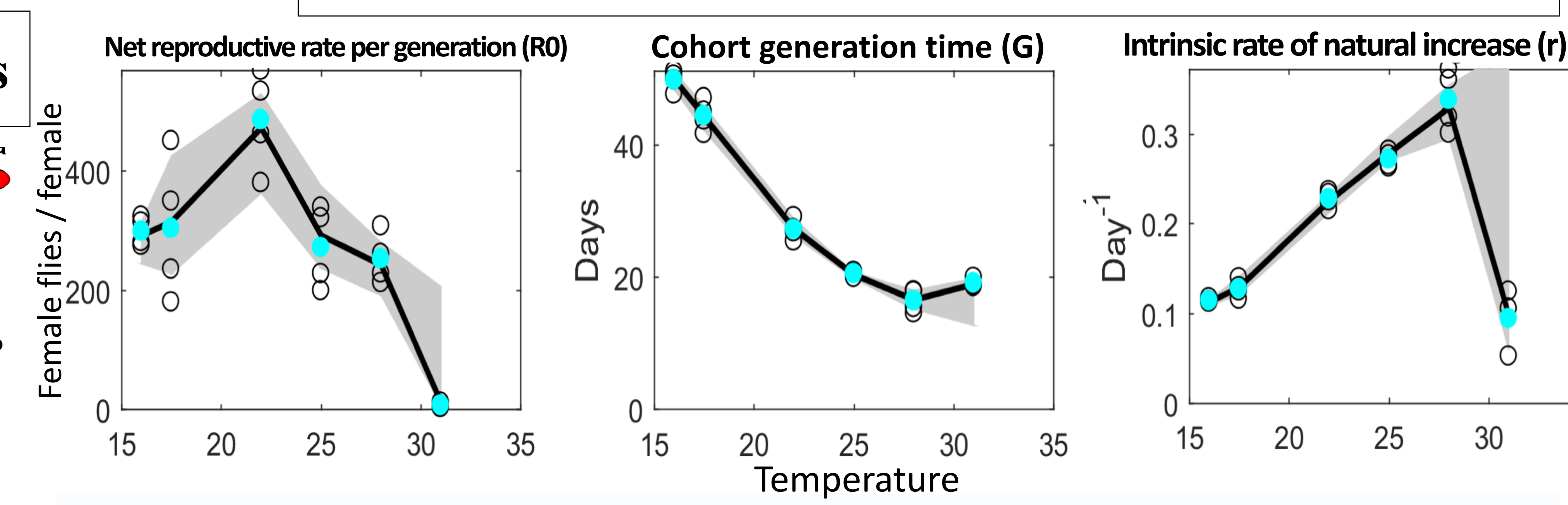

Fig. 4: TPCs of population parameters: Each point represents a cohort of individuals started from 30 eggs with 4 cohorts/temp. Life-table parameters were calculated using adult females per female as our measure of offspring produced. Curves are estimated by fitting a spline, bootstrapped 200 times, with the median of the bootstraps shown as the overall fit (black line) and the gray area represents the 95% CI across bootstraps. Groups according to probability of means differences and alpha level(0.05). Means that do not share a letter are significantly different across temperatures.

The highest intrinsic rate of natural increase was at 28 C where flies have maximum developmental rate and shortest generation time. The highest net reproductive rate, greatest reproductive fitness, and best juvenile survivorship coincide with the highest O_2-coupled ATP production at 22 C. At higher temps, the mitochondrial RCR declines.

CONCLUSION

Temperature differentially affected performance across levels of biological organization. The highest net reproductive rate, greatest reproductive fitness, and best juvenile survivorship coincided with the highest O_2-coupled ATP production at 22 C, which is the temperature at which the population has been maintained. At higher temps, the mitochondrial RCR declines, as does the organismal metabolic rate and quality of offspring produced by females over their lifespan. Low survivorship of females and their offspring at higher temperatures leads to a sharp decline in the population growth rate (*r*).

Future direction: We have been evolving this population to 16C and 28C for one year and will now test how the TPCs at different levels respond to thermal lab acclimation and adaptation.

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