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Digital supplementary material

Digital supplementary material to

WILLOT, Q., ØRSTED, M., DAMSGAARD, C. & OVERGAARD, J. 2022: Thermal-death-time model as a tool to analyze heat tolerance, acclimation, and biogeography in ants. – Myrmecological News 32: 127-138.

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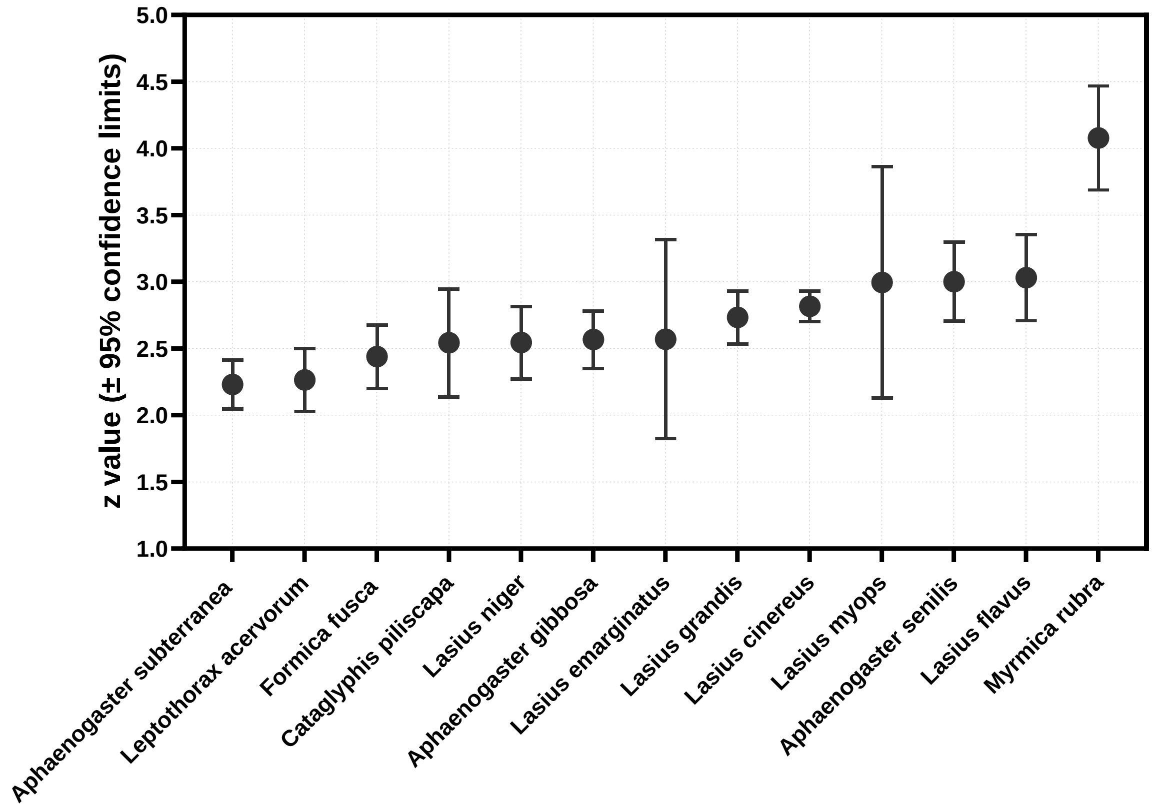


**Figure S1.** Species-specific z values (defined as -1/slope of the of species-specific TDT curves, Fig. 2, Tab. 1) with ± 95% confidence limits. Z values ranged from 2.23 (*Aphaenogaster subterranea*) to 4.08 (*Myrmica rubra*, Tab. 1). Lower values indicative of greater thermal sensitivity (*i.e.,* an increased change in knockdown proportion with a change in exposure temperature).

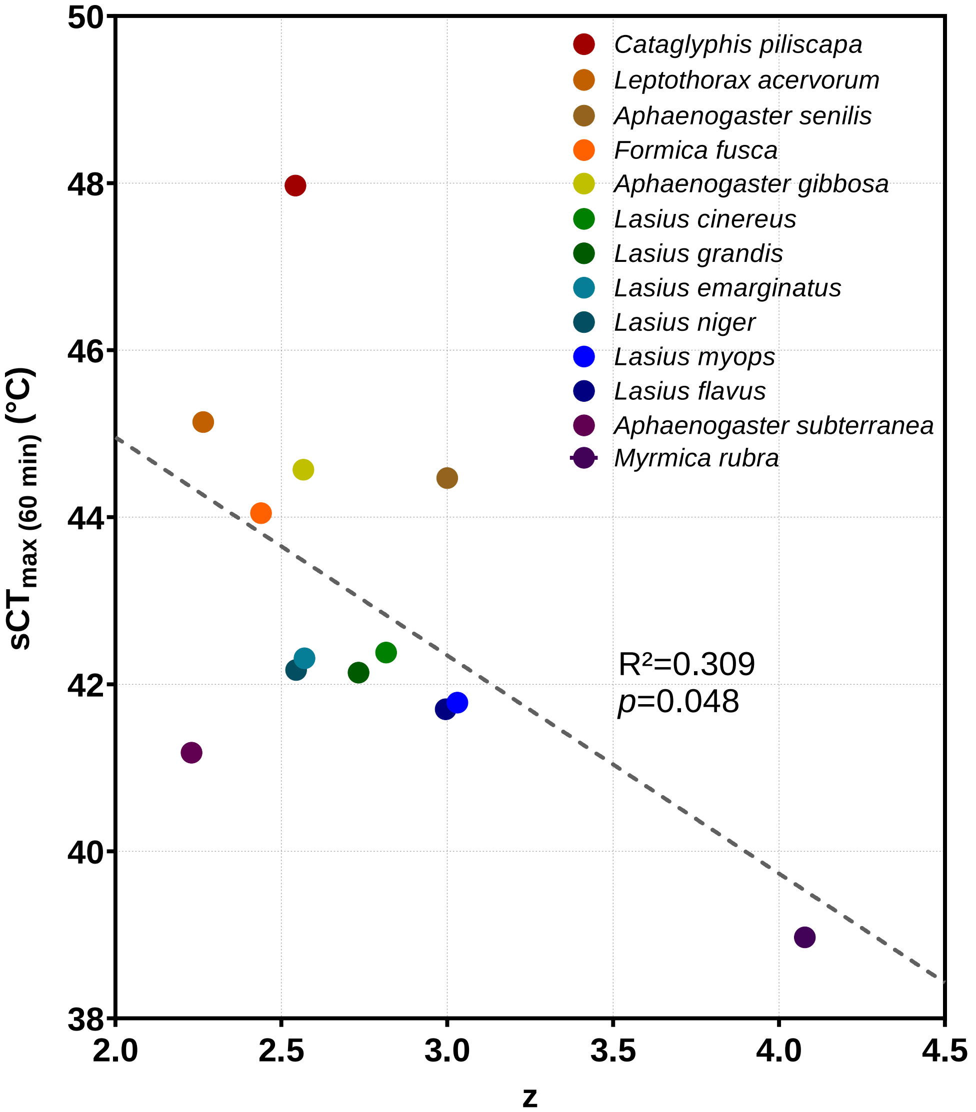


**Figure S2.** Correlation between the coefficient of thermal sensitivity z (defined as -1/slope of the of species-specific TDT curves, Fig. 2, Tab. 1) and $sCT_{max\ (10\ min)}$ across species. $sCT_{max}$ was overall a poor predictor of thermal sensitivity ($R^2$=0,309, *p*=0,048). This indicates only little to moderate negative relationship between basal heat-tolerance and the ability to better tolerate further additional augmentation of temperature. Species are color coded from higher (red) to lower (dark purple) heat-tolerance.

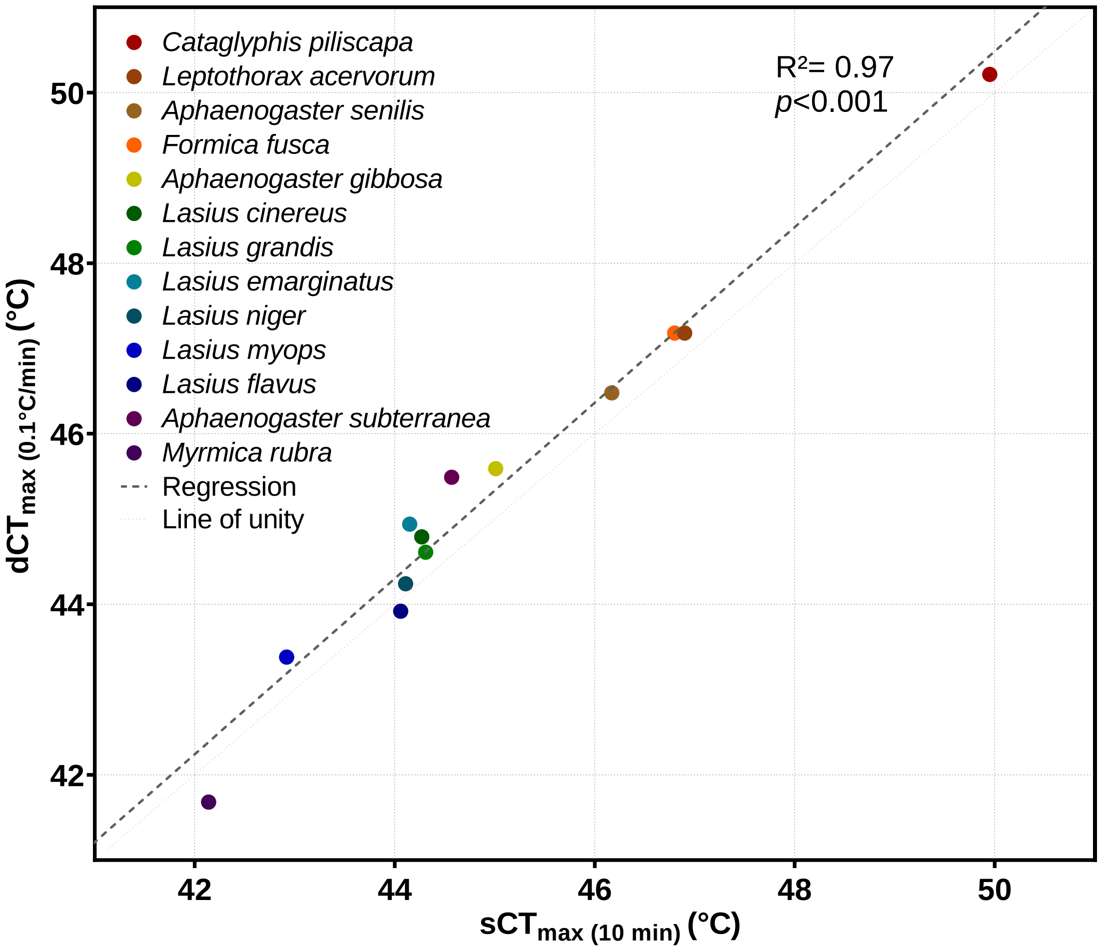


**Figure S3.** $dCT_{max}$ values at a ramping rate of 0.1°C/min plotted against sCTmax values at 10 minutes derived from TDT curves parameters, for the 13 species included in our analysis (values are provided in Tab. S2). Temperatures inducing knockdowns after 10min in workers were close to the temperatures causing knockdowns in ramping assays at 0.1°C/min (within 0.25°C on average of the line of unity), which provide an opportunity for a quick comparison of results between the two types of assays. Species are color coded from higher (red) to lower (dark purple) heat-tolerance.

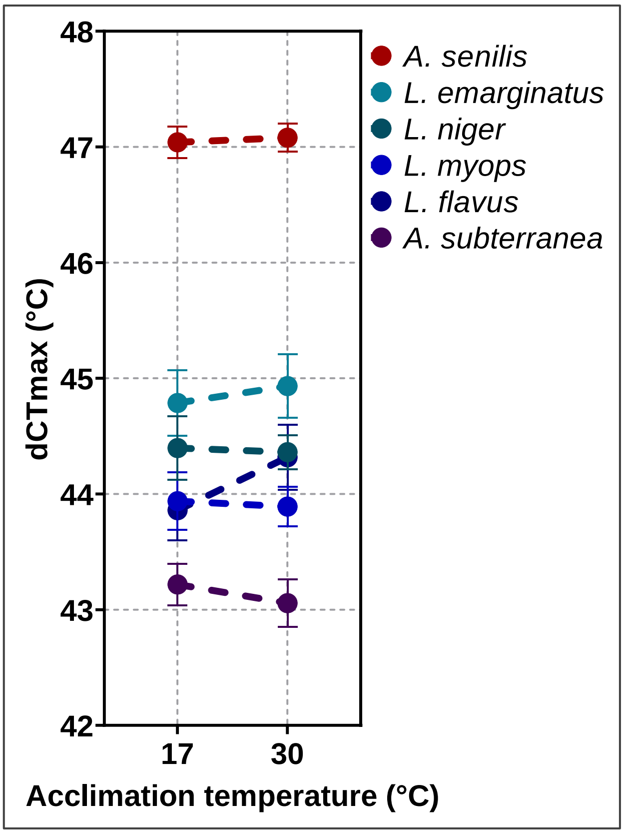


**Figure S4.** Impact of acclimation temperature (17 and 30°C) on $dCT_{max}$ for six species of ants. $dCT_{max}$ was plotted against acclimation temperature. There was a minor but significant interaction between species and acclimation treatment, although acclimation alone did not have a statistically significant effect on $dCT_{max}$ (Tab. S4).

| Species | $dCT_{max}$ parameters (°C/min) | | |
|---|---|---|---|
| | Ramping rate (°C/min) | Observed | Predicted from TDT curves |
| *Cataglyphis piliscapa* | 0.1 | 50.2 | 49.7 |
| | 0.25 | 51.3 | 50.7 |
| *Leptothorax acervorum* | 0.1 | 47.2 | 46.9 |
| | 0.25 | 47.8 | 47.8 |
| *Aphaenogaster senilis* | 0.1 | 47.2 | 46.5 |
| | 0.25 | 48.5 | 47.7 |
| *Formica fusca* | 0.1 | 46.5 | 46.0 |
| | 0.25 | 47.3 | 47.0 |
| *Aphaenogaster gibbosa* | 0.1 | 45.6 | 44.8 |
| | 0.25 | 46.6 | 45.9 |
| *Lasius cinereus* | 0.1 | 45.5 | 44.3 |
| | 0.25 | 46.5 | 45.4 |
| *Lasius grandis* | 0.1 | 44.8 | 44.0 |
| | 0.25 | 45.7 | 45.1 |
| *Lasius emarginatus* | 0.1 | 44.6 | 44.0 |
| | 0.25 | 45.5 | 45.0 |
| *Lasius niger* | 0.1 | 45.0 | 43.9 |
| | 0.25 | 45.9 | 45.0 |
| *Lasius myops* | 0.1 | 44.2 | 43.2 |
| | 0.25 | 45.0 | 44.4 |
| *Lasius flavus* | 0.1 | 44.0 | 43.8 |
| | 0.25 | 45,0 | 45.0 |
| *Aphaenogaster subterranea* | 0.1 | 43.4 | 43.0 |
| | 0.25 | 44.6 | 43.9 |
| *Myrmica rubra* | 0.1 | 41.7 | 41.3 |
| | 0.25 | 43.9 | 42.9 |

| | Equation | Z | $R^2$ | *p* value |
|---|---|---|---|---|
| Global dataset | y=1.008*x - 1.021 | -0.991 | 0.978 | $<1*10^{-4}$ |

**Table S1.** Experimental $dCT_{max}$ values compared to predicted $dCT_{max}$ values derived from TDT curve parameters at two ramping rates (0.1 and 0.25°C/min).

| Species | $sCT_{max\ (10\ min)}$(°C) | $dCT_{max\ (0.1°C\ /min)}$ (°C) |
|---|---|---|
| *Cataglyphis piliscapa* | 50.0 | 50.2 |
| *Leptothorax acervorum* | 47.0 | 47.2 |
| *Aphaenogaster senilis* | 46.8 | 47.2 |
| *Formica fusca* | 46.2 | 46.5 |
| *Aphaenogaster gibbosa* | 45.0 | 45.6 |
| *Lasius cinereus* | 44.6 | 45.5 |
| *Lasius grandis* | 44.3 | 44.8 |
| *Lasius emarginatus* | 44.3 | 44.6 |
| *Lasius niger* | 44.2 | 44.9 |
| *Lasius myops* | 44.1 | 44.2 |
| *Lasius flavus* | 44.1 | 43.9 |
| *Aphaenogaster subterranea* | 43.0 | 43.4 |
| *Myrmica rubra* | 42.1 | 41.7 |

**Table S2.** Experimental $sCT_{max(0.1°C/min)}$ and $dCT_{max\ (10\ min)}$ values for the 13 species included in our model system. These values correspond to the constant temperature causing 50% of knockdown in workers after 10 minutes exposure derived from TDT curve parameters (Fig. 2), and the average temperature at which workers stop exhibiting movement when exposed to a ramping temperature of 0.1°C /min (N=10). $sCT_{max(0.1°C/min)}$ and $dCT_{max\ (10\ min)}$ showed good correlation ($R^2$ = 0.97), close to the line of unity (Fig. S3) and allowed for a consistent ranking of species according to their respective heat-tolerance.

| Species | Acclimation (°C) | Regression parameters | | | | GLM | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Experimental temperature | | Acclimation | | Experimental temperature x acclimation | |
| | | Equation | Z | $R^2$ | *p* value | t-value | *p value* | t-value | *p value* | t-value | *p value* |
| *Aphaenogaster senilis* | 17 | y= -0.3575*x + 17.63 | 2.797 | 0.968 | $4*10^{-4}$ | | | | | | |
| | 26 | y= -0.3333*x + 16.60 | 3.000 | 0.995 | $<1*10^{-4}$ | -7.157 | $4.8*10^{-6}$ | -0.513 | 0.61 | -0.579 | 0.57 |
| | 30 | y= -0.3456*x + 17.18 | 2.893 | 0.995 | $<1*10^{-4}$ | | | | | | |
| *Aphaenogaster subterranea* | 17 | y= -0.4240*x + 19.39 | 2.358 | 0.984 | $<1*10^{-4}$ | | | | | | |
| | 26 | y= -0.4444*x + 20.12 | 2.250 | 0.993 | $<1*10^{-4}$ | -7.744 | $2.0*10^{-6}$ | 0.105 | 0.92 | -0.232 | 0.82 |
| | 30 | y= -0.4259*x + 19.33 | 2.348 | 0.992 | $<1*10^{-4}$ | | | | | | |
| *Lasius emarginatus* | 17 | y= -0.4376*x + 20.15 | 2.285 | 0.983 | $1*10^{-4}$ | | | | | | |
| | 26 | y= -0.5089*x + 23.14 | 1.965 | 0.927 | $2*10^{-3}$ | -3.355 | $4.7*10^{-3}$ | -0.356 | 0.73 | 0.388 | 0.703 |
| | 30 | y= -0.3804*x + 17.87 | 2.629 | 0.939 | $1.4*10^{-3}$ | | | | | | |
| *Lasius niger* | 17 | y= -0.4145*x + 19.48 | 2.412 | 0.984 | $<1*10^{-4}$ | | | | | | |
| | 26 | y= -0.4410*x + 20.49 | 2.267 | 0.915 | $2.8*10^{-3}$ | -3.034 | $8.9*10^{-3}$ | 0.285 | 0.78 | -0.324 | 0.75 |
| | 30 | y= -0.4328*x + 20.17 | 2.310 | 0.947 | $1.1*10^{-3}$ | | | | | | |
| *Lasius myops* | 17 | y= -0.3991*x + 18.54 | 2.506 | 0.993 | $<1*10^{-4}$ | | | | | | |
| | 26 | y= -0.3338*x + 15.72 | 2.996 | 0.958 | $7*10^{-4}$ | -5.747 | $5.05*10^{-5}$ | -1.042 | 0.31 | 1.073 | 0.30 |
| | 30 | y= -0.3658*x + 17.21 | 2.734 | 0.983 | $1*10^{-4}$ | | | | | | |
| *Lasius flavus* | 17 | y= -0.3544*x + 16.59 | 2.822 | 0.975 | $2*10^{-4}$ | | | | | | |
| | 26 | y= -0.3299*x + 15.54 | 3.031 | 0.994 | $<1*10^{-4}$ | -5.929 | $3.68*10^{-5}$ | -0.101 | 0.92 | 0.128 | 0.90 |
| | 30 | y= -0.3567*x + 16.73 | 2.803 | 0.981 | $1*10^{-4}$ | | | | | | |

**Table S3.** Coefficient of determination ($R^2$) and their linked p-values for TDT curves of species acclimated at either 17, 26 or 30°C (Fig. 4). TDT curves of all acclimation groups were characterized by high coefficients of determination ($R^2$=0.915 to 0.995 across the dataset, Tab. S2). Overall, we detected no effect of acclimation on time to knockdown in the species tested (General Linear Model testing for the impact of experimental temperature, acclimation, and the interaction between experimental temperature and acclimation on time to knockdown, Fig. 4).

| Factor | % of total variation | *p* value | Significance |
|---|---|---|---|
| Species | 96.43 | $<1*10^{-4}$ | **** |
| Acclimation | 0.296 | 0.104 | n.s |
| Species*acclimation | 0.6279 | $6*10^{-4}$ | *** |

**Table S4.** Impact of acclimation temperature (17 and 30°C) on $dCT_{max}$ for six species of ants (Fig. S4). There was a significant interaction between species and acclimation, indicating that there were minor differences in acclimation responses between species. Overall, acclimation alone did not have a statistically significant effect on $dCT_{max}$ (2-way ANNOVA with species and acclimation as factors).