Heat stress during early development poses lasting threats to a montane stag beetle, *Lucanus maculifemoratus dybowskyi*

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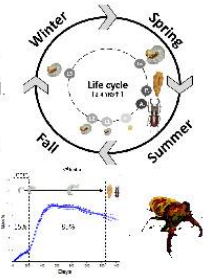
Background & Objective

- Recent climate change has raised not only average temperatures but also the frequency and intensity of extreme thermal events, such as heat waves. Exposure to extremely high temperatures imposes physiological stress that impairs insect fitness, particularly in species confined to high latitudes or altitudes. Beyond its immediate effects, heat stress experienced during development may have lasting consequences for survival and other fitness-related traits later in life.
- The Dybowski's stag beetle, *Lucanus maculifemoratus dybowskyi* (Coleoptera: Lucanidae), is an ecologically important flag species inhabiting montane regions of northeastern Asia, including Korea, Japan, and Manchuria. This cold-adapted species is unusually vulnerable even to moderately warm temperatures (e.g., 25°C), which are typically considered optimal for many other temperate insects.
- The objective of this study is to explore how early-life thermal stress affects survival, growth trajectories, and key fitness traits in larvae of *L. maculifemoratus dybowskyi*. To address this, larvae were reared at one of three temperatures (15°C, 20°C, or 25°C) during their first two instars before being transferred to a common temperature of 20°C for subsequent development.

Materials & Methods

Study insect:

- Larvae undergo three instars, with the 3rd accounting for ~85% of the total larval period.
- Adults emerge in early/mid summer and lay eggs in early fall
- Complete lifecycle in nature is not fully described.

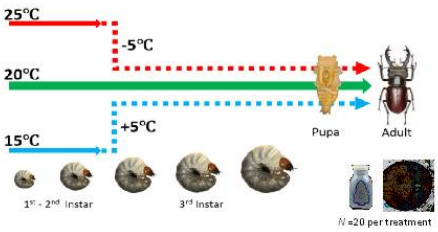


Insect collection:

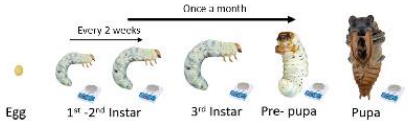
- More than 200 eggs were obtained from 16 pairs of stag beetles collected in July 2021 at multiple field-sites in Yeongwol, Korea (37.18° N, 128.47 ° E).

Experimental design:

- Larvae were reared individually on fermented sawdust at one of three temperatures (15°C, 20°C, 25°C) during the 1st and 2nd instars.
- Upon entering the 3rd instar, all individuals were switched to 20 °C and maintained until adult eclosion.



- Survival and growth were recorded across development.

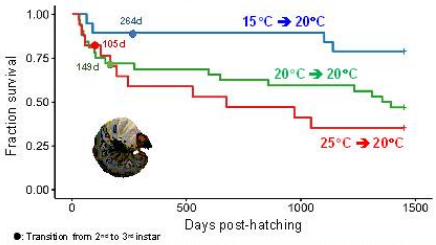


Statistical analysis:

Data	Model
Survival	• Cox regression
Growth trajectories	• Repeated measures generalized linear mixed model (GLMM)
Adult body size	• Principal component analysis (PCA); General linear model (GLM)

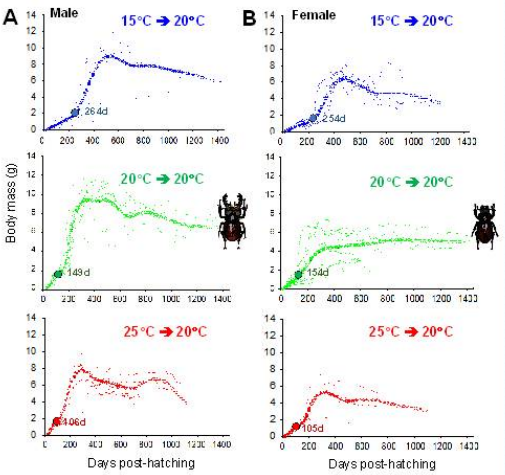
Results

Survival



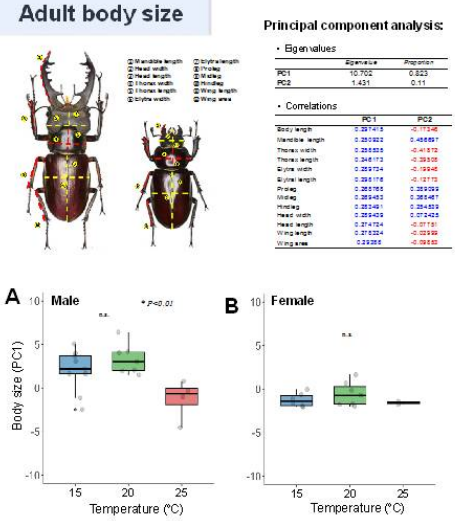
- Exposure to high temperatures during early instars (1st and 2nd) increased mortality in the 3rd instar, even after transfer to a common temperature (20°C). (Cox regression: *P<0.05*)
- Survival was highest in larvae exposed to 15°C (79%) during early development, lower at 20°C (45%), and lowest at 25°C (36%).
- Early heat stress had lasting negative effect on stag beetle's survival during development.

Growth trajectories



- Early temperature significantly affected the pattern of growth trajectories (GLMM: *P<0.01*)
- In both sexes, the maximum larval mass attained during the 3rd instar was reduced in larvae exposed to 25°C during early development (GLM: *P=0.03*).

Adult body size



- PCA results showed that PC1 represented overall adult body size.
- In males, adult body size was reduced by early exposure to 25°C. However, no such effect was observed in females.

Summary & Implications

- Exposure to high temperatures during early development reduced survival, growth, and adult body size, even after individuals were transferred to normal temperatures. This finding indicates that early heat stress can have lasting effects across the entire life cycle of this stag beetle.
- Males were more vulnerable to early heat stress than females with respect to body size, implying that early temperature may alter male dominance and the outcomes of sexual selection.
- Our results provide important insights for predicting how extreme thermal events (e.g., heat waves) will affect organismal performance, population dynamics, and the biogeograpahic distribution of cold-adapted insects, such as *L. maculifemoratus dybowskyi.*

Acknowledgements

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