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ThermAdapt report:

Consequences of temperature fluctuations for fitness-related traits in three species of dung flies

Purpose of the visit

I have investigated the effect of constant vs. fluctuating temperatures on the life history of *Drosophila spp* in the context of thermal adaptation and phenotypic plasticity. The purpose of this study was to replicate these experiments in other species with similar life histories but potentially different responses (Blanckenhorn 1999), namely in the yellow dung fly (*Scatophaga stercoraria*) and the black scavenger (or dung) flies *Sepsis (neo)cynipsea* and *S. punctum*. All these species are well researched and characterized in the laboratory of Dr. Wolf Blanckenhorn at the University of Zurich.

The stay was further meant for me to learn new methods while also working with methods that I am familiar with, and to see how other laboratories conduct these. This included working with different species but on the same subject matter. Finally, the stay should establish a stronger contact between the research groups for the future benefit of both departments.

Work carried out during the stay

A population of yellow dung flies, *Scatophaga stercoraria*, and two pairs of populations or closely related species of black scavenger flies were set up for mating and egg laying. The sepsid population pairs consisted of a Swiss and Canadian (Ottawa) population of *Sepsis punctum,* and *S. cynipsea* contrasted to the sister-species *S. neocynipsea*, both of Swiss origin. After laying, eggs were transferred to small dung containers and distributed randomly to one of five temperature regimes: constant 12°C, 18°C and 24°C and fluctuating 18°C ± 3°C and 18°C ± 6°C on a 12/12 h daily basis. The light cycle was 16/8 L/D for all regimes.

At the onset of hatching the containers were checked for emerging flies on a daily basis. These were transferred to glass vials and fed on ad libitum amounts of water saturated with sugar to investigate longevity on this diet depending on developmental temperature. Egg-to-adult survival for each container

was also scored, as was development time. Dead flies were frozen for further morphometric measurements.

The data were analyzed with linear mixed effects models using R and model simplification was attempted prior to statistical inference. Graphs were made with JMP. Data are presented for the yellow dung fly only (so far).

Sex-ratio as a function of temperature treatment was computed as a proportion and arcsine-square root transformed to improve normality. The data for sex-ratio did not deviate critically from normality (Shapiro-Wilk's test; $0.99 > P > 0.02$) so the distribution was assumed to be normal in the model.

Egg-to-adult viability (combined sexes) as a function of temperature regime was computed as a proportion and arcsine-square root transformed to improve normality. Shapiro-Wilk's test showed that the data did not deviate critically from normality ($0.48 > P > 0.01$) so the distribution was assumed to be normal in the model. The data was therefore analyzed with a linear mixed effects model with temperature as a fixed effect and family as a random effect.

Development time was not quite normally distributed (Shapiro-Wilk's test; $0.66 > P > 0.0001$), but the residuals of the linear fit were clearly homoscedastic judging from visual inspection. One exception was the residuals of the 12°C, which were more variable than in the other treatments (fig. 1). However, the observations were, on the other hand, normally distributed for the 12°C treatment (Shapiro-Wilk's test; males: $P = 0.66$, females = 0.29). Based on this information the data were analysed with a linear mixed effects model with temperature treatment and sex as fixed effects and family as a random effect.

As for development time, the data for longevity was also not quite normally distributed (Shapiro-Wilk's test; $0.33 > P > 0.005$) but the residuals of the linear fit were clearly homoscedastic judging from visual inspection. Adult longevity was therefore also investigated with a linear mixed effects model. Temperature and Sex were entered as fixed effects and Family as random (Sex nested in Family). Tukey's HSD test was applied to test for pair wise differences in longevity between temperatures and sexes. Permutation t-tests with correction for multiple related tests (Bonferroni) were also conducted to check the results from the linear model.
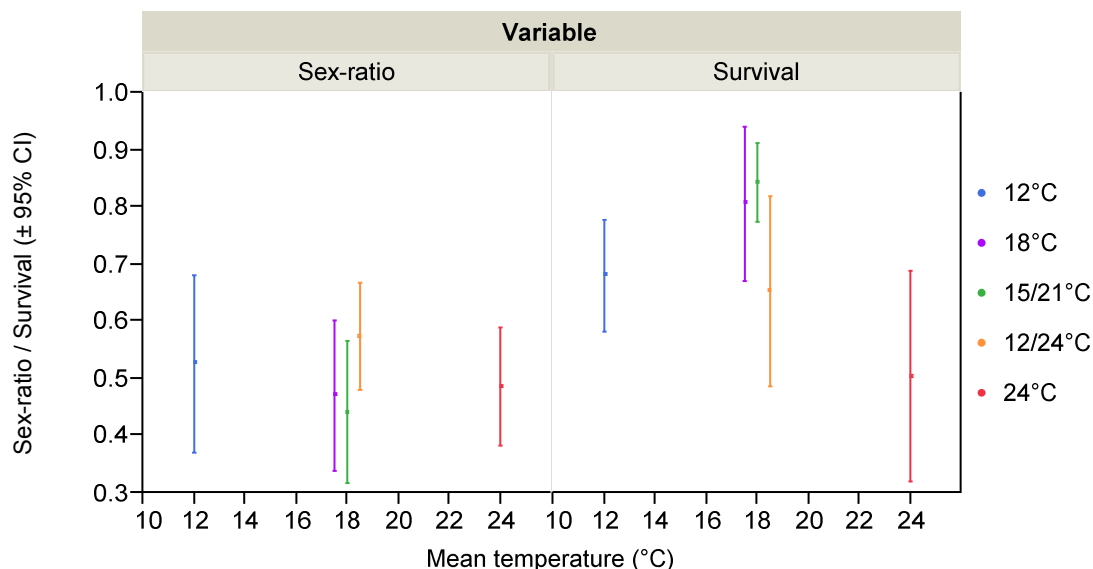
Figure 1. Sex-ratio and egg-to-adult survival as a function of temperature treatment with 95% confidence limits. Sex-ratio is given as the proportion of males relative to the total number of hatched individuals. Survival is given as males and females combined.

Preliminary results

Sex-ratio

"Family" was removed from the model (AIC: 9.32 vs. 9.62; P = 0.13), but this did not change the fact that there was no effect of temperature on sex-ratio (P = 0.39)(Tab. 2, Fig. 1).

Egg-to-adult viability

An attempt to simplify the model by taking out the random factor "Family" was rejected as this resulted in a significant loss of information (AIC: 41.79 vs. 43.84; P = 0.044). Hence the full model was retained.

Temperature regime had a significant overall effect on survival (P = 0.004; Tab. 1, Fig. 1) which was mainly caused by decreased survival at the constant high 24°C treatment.

Development time

Both temperature and sex effects, as well as their interaction, were highly significant (P < 0.001; Tab. 2, Fig. 2). Notably development was prolonged for the fluctuating temperature treatments relative to the constant 18°C of same mean. The size of the difference scaled in proportion with the amplitude of the fluctuations. Sex differences were evident in all temperatures but 24°C.

Trait	12°C (n) Mean±S.E.	18°C (n) Mean±S.E.	24°C (n) Mean±S.E.	15/21°C (n) Mean±S.E.	12/24°C (n) Mean±S.E.	Source of variation	D.F.	ANOVA F-value	P	Tukey´s pairwise Comparison
Egg-to-adult viability	(11) 0.68 ± 0.04	(11) 0.81 ± 0.06	(11) 0.50 ± 0.06	(11) 0.84 ± 0.03	(11) 0.65 ± 0.07	Between Within	4 40	4.56	0.004	(18 > 24)*, (15/21 > 24)*
Sex-ratio	(11) 0.53 ± 0.07	(11) 0.47 ± 0.06	(11) 0.44 ± 0.06	(11) 0.44 ± 0.06	(11) 0.57 ± 0.04	Between Within	4 50	1.05	0.39	

Table 1. Summary statistics for egg-to-adult viability and sex-ratio. Sample sizes (n) and means with standard errors are given for each temperature treatment. ANOVA values are given for overall significance of temperature treatment along with Tukey's pair wise comparison.

Longevity

The linear mixed effects model showed that there was a significant effect of temperature on longevity (P < 0.001; Tab. 2, Fig. 3) as well as of sex (P = 0.025). The interaction was, however, only marginally significant (P = 0.052). Since the interaction term was very close to significant, the full model was not reduced by removing this.

Tukey's HSD test revealed that longevity was highly significantly reduced at constant 24°C development compared to all other temperature treatments (Tab.2). It also revealed that females survived longer than males when developing at 12°C (P < 0.001).

Trait	Temperature Regime	Sex (n) ♂	Sex (n) ♀	Tukey's HSD Sex	Tukey's HSD Temperature Treatment
Development time	12°C	(34) 49.4 ± 0.49	(35) 44.6 ± 0.44	(♂ > ♀)***	(> 18, 24, 15/21, 12/24)***
	18°C	(42) 24.5 ± 0.19	(45) 22.6 ± 0.14	(♂ > ♀)***	(> 24)***; (<12, 15/21, 12/24)***
	24°C	(24) 20.0 ± 0.16	(30) 19.0 ± 0.20	N.s.	(<12, 18, 15/21, 12/24)***
	15/21°C	(41) 26.3 ± 0.13	(49) 24.7 ± 0.14	(♂ > ♀)***	(> 18, 24)***; (< 12, 12/24)***
	12/24°C	(40) 27.3 ± 0.16	(30) 25.7 ± 0.20	(♂ > ♀)***	(> 18, 24, 15/21)***; (< 12)***
Longevity	12°C	(36) 25.9 ± 1.71	(35) 35.1 ± 1.43	(♀ > ♂)***	(> 24)***
	18°C	(42) 26.0 ± 1.83	(45) 27.1 ± 1.70		(> 24)***
	24°C	(24) 14.7 ± 1.21	(30) 17.7 ± 1.38		(< 18, 15/21, 12/24)***
	15/21°C	(41) 28.2 ± 1.29	(48) 29.1 ± 1.48		(> 24)***
	12/24°C	(40) 27.1 ± 1.39	(30) 29.6 ± 1.54		(> 24)***

Table 2. Summary statistics for development time and adult longevity. Sample sizes (n) and means with standard errors are given for each temperature treatment and each sex along with Tukey's pair wise comparison for differences between sex and temperature treatment.
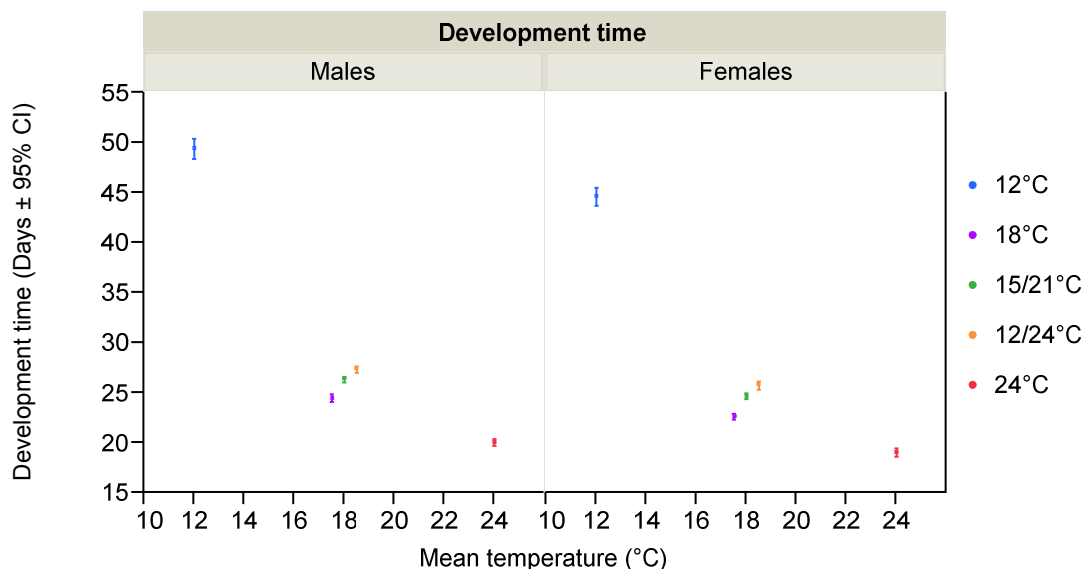
Discussion

The main goal of this project was to investigate the effect of temperature fluctuations on fitness related traits. Based on the shape of the reaction norms of the constant temperatures it should be possible to predict the response of the fluctuating temperature regimes (Jensen's inequality; Jensen 1906, Ruel & Ayres 1999). If the reaction norm is accelerating with temperature then fluctuations would be predicted to elevate the response variable compared to a constant temperature treatment with the same mean. Conversely, a decelerating reaction norm would depress the response variable relative to the constant temperature treatment. A linear relationship with temperature would predict no difference between a fluctuating regime and the constant with the same mean.

The preliminary analyses showed that there was no temperature effect on the sex-ratio, and this did not change with temporal variance as predicted by Jensen's inequality. Egg-to-adult viability, on the other hand, was affected by temperature – namely constant high temperature resulted in lowered survival. One would therefore expect this to be manifested in the fluctuating regimes as lower survival compared to the constant 18°C. This was not quite what was observed, as the 15/21°C treatment displayed slightly higher survival than the constant 18°C. The higher amplitude 12/24°C treatment did, however, display reduced survival, though not significantly so. This suggests that exposure to extreme temperatures could be more important than temperature variation per se (Jentsch *et al.* 2007). The confidence limits and the sample size (n = 11) here were, however, insufficient to establish this relationship statistically.
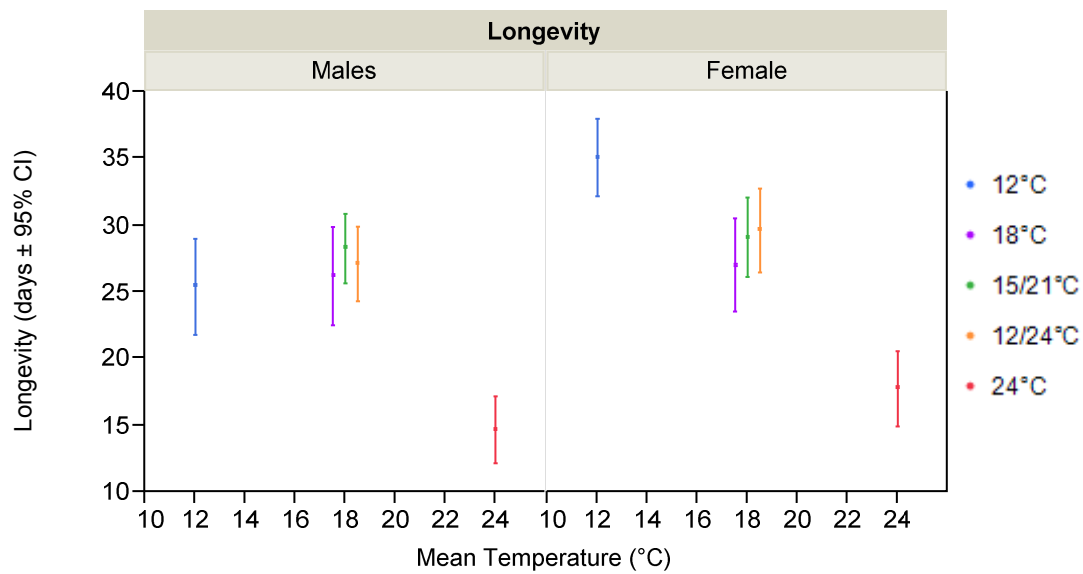
Figure 3. Adult longevity as a function of temperature treatment given in days with 95% confidence limits.

Development time or rate showed the pattern expected from Jensen's inequality in that the large effect of low temperature resulted in longer development. Longevity or life-span showed a sex-specific pattern attributable to large sex differences in longevity at 12°C. The response to temperature fluctuation, however, did not differ qualitatively even though the shapes of the reaction norms of the constant temperature treatments were quite different (polynomial and linear respectively).

In conclusion, it seems that there are other things going on during development than what is expected from the mathematical properties associated with the reaction norms of development at different constant temperatures. An important aspect is probably the capacity (or lack hereof) of an organism to acclimate to a change in temperature and the associated time lag to optimal performance at the given temperature (Gabriel 2005). Furthermore, these time lags are not necessarily symmetric, meaning that the induction of a response to one temperature may not mirror the induction of the reversed case. Heat shock proteins are, for instance, induced at stressful temperatures at a faster rate than they disappear upon reversal to a more benign temperature (Sørensen *et al.* 2003).

Analyses including morphometric traits will shed further light on the patterns presented here and they will allow for growth rates to be estimated. It will be interesting to contrast the results obtained for the yellow dung flies with those of the sepsid flies since the latter are more heat tolerant and their optimum temperature is rather close to the 24°C treatment.

Other outcomes of the project

The data obtained from the project are expected to result in one or two publications. The stay also prompted me to apply for funding to work at the University of Zürich for a two year period as Post Doc. This will further strengthen the collaboration between my current research affiliation in Denmark and the Invertebrate Behavioral and Evolutionary Ecology group at the University of Zürich.

References

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