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Authors' contributions

Yuan-Jie Li: Investigation; Formal analysis; Writing – original draft; Writing – review & editing, **Si-Yang Chen:** Investigation, **Lisa Bjerregaard Jørgensen:** Formal analysis; Writing – review & editing; **Johannes Overgaard:** Formal analysis; Writing – review & editing, **David Renault:** Funding acquisition; Supervision; Writing – review & editing; **Hervé Colinet:** Conceptualization; Funding acquisition; Supervision; Writing – review & editing, **Chun-Sen Ma:** Conceptualization; Formal analysis; Funding acquisition; Supervision; Writing – original draft; Writing – review & editing

1 Interspecific differences in thermal tolerance landscape explain aphid community 2 abundance under climate change

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14 Abstract

15 A single critical thermal limit is often used to explain and infer the impact of climate change on
16 geographic range and population abundance. However, it has limited application in describing the
17 temporal dynamic and cumulative impacts of extreme temperatures. Here, we used a thermal
18 tolerance landscape approach to address the impacts of extreme thermal events on the survival
19 of co-existing aphid species (*Metopolophium dirhodum*, *Sitobion avenae* and *Rhopalosiphum*
20 *padi*). Specifically, we built the thermal death time (TDT) models based on detailed survival
21 datasets of three aphid species with three ages across a broad range of stressful high (34~40°C)
22 and low (-3~-11°C) temperatures to compare the interspecific and developmental stage variations
23 in thermal tolerance. Using these TDT parameters, we performed a thermal risk assessment by
24 calculating the potential daily thermal injury accumulation associated with the regional
25 temperature variations in three wheat-growing sites along a latitude gradient. Results showed that
26 *M. dirhodum* was the most vulnerable to heat but more tolerant to low temperatures than *R. padi*
27 and *S. avenae*. *R. padi* survived better at high temperatures than *Sitobion avenae* and *M.*
28 *dirhodum* but was sensitive to cold. *R. padi* was estimated to accumulate higher cold injury than
29 the other two species during winter, while *M. dirhodum* accrued more heat injury during summer.
30 The warmer site had higher risks of heat injury and the cooler site had higher risks of cold injury
31 along a latitude gradient. These results support recent field observations that the proportion of *R.*
32 *padi* increases with the increased frequency of heat waves. We also found that young nymphs
33 generally had a lower thermal tolerance than old nymphs or adults. Our results provide a useful

34 dataset and method for modelling and predicting the consequence of climate change on the
35 population dynamics and community structure of small insects.

36 **Keywords:** Extreme temperature, Thermal death time curve, Cereal aphids, *Sitobion avenae*,
37 *Rhopalosiphum padi*, *Metopolophium dirhodum*

38 Introduction

39 Climate change leads to more frequent extreme temperature events (Perkins-Kirkpatrick and
40 Lewis 2020, Arias et al. 2021), which trigger consequences from individual physiology to
41 ecosystem functions (Ma et al. 2021; Secretariat et al. 2021). Insects' body temperature is mainly
42 determined by the microclimates of their habitats, making them highly sensitive to temperature
43 changes (Pincebourde and Woods 2020). Exposure to a short-term extreme temperature that
44 reaches or exceeds critical thermal limits can cause an immediate effect on fecundity, fertility and
45 even mortality (Zhang et al. 2013; Liang et al. 2014; Sales et al. 2021). Therefore, thermal
46 tolerance is often correlated with population abundance, species distribution, and phenology, and
47 thus plays an important role in predicting the effect of climate change (Sunday et al. 2012;
48 Amundrud and Srivastava 2020; Alruiz et al. 2022).

49 A single temperature estimated from static or dynamic assays has been widely adopted as an
50 upper or lower thermal tolerance limit (Winther Bak et al. 2020). However, the critical thermal limit
51 depends on the methodological context including the exposure time (Rezende et al. 2014;
52 Terblanche et al. 2007). Moreover, this limit, which represents a static snapshot of thermal
53 tolerance, is not able to describe the temporal dynamic and cumulative impact of environmental
54 extreme temperatures on survival (Alruiz et al. 2022).

55 Thermal Death Time (TDT) curve, which describes that the tolerable duration at stressful
56 temperatures exponentially decreases with increasing stress intensity, can fully and accurately
57 characterize species' thermal tolerance (Bigelow 1921; Fry et al. 1946; Nedvěd et al. 1998;
58 Colinet et al. 2011; Rezende et al. 2014; Jørgensen et al. 2019; Tarapacki et al. 2021). The TDT
59 framework has been recently re-examined and parameterized to enable the analysis of the
60 combined impact of extreme temperatures and exposure times on survival, which is now referred
61 to as the "thermal tolerance landscape". (Rezende et al. 2014; Jørgensen et al. 2019; Jørgensen
62 et al. 2021; Ørsted et al. 2022). Furthermore, the injury accumulation rate increases exponentially
63 with temperature and the injury sustained at different static temperatures can be completely
64 additive (Jørgensen et al. 2021; Ørsted et al. 2022). Thus, through the TDT model, we can not
65 only get a holistic view of how thermal tolerance duration varies with temperatures (Tarapacki et
66 al. 2021; Willot et al. 2022), but also link the thermal tolerance to the injury accrued under natural

67 temperatures. However, few studies employ this approach to extrapolate the thermal tolerance
68 estimated in the laboratory to natural settings.

69 The same dose of exposure to thermal extremes might lead to different reactions among co-
70 existing species (Ma et al. 2015a; Ma et al. 2015b) because some species can be more stress-
71 resilient than others (Kaspari et al. 2015; Buckley and Huey 2016). The interspecific differences
72 in thermal tolerance can shift the community structure through cascading effects (Harvey et al.
73 2020; Filazzola et al. 2021), altering the relative abundance of coexisting insect species (Ma et al.
74 2015b; Birkett et al. 2018; Zhu et al. 2021). This is particularly important for practice in agricultural
75 pest management (Mohammed et al. 2019). In addition, the natural population of insects
76 commonly consists of multiple life stages or age cohorts that have different thermal tolerance
77 (Bowler and Terblanche 2008; Zhang et al. 2015; Zhao et al. 2019), even the stages living in the
78 same microhabitat also show different heat tolerance (Zhao et al. 2017). When the mixed-stage
79 population experiences extreme temperature events, the most sensitive stages are likely to be
80 injured and die, whereas the most tolerant may survive and develop to the adult stage for
81 reproduction during the following mild phases, thereby contributing to population recovery (Ma et
82 al. 2021). Therefore, clarifying the thermal tolerance in different developmental stages is essential
83 for understanding the ecological consequences of extreme temperature events.

84 Here we used three species of co-existing global pests (family Aphididae) in cereal crops, *Sitobion*
85 *avenae* (Fabricius, 1775), *Rhopalosiphum padi* (Linnaeus, 1758), and *Metopolophium dirhodum*
86 (Walker, 1849) as focus species. We aimed to answer the following questions: 1) can the change
87 in aphid community abundance be related to interspecific differences in the basal thermotolerance
88 of the three aphid species? 2) do older aphids suffer less than younger ones from heat/cold stress?
89 To answer these questions, we conducted an elaborate study to characterize the interspecific
90 variation in thermal tolerance among the three cereal aphid species using the TDT framework at
91 both high and low temperatures. Then, we examined the effect of developmental stage/age on
92 the intraspecific variations in thermal tolerance. Finally, we used the TDT model parameters to
93 perform a risk assessment predicting the potential injuries under the natural environments for
94 three aphid species in three sites along a latitude gradient in China.

95 **2. Material and methods**

96 **2.1 Aphid stock rearing**

97 Populations of three common aphid species developing on winter wheat, *S. avenae*, *M. dirhodum*
98 and *R. padi* were originally collected in May 2010 from wheat fields near Beijing (39°48 N, 116°28
99 E) (Zhao et al. 2014). The colonies were then refreshed annually with field-collected aphids. The

100 aphids were reared in screened cages (60 × 60 × 60 cm) at 22 ± 0.5 °C, RH 70%, and a
101 photoperiod of 16 L: 8 D. Aphids were fed on 10 –20 cm tall winter wheat seedlings that were
102 renewed weekly. Aphids used for experiments were prepared by placing newly molted adult
103 aphids in a pot with wheat seedlings at 20 °C. After 24 hours, the apterous adult aphids were
104 removed so that only newly-born nymphs remained. Subsequently, these nymphs were allowed
105 to develop for 2, 6, 12 days to generate three different stages: 2nd, 3rd or 4th instar (depending on
106 species) and adult, respectively.

107 **2.2 Tests of heat and cold survival**

108 For heat tolerance tests, a multifactorial experiment involved the three aphid species × three ages
109 (2, 6, 12 days) × seven temperature regimes (34, 35, 36, 37, 38, 39, 40°C) × \geq five exposure
110 times (ranging from 0 to 2000 min depending on the temperature) was conducted. Similarly, a
111 multifactorial experiment for cold tolerance included the three aphid species × three ages (2, 6,
112 12 days) × nine temperature regimes (-3, -4, -5, -6, -7, -8, -9, -10, -11°C) × \geq five exposure times
113 (ranging from 0 to 10162 min depending on the temperature) was conducted. All the treatments
114 were replicated at least 3 times, and for each replicate, 7-12 aphids were tested (Table S1). To
115 obtain a reliable survival curve at each temperature, we tried to find and test the exposure times
116 that result in survival rates close to 0%, 25%, 50%, 75%, and 100%, respectively. To reduce
117 handling stress and provide food as well as a water source, we cut off the wheat leaves with
118 aphids and then transferred them directly into glass vials (10 mL). Then, we closed each vial with
119 a dry sponge and placed a rack with vials into a glycol bath (Ministat 230-cc-NR; Huber Ltd.,
120 Germany, accuracy ± 0.01 °C) pre-set at the experimental temperature. For each temperature
121 and exposure time, the exposed aphids were removed from the bath and allowed to recover at
122 20 °C for 24h before checking the survival of each individual. Aphids were considered alive if the
123 individual moved following a gentle tactile stimulation with a brush. In total, 14980 and 14633
124 individuals were tested, for heat and cold tolerance, respectively, corresponding to 1502 and 1536
125 replicates.

126 **2.3 Survival curves, Lt_{50} , and Thermal Death Time (TDT) curves**

127 For each tested temperature, the survival proportion was fitted to a logistic regression model using
128 the *glm* function and the Lt_{50} (i.e. the temperature-specific exposure duration resulting in 50%
129 mortality) was estimated using the *dose.p* function in the R package "MASS" (Ripley et al. 2013).
130 TDT curves were generated by regressing $\log_{10}(Lt_{50})$ against test temperature for each
131 species/age-group combination, at both high and low temperatures. The output of the TDT

132 analysis is typically given by model estimates of temperature causing 50% mortality after a
 133 specific time (e.g., the temperature that causes 50% mortality after 1 hour), as well as the thermal
 134 sensitivity, which is often expressed from z ($-1/\text{slope}$). The latter parameter reveals how many
 135 degrees are required to change tolerance duration 10-fold (Rezende et al. 2014). Note that for
 136 cold tolerance, thermal sensitivity is expressed by z' which corresponds to $1/\text{slope}$.

137 **2.4 Examining effects of species and age**

138 The effect of test temperature, species, and age on Lt_{50} was examined in a three-way ANCOVA
 139 including all interaction terms, with test temperature as a continuous variable and species and
 140 age as categorical variables. Interspecific differences in heat or cold tolerance were examined
 141 independently for each age group by comparing differences in the parameters of the TDT curves.
 142 Specifically, slopes of the TDT curves (thermal sensitivity) were first compared pairwise using
 143 the functions *emtrends()* and *pairs()* in the R package “emmeans” (Lenth, 2019), which are based
 144 on least-squares means. Further, we tested whether the intercepts (Log-transformed Lt_{50} estimate)
 145 differed between species by comparing the intercept at 37 °C for heat tolerance and -7 °C for cold
 146 tolerance using the function *emmeans()* in “emmeans” package (Lenth, 2019). These
 147 temperatures were chosen as they represented intermediate values in the range of temperatures
 148 used to assess heat and cold tolerance, respectively. A similar approach, comparing slopes and
 149 intercepts, was used to examine the effects of aphid age on both heat and cold tolerance for each
 150 species. All data analyses were performed in R version 4.1.2.

151 **2.5 Relating TDT curves to natural temperature regimes**

152 Following the method described by Jørgensen et al. 2021, we used parameters of TDT curves
 153 (slope and intercept) to estimate the cumulative daily thermal injury experienced by aphids during
 154 natural thermal fluctuations. This basic risk analysis has of course some limits, for instance, it
 155 ignores the importance of microhabitat choice and behavioral thermoregulation by using the
 156 simple assumption that aphid body temperature is equal to air temperature. Further, this analysis
 157 does not consider if cold tolerance changes seasonally due to acclimatization to winter or summer
 158 conditions. Despite these caveats, this simple approach can provide a raw proportional risk
 159 assessment to compare species or regions based on both the intensity and duration of thermal
 160 extremes. Specifically, it calculates, minute by minute, the accumulation of thermal stress over 24
 161 hours (1440 min) using the formula:

$$162 \quad \text{Accumulated injury} = \sum_{i=1}^{i=1440} \frac{100 \cdot (t_{i+1} - t_i)}{10^{(\beta \cdot \max(T_i; T_{i+1}) + \alpha)}}$$

163 where α is the slope and β is the intercept of the TDT curve, t_i and t_{i+1} , are the i minute and the
 164 $i+1$ minute, respectively, $\max(T_i; T_{i+1})$ is the maximum temperature between the i and $i+1$ minute;
 165 for winter, it is the $\min(T_i; T_{i+1})$, the minimum temperature between the i and $i+1$ minute. Note that
 166 thermal injuries only accumulate in the stressful temperature range (above a critical temperature
 167 T_c for heat stress or below a critical temperature T_c' for cold stress) (Jørgensen et al. 2021, Ørsted
 168 2022). We defined T_c or T_c' as the temperature estimated to cause 50% mortality (Lt_{50}) in one
 169 week. Specifically, we calculated this T_c or T_c' from the TDT curves. For 6-day-old *M. dirhodum*,
 170 *R. padi* and *S. avenae*, T_c was estimated as 25.29°C, 29.34 °C and 28.82 °C, respectively and
 171 cold T_c' was -5.15°C, -1.81 °C and -4.79°C, respectively.

172 To associate thermal tolerance measures to natural climate conditions, we downloaded hourly
 173 temperature data for winter (from November to February, 2016/2017) and summer (from May to
 174 August, 2016) months from three sites in China where grow winter wheat infested by cereal aphids:
 175 southern site (Wuhan, N 30.78°, E 114.21°), intermediate site (Xinxiang, N 35.30°, E 113.92°) and
 176 northern site (Beijing, N 40.07°, E 116.58°) (Figure 4A). Data were downloaded from a global
 177 weather API (<https://www.visualcrossing.com/weather-data>) in May 2022. We calculated minutely
 178 temperature by linear interpolation from the hourly temperature using the `na.approx` function in
 179 the “zoo” package (Zeileis et al. 2014). The accumulated daily heat stress dose was calculated
 180 from midnight to midnight and the accumulated daily cold stress dose from noon to noon. In total,
 181 this analysis produced 122 estimates of daily heat stress doses and 120 estimates of daily cold
 182 stress doses for each species and region.

183 We used Kruskal-Wallis Rank Sum Tests followed by Pairwise Wilcoxon Rank Sum Tests with a
 184 Benjamini-Hochberg correction to compare the differences in the calculated daily accumulated
 185 injury between species within sites and sites with the same species. Note that to avoid comparing
 186 a lot of days without any injury accumulation, only days with injury accumulation were compared.

187 3. Results

188 3.1 Survival curves, TDT curves: estimation of critical thermal limits

189 The survival curves generally fitted the logistic function well, with a median R^2 of 0.81 (Fig. 1A
 190 and B, 74% of assays had McFadden’s pseudo- $R^2 > 0.70$, See Table S2). For both heat and cold
 191 tolerance, the estimated Lt_{50} decreased exponentially with increasing stress intensity
 192 (temperature) as predicted by the TDT curves (Figure 1C and D). Linear regressions of $\log_{10}(Lt_{50})$
 193 against temperature generated nine TDT curves (three species \times three ages) for high and low
 194 temperatures respectively. All curves had a high determination coefficient (R^2) ranging from 0.78
 195 to 0.99 ($P < 0.01$), especially for those at high temperatures (see Table 2 and Table 3).

196 Here, we used the temperature causing 50% mortality after 1 hour ($CT_{\max(1h)} / CT_{\min(1h)}$) as the
197 upper or lower critical thermal limit. *R. padi* ranked first in $CT_{\max(1h)}$ (36.76°C ~ 37.29°C in different
198 ages), 0.97~1.94°C higher than *M. dirhodum* (Table 1). The thermal sensitivity at high
199 temperatures ranged from 3.19 to 4.17 (Table 1). $CT_{\min(1h)}$ fell between -8.24 ~ -2.84 °C (Table 2).
200 Aphids had high cold sensitivity as the lower z' values indicated greater thermal sensitivity (i.e., a
201 larger change in Lt_{50} with a change in exposure temperature).

202 **3.2 Interspecific variation in thermal tolerance**

203 The effects of temperature, species, and age on Lt_{50} were examined in a three-way ANCOVA
204 including all interaction terms. This analysis found that species interacted with both age and
205 temperature at both high and low temperatures (Table S3 and S4). Accordingly, we split the
206 dataset according to age so that TDT slopes and intercepts from different species could be
207 compared. *R. padi* generally had the highest heat tolerance, as depicted by the significantly higher
208 $\log_{10}(Lt_{50})$ value (1.71~1.86) at 37 °C for all age groups, followed by *S. avenea*, and then *M.*
209 *dirhodum* (Table 1, see p-values in Table S5). The thermal sensitivity (z) showed few interspecific
210 differences; *M. dirhodum* was characterized by a lower thermal sensitivity (higher z) than *R. padi*
211 and *S. avenea* (Table 1, See p-values in Table S6).

212 For cold tolerance, *R. padi* showed a significantly lower $\log_{10}(Lt_{50})$ at -7°C for all age groups than
213 the other two species (Table 2, see p-values in Table S7). The difference between *M. dirhodum*
214 and *S. avenae* was relatively small. The thermal sensitivity (z') did not differ significantly among
215 species, except for 12-day adults of *R. padi* that had a statistically higher z' (lower thermal
216 sensitivity) than *M. dirhodum*. (Table 2, See p-values in Table S8).

217 **3.3 Developmental stage variation in thermal tolerance**

218 Adults (12-d-old) or old nymphs (6-d-old) generally had higher thermal tolerance than young
219 nymphs(2-d-old). For *M. dirhodum* and *S. avenea*, adults (12-d-old) had a lower z -value (higher
220 thermal sensitivity) and longer $\log_{10}(Lt_{50})$ at 37 °C than younger life stages, although this was only
221 statistically significant for *M. dirhodum* (Table 1). For *R. padi*, old nymphs (6-d-old) were the most
222 tolerant among all life stages (Table 1).

223 For cold tolerance, none of the species displayed a significant difference in thermal sensitivity (z')
224 between ages. Adult (12-d-old) *R. padi* showed a significantly lower $\log_{10}(Lt_{50})$ at -7 °C than
225 nymphs (Table 2, see p-values in Table S7).

226 **3.4 Relating TDT curves to natural temperature regimes**

227 To associate thermal tolerance measures with natural climate conditions, we calculated the
 228 accumulated thermal injury of three aphid species in three age groups using the TDT model and
 229 regional temperatures. Results were similar for all three age groups (2-d-old, 6-d-old, 12-d-old,
 230 see Figure S3-6), thus here we only present the result of 6-day-old aphids (Figure 2-4).

231 In each site, the estimated heat injury differed among species ($\chi^2 = 53.3$, $df = 2$; $p < 1*10^{-11}$ in
 232 Beijing; $\chi^2 = 36.0$, $df = 2$; $p < 1*10^{-7}$ in Xinxiang; $\chi^2 = 9.0$, $df = 2$; $p < 0.05$ in Wuhan, Figure 2B). *M.*
 233 *dirhodum* always accumulated more heat injury than the two other species in Beijing and Xinxiang
 234 (Pairwise Wilcoxon Rank Sum Tests, $p < 0.05$).

235 According to the calculation, *R. padi* might suffer cold injury across the whole winter months while
 236 the two other species might be affected only during the coldest days (Figure 3A). Estimated
 237 accumulated cold injury differed among species in Beijing ($\chi^2 = 8.5$, $df = 2$; $p < 0.05$; Figure 3B).
 238 Specifically, *R. padi* had higher daily injury accumulation than *S. avenae* in Beijing (Pairwise
 239 Wilcoxon Rank Sum Tests, $p < 0.05$), while no significant interspecific difference was found in
 240 Xinxiang. *M. dirhodum* and *S. avenae* might not accumulate any injury in Wuhan during winter
 241 (Figure 3B).

242 According to the calculation, heat injury of three species might differ with sites ($\chi^2 = 6.4$, $df = 2$; p
 243 < 0.05 for *M. dirhodum*; $\chi^2 = 9.9$, $df = 2$; $p < 0.01$ for *S. avenae*; $\chi^2 = 10.6$, $df = 2$; $p < 0.01$ for *R.*
 244 *padi*, Figure 4C). In Beijing, the northernmost site, aphids accumulated less injury during summer
 245 (Pairwise Wilcoxon Rank Sum Tests, $p < 0.05$, Figure 4C) but more injury during winter than the
 246 other two sites (Pairwise Wilcoxon Rank Sum Tests, $p < 0.05$, Figure 4D).

247 **Discussion**

248 The previous studies often inferred the impact of climate change on the coexisting species based
 249 on a single estimate of thermal tolerance, such as CT_{max} and CT_{min} , or LT_{50} and Lt_{50} (Warren and
 250 Chick 2013; Birkett et al. 2018). High-tolerant species will increase their population or expand
 251 their range, while low-tolerant species will decrease their population or shrink their range under
 252 extreme climate scenarios. However, thermal tolerance is not constant but varies with the dose
 253 determined by temperature and exposure duration as shown in our study. In nature, extreme high
 254 and low temperatures occur regularly in a diurnal and seasonal cycle, and irregularly across time
 255 and space, thereby altering the mortality of insects both dynamically and cumulatively. The
 256 thermal tolerance represented with a single temperature value can be hardly used to calculate
 257 the dynamic and cumulative thermal injury. Studies that assess demographic performances of co-
 258 existing species under limited temperature scenarios (e.g., Ma et al. 2015b) may not fully reflect

259 the abundance responses to arbitrary fluctuating temperatures in nature. Here we measured the
260 mortality under a broad range of extreme high/low temperatures and multiple exposure durations,
261 thus providing a solid dataset to build the TDT model or to do other analyses for the impact of
262 extreme climate on cereal aphid species. Through the group of survival curves and TDT model,
263 we can have a holistic and comprehensive view of the interspecific and ontogenetic variations in
264 thermal tolerance. Moreover, using the values of TDT parameters, we made a thermal stress risk
265 assessment of three aphid species in natural temperature regimes. Our data and approach
266 provide a framework for calculating the cumulative thermal mortality of aphids at different ages at
267 a given time (from one day to a year), thus could quantitatively estimate the relative abundance
268 of co-existing species.

269 ***TDT curves, interspecific and ontogenetic variations in thermal tolerance***

270 Here we aimed to examine if/how both thermal stress intensity and duration affected the survival
271 of three cereal aphid species. We found strong linear relationships between high/low
272 temperatures and log-transformed Lt_{50} across different ages of three aphid species as in other
273 ectotherms (Jørgensen et al. 2022). The steep slope (low z' value) of TDT curves from our data
274 at low temperatures is similar to the typical freezing time-temperature curve (Salt 1966), which
275 indicates that the cold tolerance we measured might reflect the susceptibility to freezing rather
276 than chill injury (Tarapacki et al., 2021).

277 It is intuitive to compare interspecific tolerance using a single value, such as CT_{max} , CT_{min} , LT_{50} ,
278 or Lt_{50} . However, we should realize that interspecific differences in thermal tolerance are not
279 constant but change with the thermal dose (intensity and exposure duration). The TDT curves of
280 three aphid species at high temperatures converge due to their different slopes (thermal sensitivity)
281 (Figure 1C), which means the Lt_{50} values of the three species are getting closer with increasing
282 intensity. Even, the interspecific differences in tolerate duration can reverse depending on the
283 temperature, for example, at temperatures below $-9^{\circ}C$, *S. avenae* could sustain a longer duration
284 than *M. dirhodum* (Figure 1D). However, our results generally showed that *R. padi* survived longer
285 at high but shorter at low temperatures, indicating it was the most heat-tolerant but cold-vulnerable
286 species. Whereas *M. dirhodum* performed as the most heat-vulnerable but cold-tolerant species.
287 These results are supported by the previous studies under lifetime constant temperature
288 exposures or the static thermal tolerance experiments (Asin and Pons 2001; Alford et al. 2014;
289 Alford et al. 2016).

290 We found that the effect of the developmental stage was similar at both high and low temperatures.
291 The old nymphs survived longer than young nymphs at either high or low temperatures in the

292 three aphid species. However, the thermal tolerance of adults was species-specific. For *S. avenae*
293 and *M. dirhodum*, adults (12-d-old) showed high tolerance, but the tested adults of *R. padi* were
294 extremely sensitive to cold stress. This pattern was also found by Alford et al 2014. Typically,
295 thermal tolerance is highest at eclosion and then declines during the early days of adult life
296 (Bowler and Terblanche 2008; Colinet et al. 2013). In our case, the development rate of *R. padi*
297 was faster than the other two species, thus 12-d-old *R. padi* was at the 3rd or 4th day of adult life
298 (but 1st or 2nd day for *S.avenae* and *M.dirhodum*). Therefore, the intolerant 12-d-old *R. padi* might
299 be resulting from a rapid decline of thermal tolerance in adult life, as observed in other insects
300 (Bowler 1967, Davison 1969, Colinet et al. 2013).

301 **Relating TDT curves to natural temperature regimes**

302 The injury risk analysis showed that *R. padi* was the least affected by high temperatures during
303 summer, which seems to fit the field observation showing that the relative abundance of *R. padi*
304 increased with extremely high-temperature frequency in south area (Ma et al. 2015b, Zhu et al.
305 2021). In Wuhan, *S. avenae* has the largest proportional abundance (78%) in 2008 when hot-day
306 (daily mean temperature ≥ 30 °C) frequency was low (Ma et al. 2015b). However, *R. padi*
307 became the most abundant species (79%) in the aphid community in 2011 when hot days
308 occurred more frequently (Ma et al. 2015b). In the middle region (near Xinxiang), the proportional
309 abundance of *R. padi* has increased from 13% in 1998 to 83% in 2012 (Zhu et al. 2021). While in
310 north sites (near Beijing), *S. avenae* was still the most abundant species which accounted for
311 89.8% in 2015 and 73.0% in 2016, followed by *R. padi* (9.26% and 14.3% in 2015 and 2016) and
312 *M. dirhodum* (0.01% and 11.81% in 2015 and 2016) (Yang et al. 2017). *R. padi* might become
313 the most abundant species, outcompeting *S. avenae* in a warmer future (Zhu et al. 2021).
314 Conversely, *M. dirhodum* is the least abundant species (Yang et al. 2017) owing to its lower
315 tolerance to high temperatures. It appears in the early spring and leaves the wheat field when the
316 temperature warms up (Chen et al. 1994).

317 It is also interesting to see the differences in the calculated accumulated injuries of aphids
318 between the three sites, which represent a climatic gradient from cold to hot climates. Aphids
319 accumulated more heat injuries in Wuhan, the southmost site, than Xingxiang and Beijing owing
320 to the longest duration of exposure to high temperatures during summer months (the durations \geq
321 30 °C in Wuhan, Xinxiang, and Beijing were 868h, 736h, and 611h, respectively, see supporting
322 information Table S9). To some extent, this result underlines the importance of exposure duration
323 to stressful temperatures when assessing the impact of thermal stress.

324 Winter temperature varied greatly in the three sites: Beijing, the northmost site, had a minimum
325 temperature of -13°C and an average daily temperature of 0.6°C whereas winter in Wuhan was
326 mild with an average daily temperature of 8.7°C (Table S9). Aphids could suffer from cold injury,
327 manyfold the dose resulting in 50% mortality in Beijing, which implied that the three species may
328 not be able to overwinter as nymphs in the winter wheat field here. Indeed, it had been reported
329 that the geographical overwinter limit for *S. avenae* and *R. padi* was the 0°C isotherm in January
330 ($\text{N } 35^{\circ}$, near Xinxiang) (Yang and Yang 1991, Luo et al. 1994). Instead, they might be able to
331 overwinter in Wuhan where they hardly get injured during winter and then migrate to the north
332 region at the start of spring (Li et al. 2014).

333 Over the past 5 decades (1961–2013), China's temperature increased by 1.44°C (90%
334 confidence interval $1.22\text{--}1.66^{\circ}\text{C}$) (Sun et al. 2016). There was a general increase in extreme
335 warm indices (such as warm days, warm nights, T_{max}) and a significant reduction of cool nights
336 and cool days during winter (Sun et al. 2016). Thus, the changes in aphid community abundance
337 that had taken place in the south area would probably push northward in the future.

338 **Limitations**

339 Although the risk analysis shows very coherent patterns that seem to match with field
340 observations, it is, however, important to recognize that such a model has some limitations, in
341 particular, it does not consider i) the difference between air and insects' body temperature and ii)
342 the capacity of insects to relocate into more permissive microclimates (i.e. avoidance) (Ma and
343 Ma 2012a, b, Woods et al. 2015, Pincebourde and Suppo 2016, Ma et al. 2018b). In addition, the
344 approach does not consider if thermal tolerance changes seasonally with acclimation (Overgaard
345 et al. 2008, Bujan et al. 2020), nor the possibility that changes in feeding behavior could be altered
346 to provide on-time nutritional adjustments aiding physiological changes (for instance: synthesis of
347 compatible solutes, acquisition of antioxidant molecules). Furthermore, we calculated the daily
348 injury accumulation with the assumption that aphids that survived the thermal stress could recover
349 quickly during non-stressful temperature regimes (cooler nights for heat injury and warm noon for
350 cold injury). While this recovery phase is true and well described (see discussion in Colinet et al.
351 2015), the capacity to fully recover depends on the level of thermal injury and the repair
352 temperature and duration (Colinet et al. 2011, Ma et al. 2018a, Bai et al. 2019, Zhu et al. 2019,
353 Ørsted et al. 2022). Advanced models that include the temperature-dependent repair function
354 have recently been developed to incorporate this recovery capability (Ørsted et al. 2022). Despite
355 these pitfalls, this simple approach based on basal thermal tolerance and cumulative daily thermal
356 stress proved to be rather efficient in discriminating both the most susceptible species and regions.

357 It could be further developed into a fine model to predict the population dynamics of aphid species
358 in the field if integrating the effect of thermal dose on fertility in the future.

359 **Conclusion**

360 Our results answered the three questions: 1) interspecific differences in basal thermal tolerance
361 can be a factor driving the community abundance change in aphid communities. The risk
362 assessment under the natural temperature profile seems to fit the field observations that the more
363 tolerant species is becoming more abundant with increasing temperature in the context of climate
364 change. 2) Although the effect of age was not consistently observed in the three aphid species,
365 we found that younger nymphs tended to have a lower thermal tolerance.

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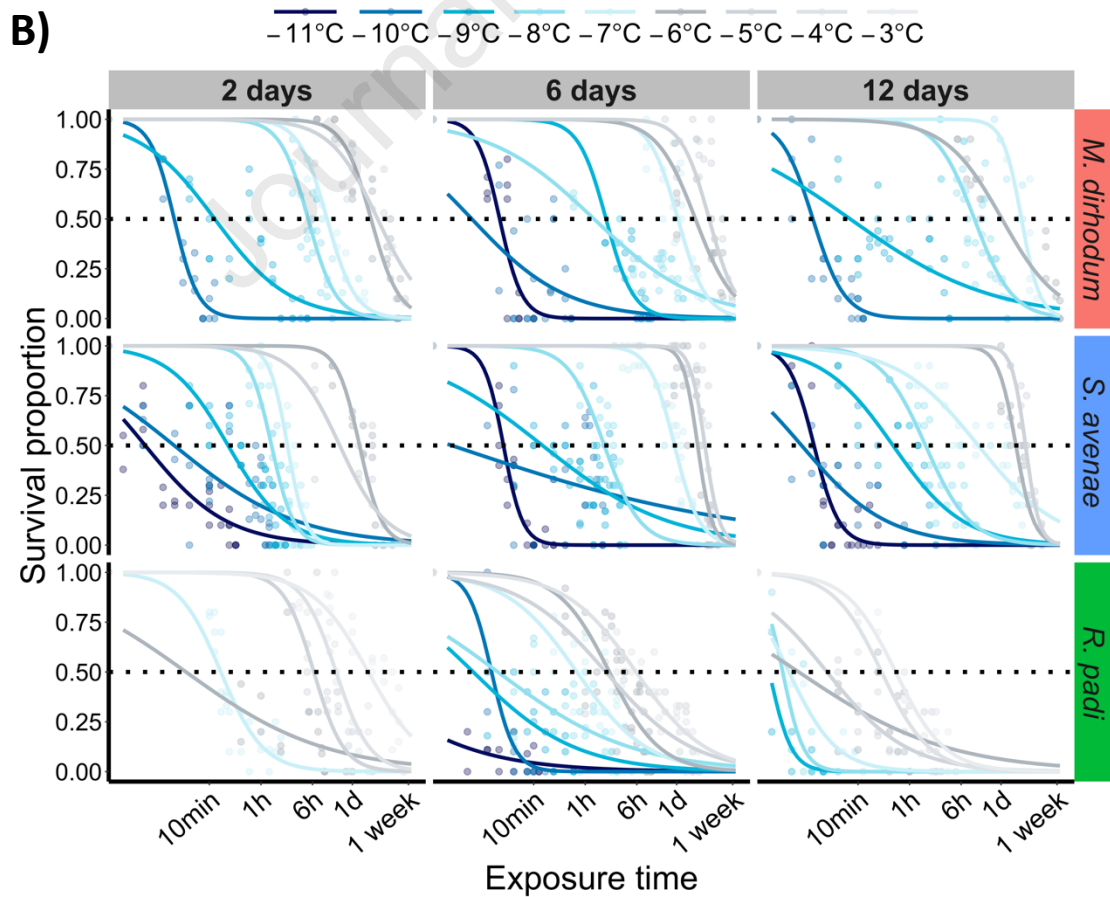
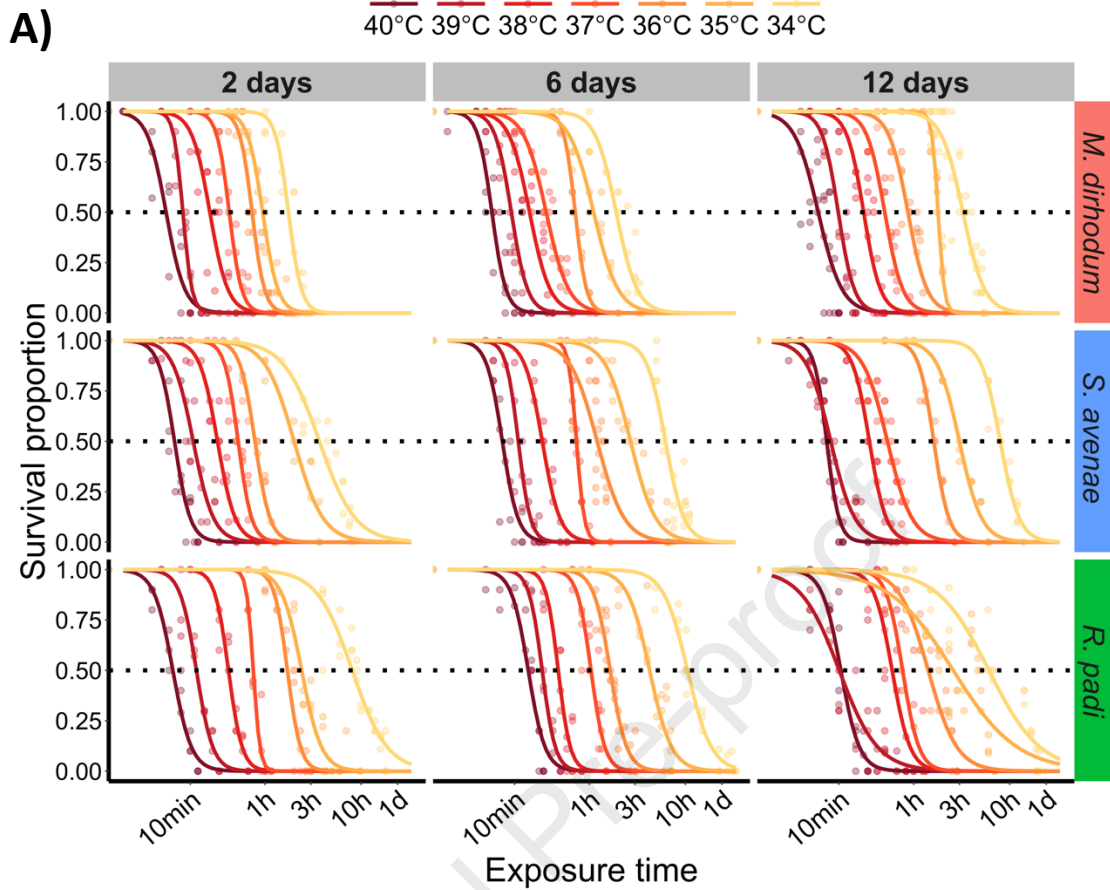
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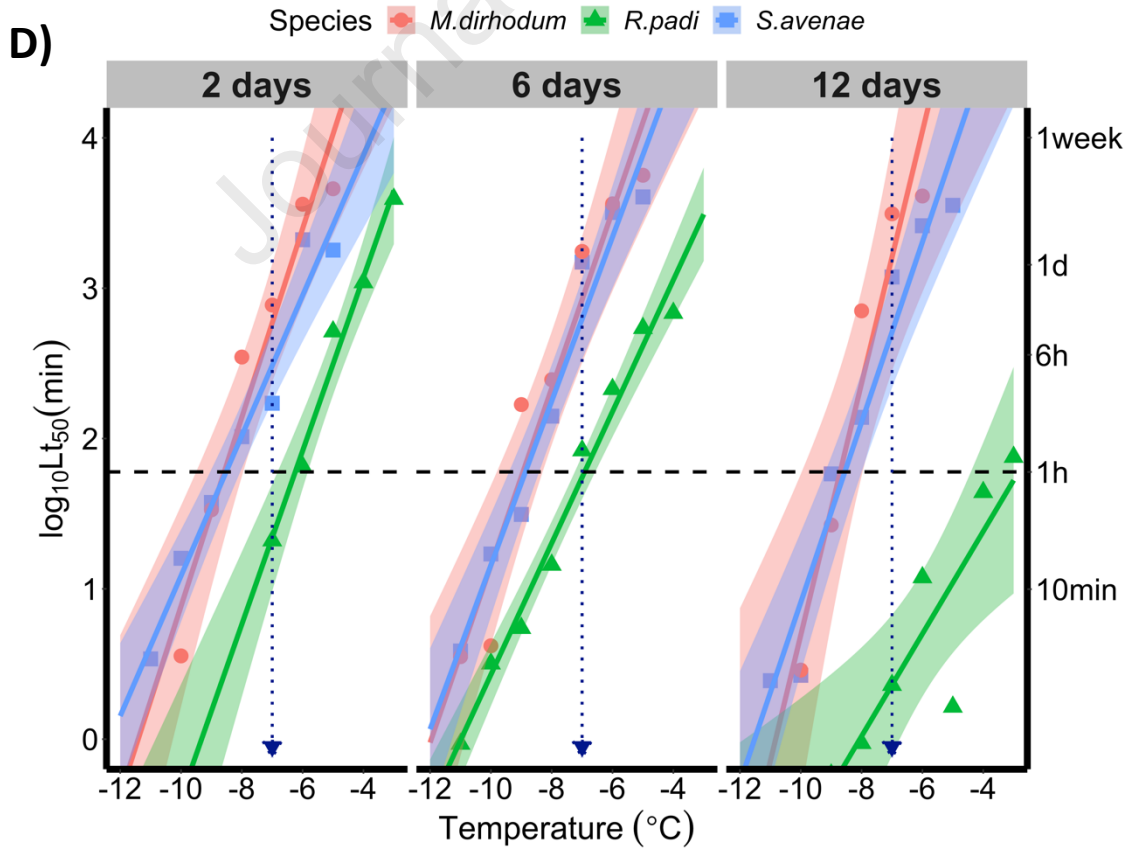
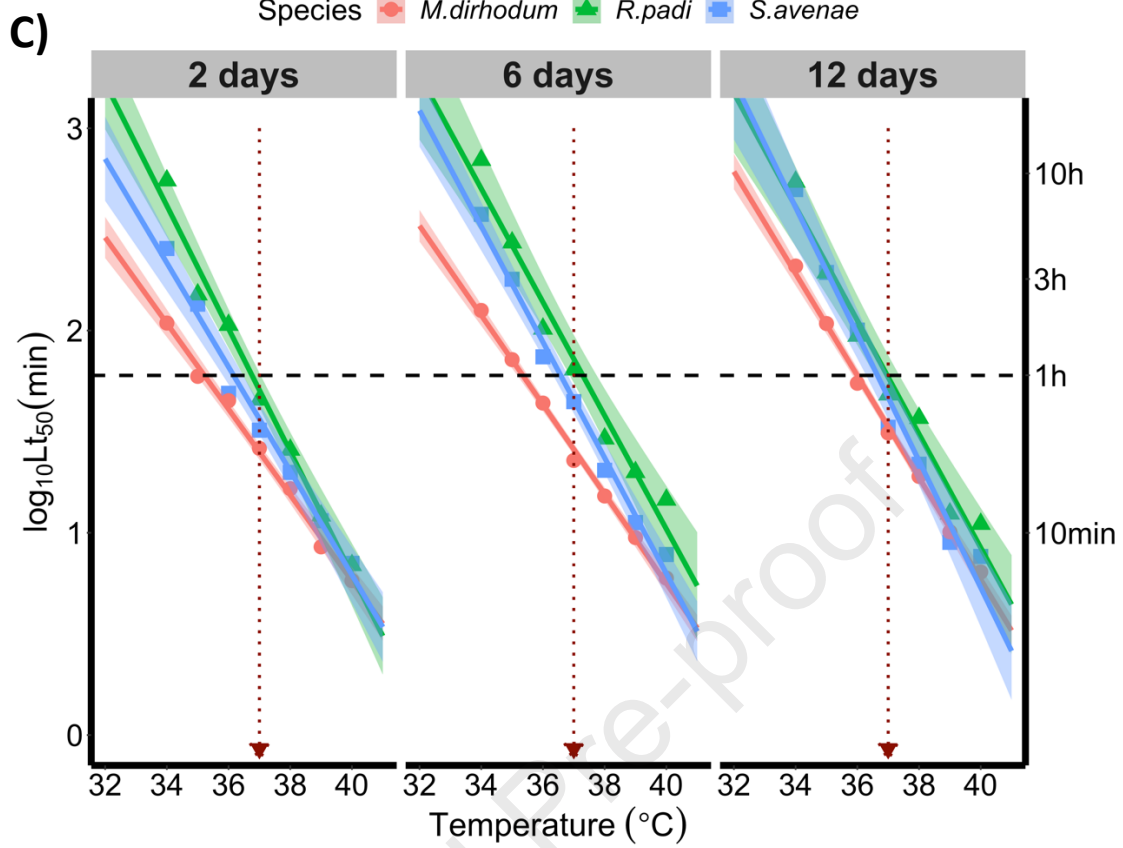


Figure 1. Survival curves (panel A for heat, B for cold) and thermal death time curves (panel C for heat, D for cold) for the three ages (2, 6, and 12 days) of *M. dirhodum*, *S. avenae*, and *R. padi*. The curves in different colors (A and B) were fitted by the logistic regression model at each temperature. Each point in panels A and B represented the survival proportion of aphids in a single replicate and each point in panels C and D represented the $\log_{10}(Lt_{50})$ value estimated from the survival curve at a tested temperature. Lines and shades in C and D illustrated the linear regression lines and the corresponding 95% confidence intervals. CT_{max} and CT_{min} were the intercepts with the black dashed line ($Lt_{50} = 1$ hour). Thermal sensitivity (z or z') was derived from the slope of the line ($z/z' = -1/\text{slope}$). The $\log_{10}(Lt_{50})$ at 37°C and $\log_{10}(Lt_{50})$ at -7°C where red and blue dashed arrows pointed were pairwise compared between species and ages.

Table 1. Parameters of thermal death time curves for each age group of the three species at high temperatures. Superscript lowercase letters in thermal sensitivity (z) and $\log_{10}(Lt_{50})$ at 37°C marked significant differences in slope and intercept between species with the same age ($p < 0.05$). Superscript uppercase letters marked significant differences between age groups for the same species ($p < 0.05$).

Species	R ²	P value	Thermal sensitivity (z)	CT _{max} (°C)	$\log_{10}(Lt_{50})$ at 37°C
2 days					
<i>M. dirhodum</i>	0.99	<1*10 ⁻⁴	4.71 ^{B, b}	35.21	1.40 ^{A, a}
<i>S. avenae</i>	0.98	<1*10 ⁻⁴	3.89 ^{A, a}	36.16	1.56 ^{A, b}
<i>R. padi</i>	0.99	<1*10 ⁻⁴	3.29 ^{A, a}	36.76	1.71 ^{A, c}
6 days					
<i>M. dirhodum</i>	0.99	<1*10 ⁻⁴	4.52 ^{B, b}	35.35	1.41 ^{A, a}
<i>S. avenae</i>	0.99	<1*10 ⁻⁴	3.49 ^{A, a}	36.58	1.66 ^{A, b}
<i>R. padi</i>	0.97	<1*10 ⁻⁴	3.57 ^{A, a}	37.29	1.86 ^{B, c}
12 days					
<i>M. dirhodum</i>	0.99	<1*10 ⁻⁴	3.97 ^{A, a}	36.00	1.53 ^{B, a}
<i>S. avenae</i>	0.98	<1*10 ⁻⁴	3.19 ^{A, a}	36.65	1.67 ^{A, b}
<i>R. padi</i>	0.97	<1*10 ⁻⁴	3.56 ^{A, a}	36.97	1.77 ^{A, c}

Table 2. Parameters of thermal death time curves for each age group of the three species at low temperatures. Superscript lowercase letters in thermal sensitivity (z') and $\log_{10}(Lt_{50})$ at -7°C marked significant differences in slope and intercept between species with the same age ($p < 0.05$). Superscript uppercase letters marked significant differences between age groups for the same species ($p < 0.05$).

Species	R^2	P value	Thermal sensitivity (z')	$CT_{\min} (^{\circ}\text{C})$	$\log_{10}(Lt_{50})$ at -7°C
2 days					
<i>M. dirhodum</i>	0.94	$<1*10^{-2}$	1.59 ^{A, a}	-8.58	2.77 ^{A, b}
<i>S. avenae</i>	0.96	$<1*10^{-3}$	2.14 ^{A, a}	-8.52	2.49 ^{A, b}
<i>R. padi</i>	0.98	$<1*10^{-2}$	1.73 ^{A, a}	-6.24	1.34 ^{B, a}
6 days					
<i>M. dirhodum</i>	0.93	$<1*10^{-3}$	1.70 ^{A, a}	-8.94	2.92 ^{A, b}
<i>S. avenae</i>	0.96	$<1*10^{-4}$	1.83 ^{A, a}	-8.86	2.80 ^{A, b}
<i>R. padi</i>	0.98	$<1*10^{-4}$	2.29 ^{A, a}	-6.92	1.74 ^{B, a}
12 days					
<i>M. dirhodum</i>	0.93	$<1*10^{-2}$	1.19 ^{A, a}	-8.70	3.21 ^{A, b}
<i>S. avenae</i>	0.98	$<1*10^{-3}$	1.67 ^{A, ab}	-8.55	2.71 ^{A, b}
<i>R. padi</i>	0.78	$<1*10^{-2}$	2.94 ^{A, b}	-2.84	0.36 ^{A, a}

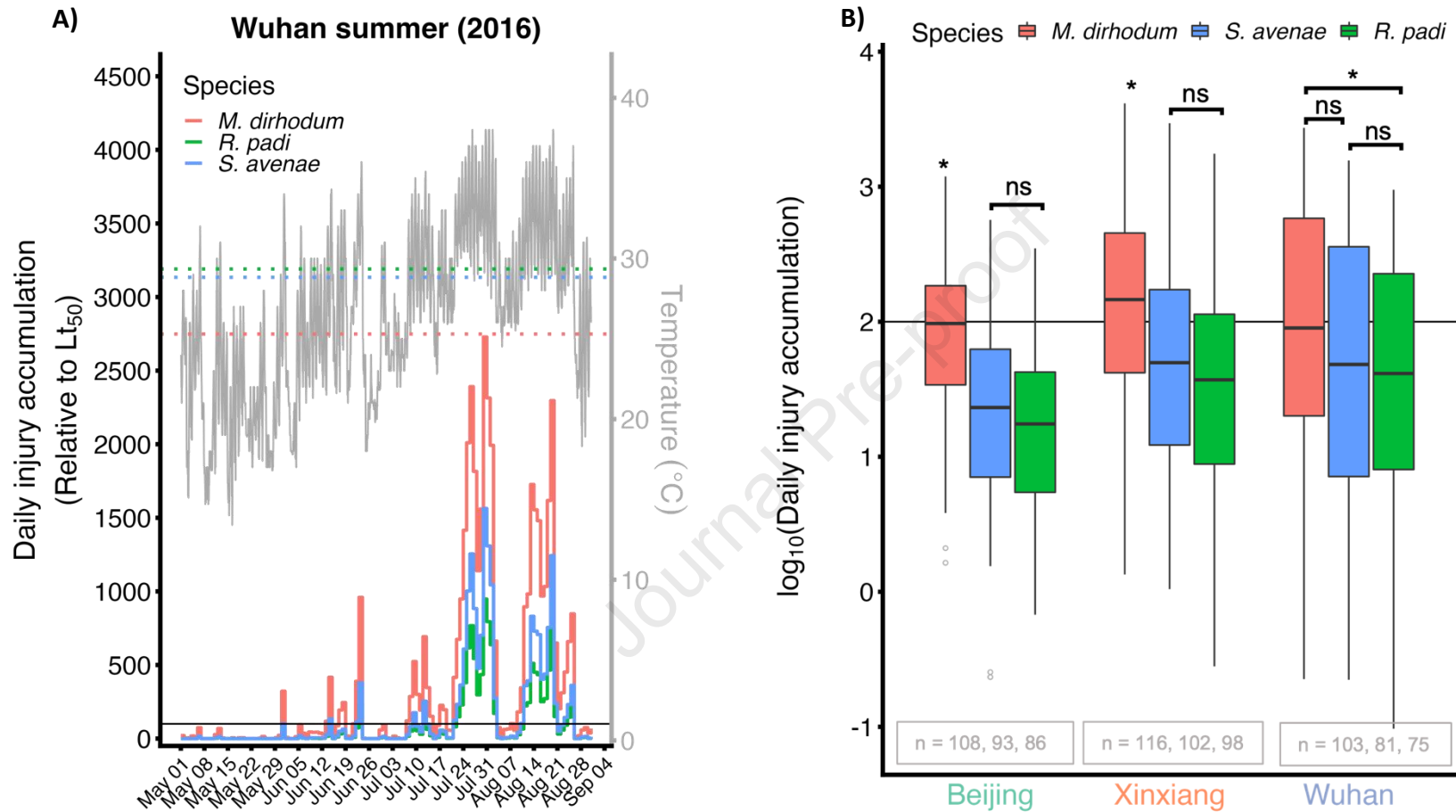


Figure 2. Predicted daily injury accumulation based on the TDT parameters for the three species (age 6 days) under natural temperature fluctuations in the summer (May-August) of 2016. A) The temperature profile (grey, secondary y-axis) was used to project the daily

accumulation of injury by use of the TDT parameters (see the formula in the method part). The species-colored lines/steps at the bottom of the graph indicated the amount of injury accumulated *per day* relative to the amount of injury that would result in half the aphid dying (here set to 100 with the black line). In the current run of this model, only temperatures above a T_c of 25.29°C (red dotted line), 29.34 °C (green dotted line) and 28.82 °C (blue dotted line) were set to result in injury for *M. dirhodum*, *R. padi* and *S.avenae*, respectively. **B)** Boxplot summarizing the data from (A) and similar analyses for Beijing and Xinxiang in the summer (see figure S1A and B). Note that the y-axis was \log_{10} -transformed and the injury accumulation resulting in median lethal (100) was marked by the line at $\log_{10}(\text{Daily injury accumulation}) = 2$. To avoid comparing a lot of days without any injury accumulation, only days with injury accumulation (indicated as n in the graph) were summarized in this plot. Comparisons among species within sites were examined using Kruskal-Wallis Rank Sum Tests followed by Pairwise Wilcoxon Rank Sum Tests with a Benjamini-Hochberg correction – results were indicated on the plot. Asterisks marked significant differences ($p < 0.05$) between two groups.

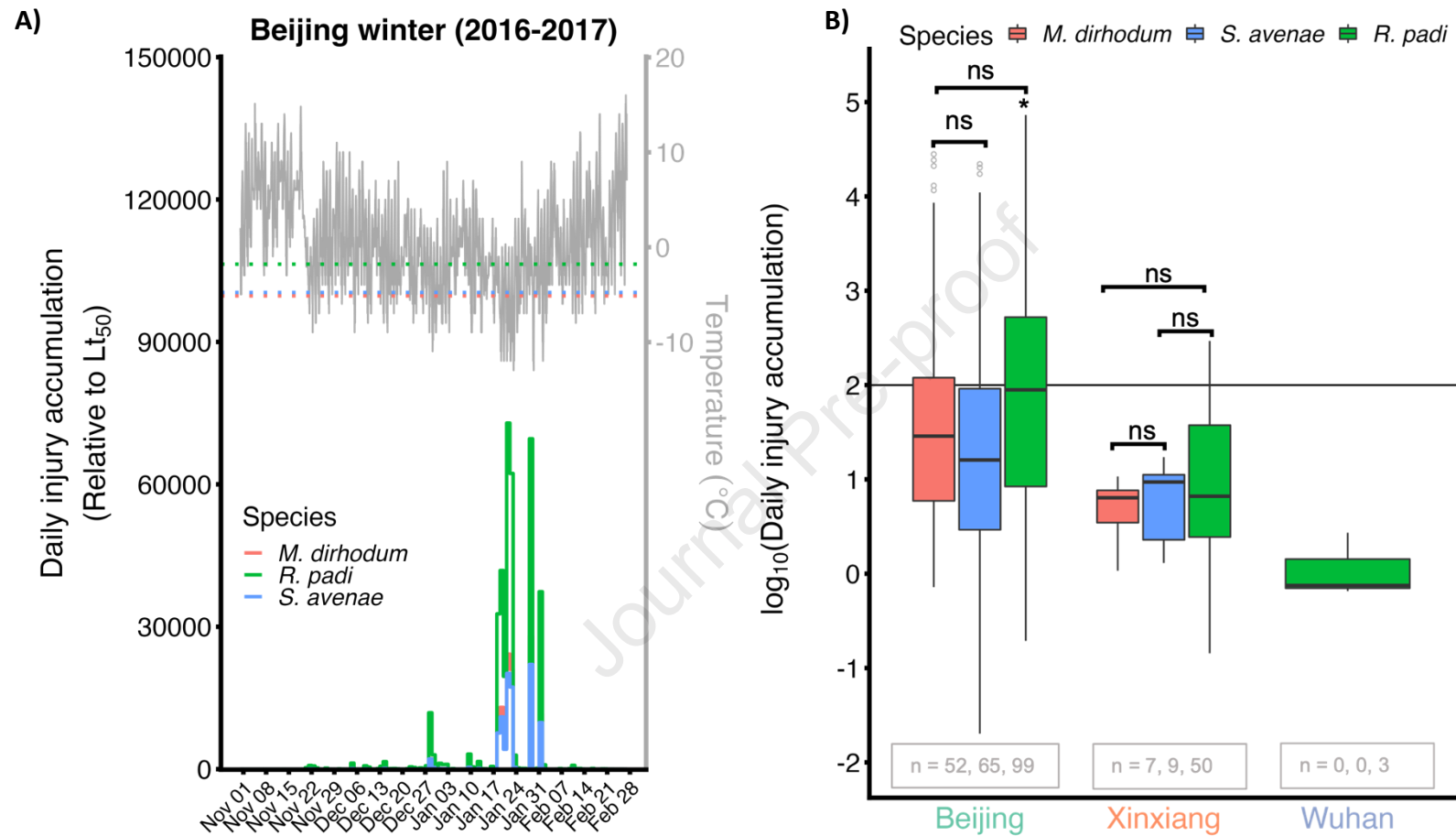


Figure3. Predicted daily injury accumulation based on the TDT parameters for the three species of cereal aphids (*M. dirhodum*, *S. avenae*, *R. padi*) (age 6 days) under natural temperature fluctuations found in the winter (November-February) of 2016-2017. A) The

temperature profile (grey, secondary y-axis) was used to project the daily accumulation of injury by use of the TDT parameters (see the formula in the method part). The species-colored lines/steps at the bottom of the graph indicated the calculated amount of injury accumulated *per day* relative to the amount of injury that would result in half the aphids dying (here set to 100 with the black line). Injuries only accumulated when temperature dropped below a T_c' of -5.15°C (red dotted line), -1.81°C (green dotted line) and -4.79°C (blue dotted line) for *M. dirhodum*, *R. padi* and *S. avenae*, respectively **B**) Boxplot summarizing the data from (A) and similar analyses for Wuhan and Xinxiang in the winter (see figure S2 A and B). Note that the y-axis was \log_{10} -transformed and the injury accumulation resulting in median lethal (100) was marked by the line at $\log_{10}(\text{Daily injury accumulation}) = 2$. To avoid comparing a lot of days without any injury accumulation, only days with injury accumulation (indicated as n in the graph) were summarized in this plot. Comparisons between species within sites were examined using Kruskal-Wallis Rank Sum Tests followed by Pairwise Wilcoxon Rank Sum Tests with a Benjamini-Hochberg correction. Asterisks marked significant differences ($p < 0.05$) between two groups.

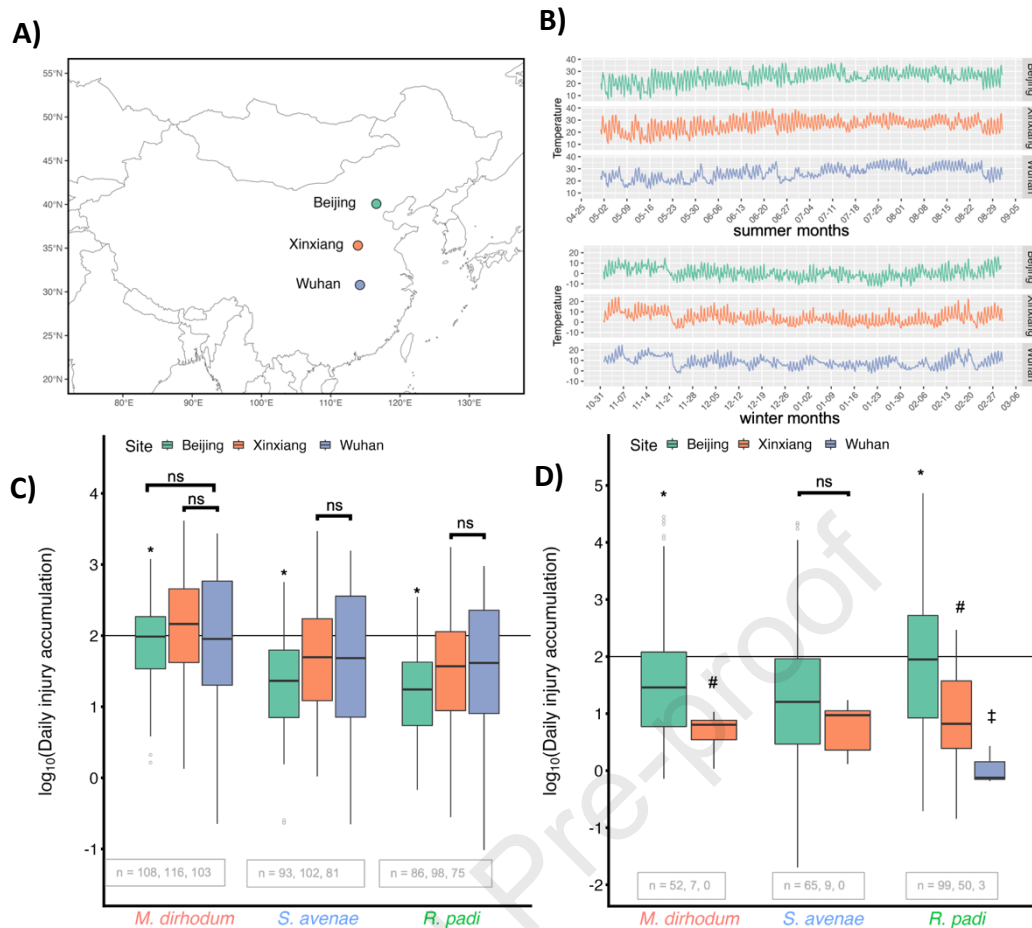


Figure 4. Temperature profiles and predicted daily injury accumulation based on the TDT parameters for the three species of cereal aphids (*M. dirhodum*, *S. avenae*, *R. padi*) (age 6 days) under natural temperature fluctuations found in three sites in the summer months (May-August) of 2016 and winter months (November-February, 2016-2017). A) Map of the geographic locations of three sites indicated in different colors. B) Overview of temperature profiles in the three sites during summer (May-August 2016) (top) and winter months (Nov-Feb, 2016-2017) (bottom). C) and D) showed a comparison of log₁₀(daily injury accumulation) between sites within species during summer months and winter months respectively. The comparisons were conducted using Kruskal-Wallis Rank Sum Tests followed by Pairwise Wilcoxon Rank Sum Tests with a Benjamini-Hochberg correction. *, #, and ‡ marked significant differences ($p < 0.05$) among sites. To avoid comparing a lot of days without any injury accumulation, only days with injury accumulation (indicated as n in the graph) were summarized in the plot.

Highlights

- Thermal tolerance landscape reflected the survivals of cereal aphids at extreme temperatures.
- *Rhopalosiphum padi* was more heat-tolerant and cold-vulnerable than *Sitobion avenae* and *Metopolophium dirhodum*.
- Young nymphs generally had lower thermal tolerance than old nymphs or adults.
- According to estimated thermal injury, *R. padi* was severely injured by cold while *M. dirhodum* was by heat.
- Interspecific differences in thermal tolerance may drive the aphid community abundance change.

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Competing interests

The authors declare that they have no conflict of interest.

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