Strain-specific thermal acclimation, but little evidence of transgenerational plasticity, in an asexual crustacean

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Abstract:

Transgenerational plasticity has been suggested as a means for species to succeed in rapidly changing environments, such as increased temperature brought on by climate change. However, the evidence for this phenomenon in animals is mixed. The freshwater crustacean *Daphnia magna* displays transgenerational plasticity in response to environmental cues such as the presence of predators or food, but studies on heat tolerance have been less conclusive. We acclimated three asexual strains of *D. magna*—originally from London, UK (“GB”), Fülöpszállás, Hungary (“HU”), and Jerusalem (“IL”)—to either 20°C (control) or 28°C (heat-acclimated) conditions; we refer to this as the parental generation. In contrast, their offspring were reared at 20°C regardless of parental treatment. We assessed acute heat tolerance (time to immobility, T_{imm}), heart rate, body size, and age at first reproduction in the parental and offspring generations. In the parental generation, heat-acclimated individuals of all strains had higher T_{imm} and reproduced earlier. The increase in heat tolerance was greatest in the southernmost strain (IL), and smallest in the northernmost strain (GB). After heat acclimation, mean heart rate increased in GB, decreased in HB, and did not change in IL. These results highlight the importance of local adaptation in thermal plasticity. Parental acclimation temperature did not affect T_{imm} in offspring; however, there was some evidence that offspring of heat-acclimated mothers reproduced later, reversing the trend observed in parents. We did not find support for transgenerational plasticity in heat tolerance, perhaps because offspring re-acclimated to the benign temperature of 20°C.

Key words: *Daphnia magna*, Cladocera, thermal plasticity, maternal effect, local adaptation

Introduction

Among the most immediate and flexible responses to rapid environmental change is phenotypic plasticity, which allows organisms to modify their physiology, behavior, or morphology in response to external stressors. Unlike genetic adaptation, plasticity can occur within a single generation, providing a critical buffer against short-term environmental extremes (Ghalambor et al 2007). In such cases, organisms can benefit from their own plastic responses as well as from transgenerational plasticity (also known as anticipatory parental effects), where parents that have experienced a stressor influence their offspring's phenotype in a beneficial way (Kuijper & Hoyle 2015; Graeve et al. 2021). This concept is especially relevant under ongoing anthropogenic environmental change, including climate change, because abrupt shifts in temperature or oxygen availability can threaten organismal function. In freshwater ecosystems, where thermal and oxygen dynamics are highly variable, plasticity plays a central role in mediating short-term resilience (Woodward et al. 2020). As a result, while increasing temperatures may hinder some species' plastic responses (Sentis et al. 2017), organisms that can non-genetically transfer plastic traits across generations are more likely to persist under those rapidly fluctuating conditions (Donelson et al. 2012).

Daphnia magna Straus, a freshwater crustacean and well-established model for environmental physiology, exhibits plastic responses to a variety of stressors, including temperature, making it a valuable system for investigating the physiological mechanisms underlying thermal tolerance. Their asexual reproduction also allows for comparison between genetically identical siblings exposed to different environmental conditions. *Daphnia* exposed to elevated temperatures often develop increased tolerance to acute heat stress, largely mediated by the upregulation of hemoglobin expression (Seidl et al. 2005). *Daphnia* have a simple hemal system (Ruppert et al. 2004), and thermal acclimation appears to improve oxygen transport efficiency through increased hemoglobin production, enhancing performance under warm, hypoxic conditions (Seidl et al. 2005). Body lipid composition and antioxidant capacity also influence heat tolerance in *Daphnia*. Individuals with high levels of polyunsaturated fatty acids, which are associated with membrane instability, have lower heat tolerance (Werner et al. 2019), whereas increased antioxidant capacity protects the body from oxidative stress and is associated with acclimation to high temperatures (Coggins et al. 2017). Plasticity of *Daphnia* in response to heat has been experimentally demonstrated by comparing heat-acclimated individuals with genetically identical siblings raised under control temperatures, revealing significantly longer knockout times (T_{imm}) in the heat-acclimated group (e.g., Williams et al. 2012; Yampolsky et al. 2014).

The vertical transmission of traits independently of DNA is broadly referred to as non-genetic inheritance, and has gained increasing attention for its evolutionary significance (Bonduriansky & Day 2009, 2020). Some researchers have argued that it warrants integration into mainstream evolutionary models alongside traditional genetic inheritance (Danchin et al. 2011; Bonduriansky & Day 2011; Bell & Hellman 2019). Non-genetic inheritance encompasses a variety of mechanisms, including epigenetic modifications, maternal nutrient provisioning, hormonal signaling, and environmental or behavioral cues, all of which can alter offspring phenotype and fitness (Bonduriansky & Day 2009, 2020). While nongenetic factors can be inherited from fathers (Rando 2012) or grandparents (Agrawal et al. 1999), factors passed from mothers to offspring, known as maternal effects, are widespread among animals and are one of the most extensively studied forms of nongenetic inheritance (Bernardo 1996). Anticipatory or adaptive maternal effects, in which mothers improve their offspring’s fitness by preparing them for specific environmental conditions, are of particular interest in evolutionary ecology. These effects are a form of transgenerational plasticity, as they represent phenotypic plasticity of the offspring in response to the parental environment (Bell & Hellman 2019). However, it is unclear how widespread such adaptive nongenetic effects are in animals (Marshall & Uller 2007; Uller et al. 2013).

In *Daphnia* species, maternal effects can influence a wide range of offspring traits. Maternal effects on morphology and life-history traits—such as offspring growth rate, size at maturity, and reproductive output—have been well documented, especially in response to environmental stressors like photoperiod and food availability (Alekseev & Lampert 2001; Coakley et al. 2017). Maternal effects on offspring defense traits, including the induction of protective morphological structures in response to predators, have also been observed (Agrawal et al. 1999; Graeve et al. 2021). Additionally, maternal exposure to pathogens or immune challenges reduces offspring susceptibility to disease (Little et al. 2003; Mitchell & Read 2005). Lastly, maternal cues can also be passed down in response to biological or metal toxins and pollutants (Gustafsson et al. 2005; Fernandez-Gonzalez et al. 2011). Under stressful environmental conditions, maternal effects have the potential to function as a form of environmental memory, allowing offspring to begin life already suited for survival in the conditions their mother experienced (Agrawal et al. 1999; Radersma et al. 2018; Graeve et al 2021).

However, whether similar transgenerational plasticity occurs in response to thermal stress in *Daphnia* remains an open question. Existing research has emphasized understanding within-generation acclimation (e.g., Williams et al. 2012; Yampolski et al. 2014), leaving room for further studies on how parental environments might influence offspring thermal responses. Previous studies show some evidence for transgenerational plasticity, where offspring of heat-acclimated mothers exhibit improvements in other physical traits, such as disease resistance and reproduction (Garbutt et al. 2014; Betini et al. 2020) despite never encountering the stressor. However, we are not aware of any studies in which maternal acclimation of *Daphnia* to high temperature yielded detectable improvement in offspring heat tolerance. Factors such as the timing of maternal exposure (early life vs. adulthood), the specific traits measured (e.g., survival vs. metabolic rate), and the genetic background of the clone may all influence whether transgenerational plasticity of heat acclimation is observed. Attention to this research gap is important, especially given the ecological relevance of temperature stress in freshwater habitats affected by global warming.

In this study, we investigate the transgenerational plasticity of heat tolerance in *Daphnia magna*. We acclimated individuals from three different strains to either a benign temperature (20 °C, "control") or a stressful higher temperature (28 °C, "heat-acclimated") and tested whether heat-acclimated individuals and their unacclimated offspring exhibited greater tolerance to an acute 37 °C heat shock. We also monitored changes in other key traits—heart rate, body size, and age at reproduction—across both generations. We predicted that unacclimated offspring of heat-exposed mothers would demonstrate greater heat tolerance compared to offspring of control mothers, suggesting a maternal transfer of beneficial traits.

Materials and Methods

Daphnia *clones and Basic Husbandry*

We used three asexual strains of *Daphnia magna* which were originally collected at three different Eurasian localities: London, United Kingdom (GB-EL75-69), Fülöpszállás, Hungary (HU-K-6), and Jerusalem (IL-MI-8). For brevity, the clones will be referred to as GB, HU, and IL, respectively. The thermal biology of each clone has previously been characterized, with GB showing relatively low acute heat tolerance and HU and IL showing relatively higher heat tolerance (e.g., Yampolsky et al., 2014; Coggins et al. 2017, 2021; Martin-Creuzburg et al. 2019). All clones have the capacity to acclimate to higher temperatures over time.

Stock populations of each *Daphnia* strain were reared in 600 mL of spring water in a Percival Model E30B growth chamber (Percival Scientific, Inc., USA) at 20 °C with a 12h:12h light–dark cycle. During the experiment, each *Daphnia* was kept individually in a glass test tube with 20 mL of spring water and fed 375,000 cells/mL of *Nannochloropsis* D.J. Hibberd algae daily (Carolina Biological Supply, USA). Algae was cultured in AlgaGro media (Carolina Biological Supply, USA) and cell concentration was estimated based on the optical density at

680 nm. Offspring were removed from tubes each day, and *Daphnia* were transferred to a new tube of fresh water twice a week.

Generational Experiment

At the beginning of the experiment, we collected ten adult females from each strain (i.e., 30 females total) and placed them in individual tubes as described above. Five of the ten were transferred to a growth chamber set to 28 °C, while the other five remained at 20 °C. These were the "grandparental" generation (i.e., the parents of our parental generation) for the heat-acclimated and control groups, respectively (Fig. 1). This ensured prenatal exposure of the parental generation to their respective temperature treatment. We collected offspring continuously from the grandparental *Daphnia* until we had three offspring from each grandmother. This number provided two *Daphnia* for measuring acute heat tolerance, body size, and heart rate (described below), plus one additional individual in case of mortality before adulthood. All *Daphnia* derived from one grandparent were considered one "family" across the experiment.

Reproduction was not synchronized among the grandparents, so not all offspring were precisely the same age. We reduced this difference as much as possible by replacing older offspring from a given grandmother with new offspring produced by the same individual, until we had a full complement of offspring for all 30 females. Individuals in the parental generation were born across a 4-day period, with the vast majority (89%) born in the final two days of that period. There were 90 parental-generation *Daphnia* in total: 45 individuals (in 15 families across three strains) acclimated to 20 °C and 45 individuals acclimated to 28 °C.

As the parental *Daphnia* began to reproduce, we removed their offspring (the F1 generation) from their tubes and recorded the number of offspring daily. All F1 *Daphnia* were kept at 20 °C regardless of parental treatment. As described above, we continued to collect F1 offspring until we had three individuals in every family. Offspring were pooled within a family, such that the three F1 *Daphnia* within a given family may have been produced by multiple mothers, but were kept separate from other families (grandmothers) of the same strain and treatment. Individuals in the F1 generation were born across a four-day period, with the exception of a single offspring (HU, 28 °C) born an additional two days earlier.

For both the parental and F1 generation, once at least three offspring had been produced by every family, a single adult female was selected from each family to assess acute heat tolerance and a second female was selected to measure body size and heart rate.

Acute Heat Tolerance

We assessed acute heat tolerance by recording time to immobilization (T_{imm}) at a constant 37 °C. Under heat stress, *Daphnia* cease to be able to swim and fall to the floor of their enclosure immobilized. T_{imm} is influenced both by evolved genetic differences among populations and plastic acclimation to elevated temperatures (Yampolsky et al 2014).

Approximately 3 hours prior to the heat tolerance assay, one adult female from each family was removed from its tube and placed in a new tube with 10 ml of clean spring water, at that individual's acclimation temperature. Test tubes were randomly arranged in test tube racks and blinded by a person other than the observer. All *Daphnia* tubes were simultaneously lowered into a 37 °C water bath such that the water in the tube was fully submerged. Every 5 minutes, observers checked each tube to assess whether the *Daphnia* had been immobilized, defined as no motion visible to the eye, excluding the beating of the internal legs.

Heart Rate and Body Size

Because environmental temperature can induce a suite of physiological and life-history changes in poikilothermic animals such as *Daphnia*, we also recorded heart rate and body size. Three hours prior to measuring heart rate, a second individual *Daphnia* from each family was selected and placed in the 20 °C incubator, regardless of treatment. After this re-acclimation period, each individual was placed in a depression slide on a stereomicroscope, and the number of heart beats in a ten-second period was counted, and this was repeated three times. The mean of the three counts was converted to beats per minute.

The height and width of each *Daphnia* was measured using ocular micrometers. Height was measured from the top of the eye to the ventral base of the caudal spine; for width, we measured the maximum width perpendicular to the spine-eye axis. All measurements were made to the nearest 0.04 mm. Finally, the approximate lateral area of the *Daphnia* was estimated using the equation for the area of an ellipse.

Statistical Analyses

All statistical analyses were performed in R 4.4.2 (R Core Team 2024). Data were visualized as box plots and Kaplan-Meier survival curves, using the R packages "ggplot2" (Wickham 2016) and "survminer" (Kassambara et al. 2024), respectively.

The data for T_{imm}, heart rate, lateral area, and age at reproduction were analyzed separately by generation. T_{imm}, heart rate, and lateral area were analyzed using linear models. T_{imm} was natural log-transformed prior to analysis to improve normality. All three models included the following variables: strain, (parental) acclimation temperature, their interaction, and observer identity. We performed type-II *F*-tests using the R package "car" (Fox & Weisberg 2019). If the effect of observer identity had a *p*-value greater than 0.1, this variable would be removed from the model; observer identity was retained in all analyses.

Age at first reproduction is a "time to event" variable, and following Coakley *et al.* (2018), we analyze these data with a Cox proportional hazards model using the R package "survival" (Therneau 2024). These models contained effects of strain, (parental) acclimation temperature, and their interaction. As above, we performed type-II Wald tests using “car”. Individuals that died before reproducing (P generation: $n = 3$; F1 generation: $n = 3$) or that did not reproduce by the date of the heat tolerance assay (P generation: $n = 10$; F1 generation: $n = 4$) were right censored.

Where there was evidence for a clone-by-treatment interaction, we investigated further with follow-up *t*-tests within each clone. We did not include observer identity in follow-up tests, as this three-level factor would substantially reduce our power to detect differences in the much smaller subsetted data set. We corrected for multiple comparisons using the Holm–Bonferroni method (Holm 1979; Rice 1989), implemented in the R package “dyplr” (Wickham et al. 2023). This procedure controls the family-wise error rate while maintaining statistical power by applying the most stringent penalty to the lowest *p*-value (multiplying it by three, equivalent to a Bonferroni correction) and decreasing penalties to the remaining *p*-values sequentially from lowest to highest. Adjusted *p*-values are reported as p_{adj}, with the raw *p*-values provided in square brackets.

We interpret *p*-values as a continuous metric of the strength of evidence, and follow the recommendations of Muff et al. (2022) in describing our results on a continuum from “no evidence” ($p > 0.1$) to “very strong evidence” ($p < 0.001$), along with graphical or written estimates of effect size.

Results

Heat Tolerance

Thermal tolerance responses to acclimation differed between generations. In the parental generation, there was very strong evidence that both strain ($F_{2,22} = 43.66$, $p < 0.001$) and temperature ($F_{1,22} = 177.45$, $p < 0.001$) affected time to immobilization. We also found strong evidence for a strain × treatment interaction ($F_{2,22} = 11.34$, $p < 0.001$; Fig. 2a). Observer identity also had an effect on measurement ($F_{2,22} = 5.68$, $p = 0.010$).

In the F1 generation, there was very strong evidence that heat tolerance differed between strains ($F_{2,22}$ = 14.59, p < 0.001), but no evidence for differences depending on parental temperature treatment ($F_{1,22}$ = 1.28, p = 0.27) or a strain × treatment interaction ($F_{2,22}$ = 0.10, p = 0.91), suggesting that transgenerational plasticity in heat tolerance was not detected (Fig. 2b). Observer identity influenced measurements ($F_{2,22}$ = 16.32, p < 0.001), indicating some observer-related variability in assessment.

Heart Rate

The influence of parental thermal acclimation on heart rate also varied across generations. In the parental generation, there was moderate evidence that heart rate differed across strains ($F_{2,22}$ = 3.68, p = 0.042). Although there was no evidence that acclimation temperature alone affected heart rate ($F_{1,22}$ = 0.006, p = 0.941), we observed moderate evidence for a strain × treatment interaction ($F_{2,22}$ = 4.45, p = 0.024), suggesting that the effect of heat acclimation varied by strain (Fig. 3a). Specifically, there was moderate evidence that GB individuals exhibited higher heart rates when reared at 28°C, with a 34.4 bpm increase in mean heart rate ($t(7.37) = 3.56$, p_{adj} = 0.025 [0.008]). We found weak evidence that heart rate of HU was lower at 28 °C, with a 55.6 bpm decrease in mean heart rate ($t(7.48) = -2.84$, p_{adj} = 0.047 [0.023]), and there was no evidence of differences between temperatures in IL, with a difference in means of only 18.8 bpm ($t(7.73) = 0.55$, p_{adj} = 0.597 [0.597]). Additionally, we found strong evidence that heart rate was influenced by measurer identity ($F_{2,22}$ = 7.97, p = 0.002).

In contrast, the F1 offspring generation exhibited no evidence of transgenerational plasticity in heart rate. The data provided no evidence that strain ($F_{2,21}$ = 0.87, p = 0.434), parental treatment ($F_{1,21}$ = 1.54, p = 0.229), nor their interaction ($F_{2,21}$ = 1.20, p = 0.320) affected heart rate (Fig. 3b). As in the parental generation, heart rate was affected by measurer identity, with very strong evidence for this effect ($F_{2,21}$ = 18.01, p < 0.001).

Body size

Thermal acclimation did not influence body size, measured as lateral area, in either generation. In the parental generation, there was no evidence that strain ($F_{2,22}$ = 2.23, p = 0.131), treatment ($F_{2,22}$ = 1.52, p = 0.230), or the strain × treatment interaction ($F_{2,22}$ = 2.22, p = 0.132) affected lateral area (Fig. 4a). However, there was very strong evidence that lateral area measurements differed by measurer ($F_{2,22}$ = 31.53, $p < 0.001$).

In the F1 generation, we again found no support for transgenerational plasticity in body size. We did not find evidence that strain ($F_{2,21}$ = 1.40, p = 0.268), parental treatment ($F_{1,21}$ = 0.02, p = 0.892), or their interaction ($F_{2,21}$ = 0.43, p = 0.657) affected lateral area (Fig. 4b). There remained strong evidence for differences in lateral area measurements between measurers ($F_{2,21}$ = 8.14, p = 0.002).

Age at Reproduction

Responses of reproductive timing to strain and treatment differed between generations. In the parental generation, there was strong evidence for a treatment effect on age at first reproduction ($\chi^2(1)$ = 20.34, $p < 0.001$), with heat-acclimated individuals reproducing earlier than controls (hazard ratio = 2.57). However, there was no evidence for a strain effect ($\chi^2(2)$ = 0.26, p = 0.88), with the reproductive timing of HU and IL being similar to the reference strain GB (hazard ratios = 0.97 and 0.72, respectively). We also found no evidence for a strain × treatment interaction ($\chi^2(2)$ = 1.83, p = 0.40), suggesting that the treatment effect on reproductive timing was consistent across strains (Fig. 5a).

In the F1 generation, there was moderate evidence for a strain effect on reproductive timing ($\chi^2(2)$ = 7.40, p = 0.025), with IL individuals reproducing later than GB (hazard ratio = 0.42), while HU and GB were similar (hazard ratio = 0.93). There was also weak evidence that heat-acclimated parents had offspring that reproduced later than offspring of control parents ($\chi^2(1)$ = 3.80, p = 0.051; Fig. 5b), suggesting a maternal effect on reproduction. However, there was no evidence for a strain × treatment interaction ($\chi^2(2) = 1.18$, $p = 0.56$).

Discussion

Environmental stimuli can influence morphology, behavior, and physiology of not only the individuals experiencing the stimulus, but potentially also their offspring. However, the nature of these changes and their fitness consequences do not always align with adaptive hypotheses. In this study, we found evidence that acclimation to high water temperature impacted the acute heat tolerance and heart rate of *Daphnia magna* in a strain-specific way, but there was no evidence that parental heat acclimation improved heat tolerance in offspring reared under benign conditions. Surprisingly, while heat-acclimated mothers reproduced at an earlier age compared to control animals, their offspring reproduced later than offspring of control mothers.

Similar to previous studies, we observed clear evidence of phenotypic plasticity in all three strains in response to acclimation temperature (Fig. 2a). Compared to animals acclimated to 20 °C, *D. magna* acclimated to 28 °C displayed greater acute heat stress tolerance (higher T_{imm}) across strains. Previous work in *D. magna* and North American *D. pulex* Leydig has identified geographic variation among strains not only in baseline heat tolerance (Geerts et al. 2014), but also in degree of phenotypic plasticity and the physiological mechanisms *Daphnia* use to withstand high temperatures (Williams et al. 2012; Yampolsky et al 2014). In our study, the degree of plasticity in heat tolerance differed among the three strains, with the smallest increase in T_{imm} observed in GB, the strain from the northernmost latitude, and the greatest increase observed in IL, the strain from the most equatorial latitude. Accordingly, for *Daphnia* populations adapted to hot localities, it appears that the capacity for plasticity is a key component of adaptation to high temperature alongside genetically determined heat tolerance.

The effect of acclimation temperature on heart rate depended on strain as well (Fig. 3a): heat-acclimated GB *Daphnia* had a higher heart rate than the control group, but heat-acclimated HU *Daphnia* had a lower heart rate than the control. Heart rates were highly, but similarly, variable in both treatment groups of IL *Daphnia*. This result suggests that our strains employed different physiological strategies in acclimating to high temperature. Previously, Yampolsky et al. (2014) reported that some *D. magna* clones acclimated to high temperatures by increasing hemoglobin concentration, while others did not. Resisting oxidative stress is another important component of heat-tolerance in *Daphnia* (Coggins et al. 2017; Zeis et al. 2019). In our study, HU individuals may have lowered their metabolic rate to reduce their oxidative stress in elevated temperatures, leading to a relatively low heart rate at 20 °C.

As expected, animals acclimated to 28 °C had their first clutch earlier than those acclimated to 20 °C (Fig. 5a). *Daphnia* are ectothermic crustaceans, so their developmental rate depends on the temperature of their environment. However, we also expected growth rate – and thus adult body size – to be higher in the 28 °C *Daphnia*, but we found no difference in lateral area between temperature treatments (Fig. 4a). Growth at 28 °C could have been limited by food availability, but based on the color of the water, it did not appear that *Daphnia* in our study were exhausting their food supply. Instead, we may have observed no difference in size because we measured individuals after reproduction. Growth of *D. magna* slows substantially around 10 days of age (Kooijman 1986), as reproduction begins. Therefore, even if heat-acclimated *Daphnia* grew faster as juveniles, it is likely that they also shifted resource allocation from growth toward egg production at earlier ages, leaving them at a similar body size as the 20 °C. Measuring juvenile body size may be necessary to detect differences in growth rate of *Daphnia*.

Although we saw clear evidence of within-generation temperature acclimation, the parental environment had only small effects on the F1 generation. There was no evidence for an effect of parental acclimation temperature on offspring acute heat tolerance (Fig. 2b), heart rate (Fig. 3b), or body size (Fig. 4b). This was somewhat surprising, transgenerational plasticity is widely reported in *Daphnia* in response to a variety of environmental cues (reviewed in LaMontagne & McCauley 2001; Agrelius & Dudycha 2025), including presence of predators (Tollrian 1995; Agrawal et al 1999), food availability (Coakley et al. 2018; Hasoon & Plaistow 2020), salinity (Mikulski & Mazurczak 2023) and temperature (Garbutt et al. 2014; Betini et al. 2020). Additionally, non-genetic transmission of heat tolerance has been previously observed in brine shrimp (*Artemia* Leach spp.), another branchiopod crustacean (Norouzitallab et al. 2014; Pais-Costa et al. 2022).

However, our results are consistent with those of other studies of temperature effects on *Daphnia*, in which elevated maternal temperature influenced some offspring traits, but did not enhance offspring heat tolerance. For example, Betini et al. (2020) found that *D. magna* acclimated to 25 °C had offspring that reproduced earlier and lived longer than offspring of 15 °C-acclimated mothers, but these differences were independent of the temperature of the offspring environment. They did not find evidence of a fitness advantage for offspring living in an environment matching that of the mother. Additionally, elevated maternal temperature has been associated with resistance to infection (Garbutt et al. 2014) and to copper toxicity (Bae et al. 2016), but also with reduced fecundity (Garbutt et al. 2014; Betini et al. 2020). Thus, the effects of maternal temperature on offspring traits in *Daphnia* are complex, and not always straightforward to predict.

We did find one potential difference between offspring of the two temperature treatments: there was some evidence that regardless of strain, offspring of 28 °C-acclimated mothers tended to reproduce later than offspring of 20 °C-acclimated mothers (Fig. 5b). This is the opposite pattern from what we observed in the P generation. If this difference is biological, there are a few possible explanations for our finding: 1) mothers reared at 28 °C may be stressed, and could pass on costly nongenetic factors to their offspring (e.g., Eriksson Wiklund & Sundelin 2001; McGhee et al. 2012); 2) F1 *Daphnia* experienced the maternal temperature environment as embryos and neonates, and the shift from 28 °C to 20 °C could have stressed the offspring; 3) because 28 °C-acclimated mothers reproduced earlier, we often used first-clutch offspring from the 20 °C mothers and later-clutch offspring from the 28 °C mothers to maintain synchronization between temperature treatments. Our data also contrast with the results of Betini et al. (2020), who found that offspring of heat-acclimated mothers reproduced at earlier ages. In addition to the factors described above, the difference between that study and ours may also reflect variation in maternal effects among strains, which has been seen previously in *Daphnia* in response to temperature (Garbutt et al. 2014) and salinity (Mikulski & Mazurczak 2023).

In conclusion, *Daphnia magna* acclimated to high temperatures developed increased tolerance to acute heat stress, in ways that were strain-specific, but their offspring reared at benign temperatures did not inherit their mothers' acquired heat tolerance. It is possible that non-genetic factors were transmitted to offspring by heat-acclimated mothers, but were "overwritten" or "ignored" by their offspring because they were not relevant to the more benign environment experienced by the offspring. Betini et al. (2020) suggested that *Daphnia* may not have evolved transgenerational effects of temperature because seasonal variation makes parental temperature a poor predictor of offspring temperature. Age at reproduction differed between offspring of the two treatments, but the adaptive significance of this is not clear. Indeed, maternal effects in animals are often not straightforwardly adaptive (Uller et al. 2013; Beyer & Hambright 2017; Coakley et al. 2018) and are sometimes costly to offspring (Eriksson Wiklund & Sundelin 2001; McGhee et al. 2012). Non-genetic inheritance is incompletely understood, and further research is required to clarify how maternal effects are transmitted and what impact they have on both parental and offspring fitness.

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Authorship Statement

CSA conceived the study and secured funding. TN and CSA collected data. SS and CSA performed statistical analysis and created figures. All authors wrote and revised the manuscript.

Disclosure Statement

The authors declare that they have no competing interests.

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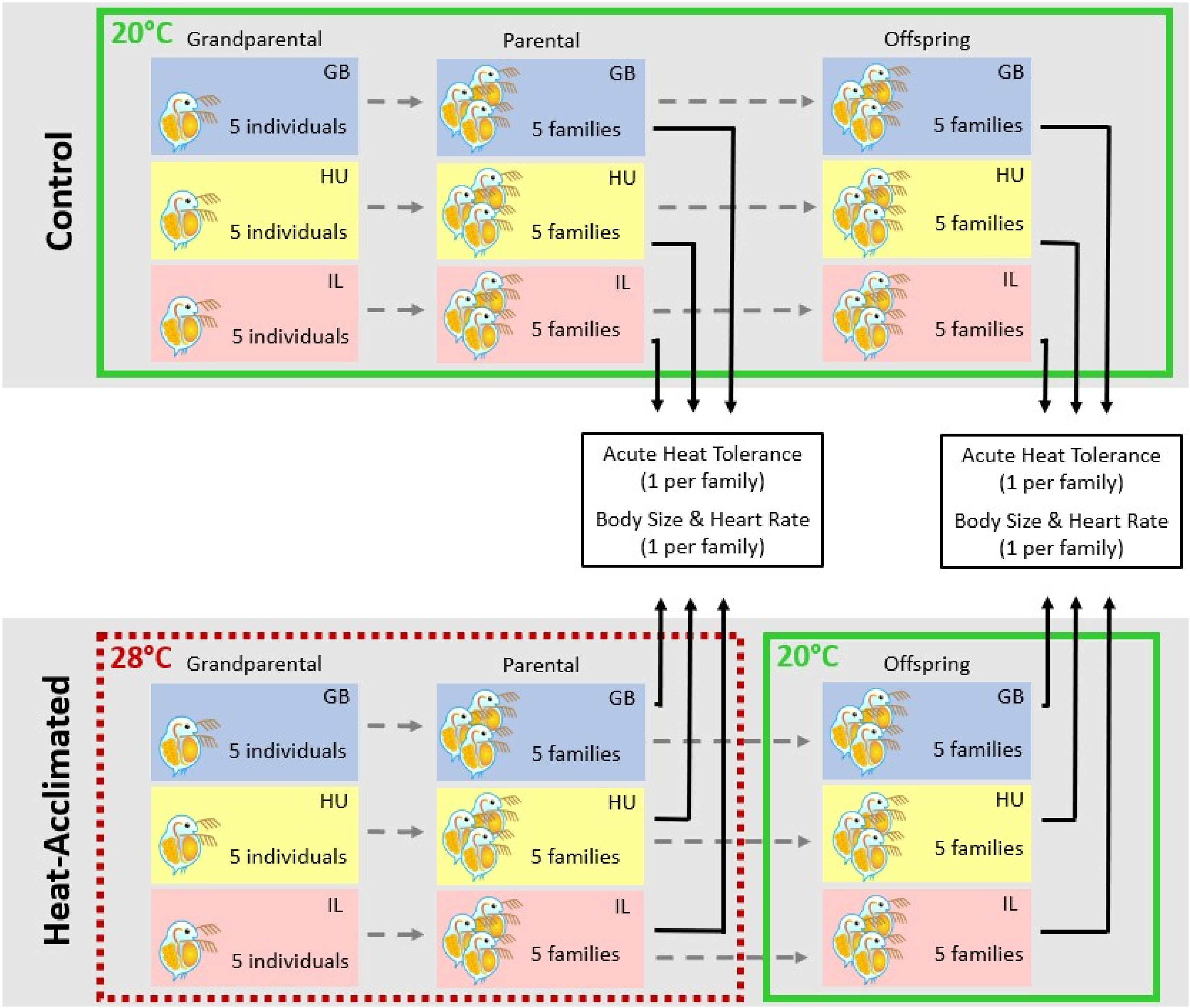

Figure 1. Schematic of experimental design. Five families (i.e., matrilines) from three asexual strains of *Daphnia magna* were subjected to one of two temperature treatments over three generations. Control: all three generations were kept in 20 °C water. Heat acclimated: the grandparental and parental generations were kept in 28 °C water, and the offspring generation was transferred to 20 °C within 24 h of birth. In adults of the parental and offspring generations of both treatments, acute heat tolerance at 37 °C, body size, and heart rate were measured within each family.

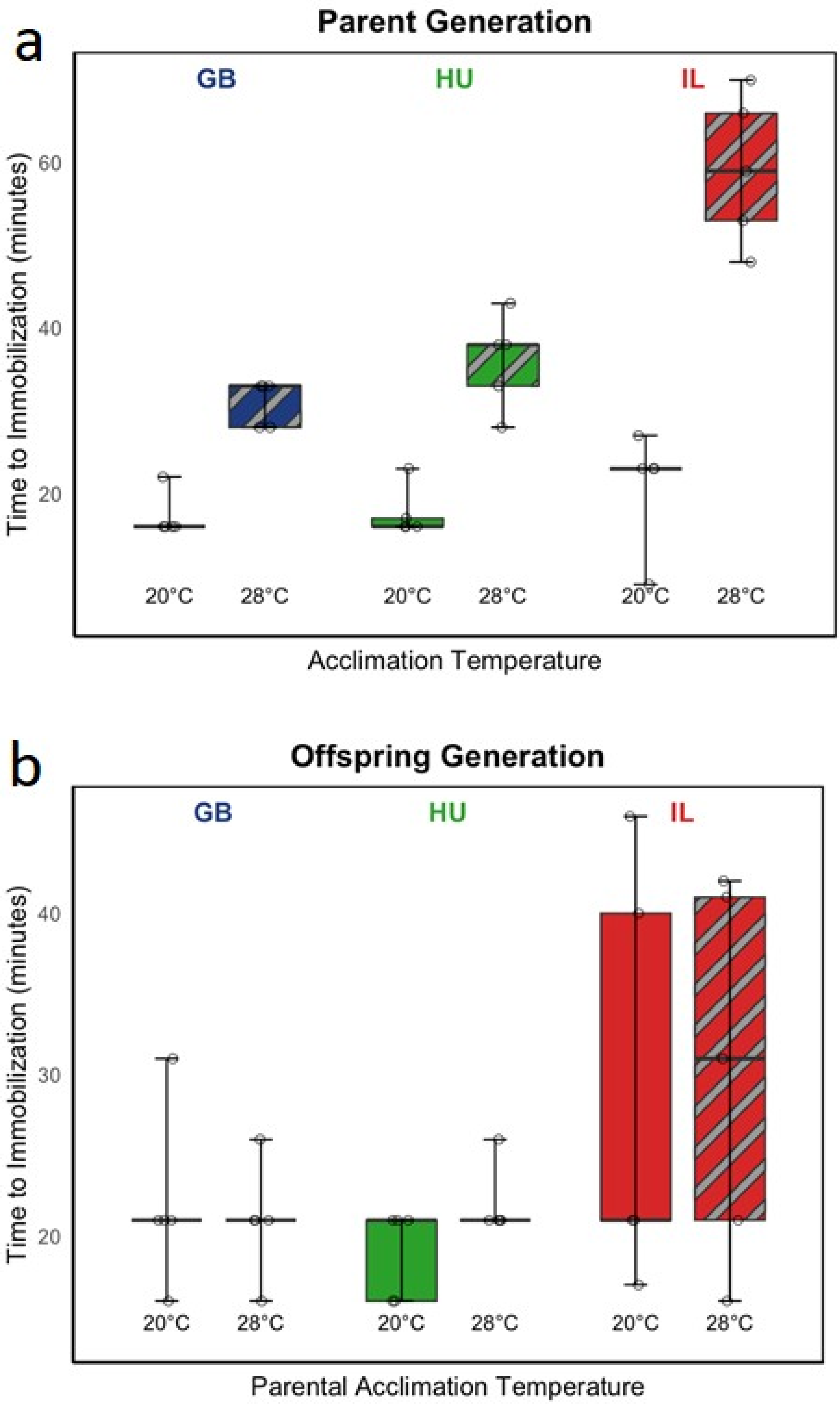
a
Parent Generation
GB
HU
IL
Time to Immobilization (minutes)
60
40
20
20°C
28°C
20°C
28°C
20°C
28°C
Acclimation Temperature
b
Offspring Generation
GB
HU
IL
Time to Immobilization (minutes)
40
30
20
20°C
28°C
20°C
28°C
20°C
28°C
Parental Acclimation Temperature

Figure 2. Responses of acute heat tolerance to parental thermal acclimatization in *Daphnia magna* across two generations. (a) Time to immobilization at 37°C of the parental generation reared at at either 20°C (control) or 28°C (heat-acclimated). (b) Time to immobilization at 37°C of the offspring generation, all reared at 20°C but whose parents were acclimated to either 20°C (control) or 28°C (heat-acclimated). Boxplots show medians, interquartile ranges, and whiskers extend to the full range of observed values and open circles representing individual data points. Strains are color-coded: GB (blue), HU (green), and IL (red), with striped fill indicating the 28°C treatment group.

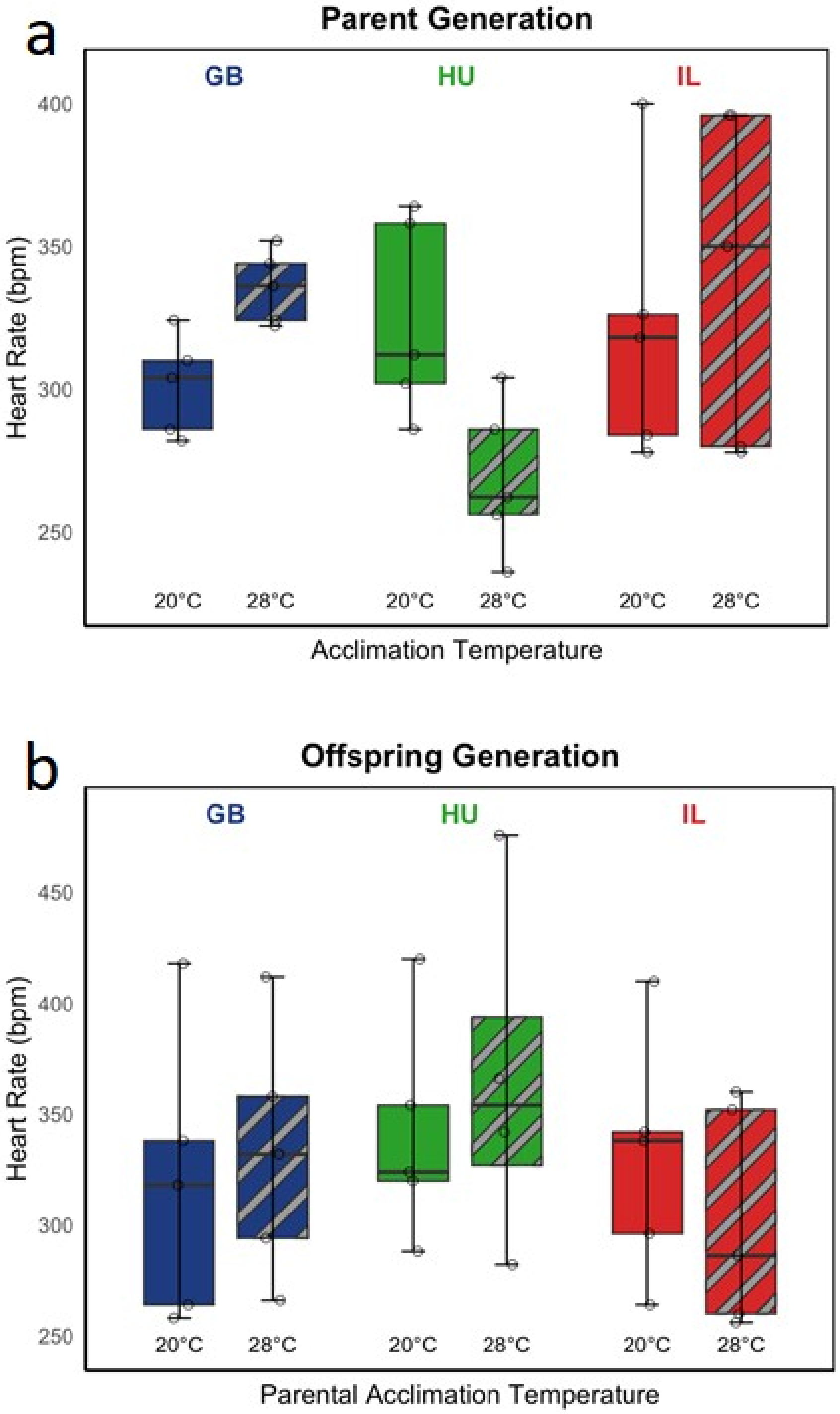

Figure 3. Responses of heart rate to parental thermal acclimatization in *Daphnia magna* across two generations. (a) Heart rates of the parental generation reared at

either 20°C (control) or 28°C (heat-acclimated). (b) Heart rates of the offspring generation, which were all reared at 20°C but whose parents were acclimated to 20°C (control) or 28°C (heat-acclimated). Heart rate (in beats per minute, bpm) was calculated as the mean of three 10-second counts taken per individual. Boxplots show medians, interquartile ranges, and whiskers extend to the full range of observed values. Strains are color-coded: GB (blue), HU (green), and IL (red), with striped fill indicating the 28°C treatment group.

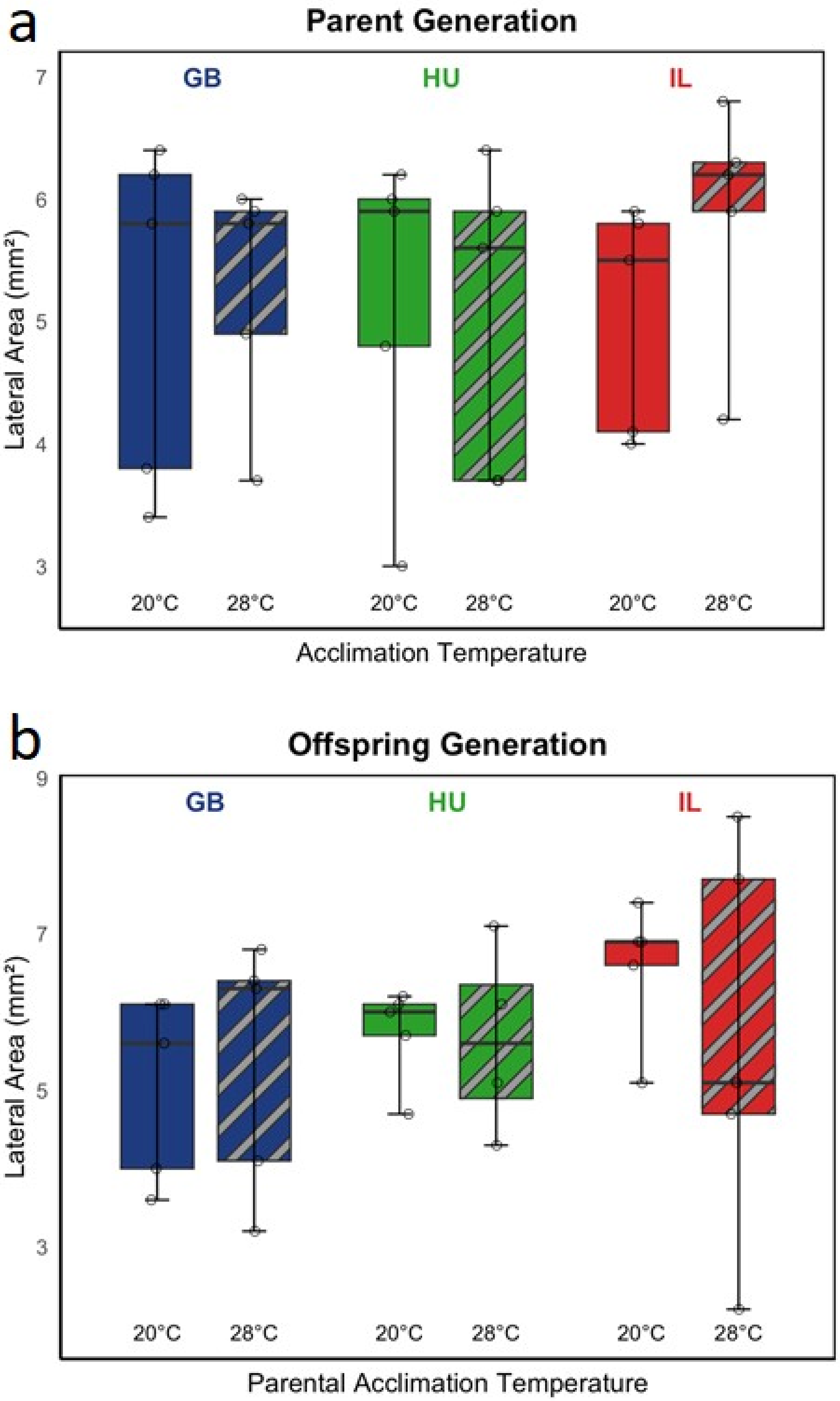
a
Parent Generation
GB
HU
IL
Lateral Area (mm²)
7
6
5
4
3
20°C
28°C
20°C
28°C
20°C
28°C
Acclimation Temperature
b
Offspring Generation
GB
HU
IL
Lateral Area (mm²)
9
7
5
3
20°C
28°C
20°C
28°C
20°C
28°C
Parental Acclimation Temperature

Figure 4. Lateral body area measurements of *Daphnia magna* from parental and offspring generations. (a) Lateral area of the parental generation reared at either 20°C (control) or 28°C (experimental). (b) Lateral area of the offspring generation, all reared at 20°C but derived from parents acclimated to either 20°C (control) or 28°C (heat-acclimated). Lateral area (in mm^2) was calculated from height and width measurements of individual Daphnia. Boxplots show medians, interquartile ranges, and whiskers extend to the full range of observed values and open circles representing individual data points. Strains are color-coded: GB (blue), HU (green), and IL (red), with striped fill indicating the 28°C treatment group.

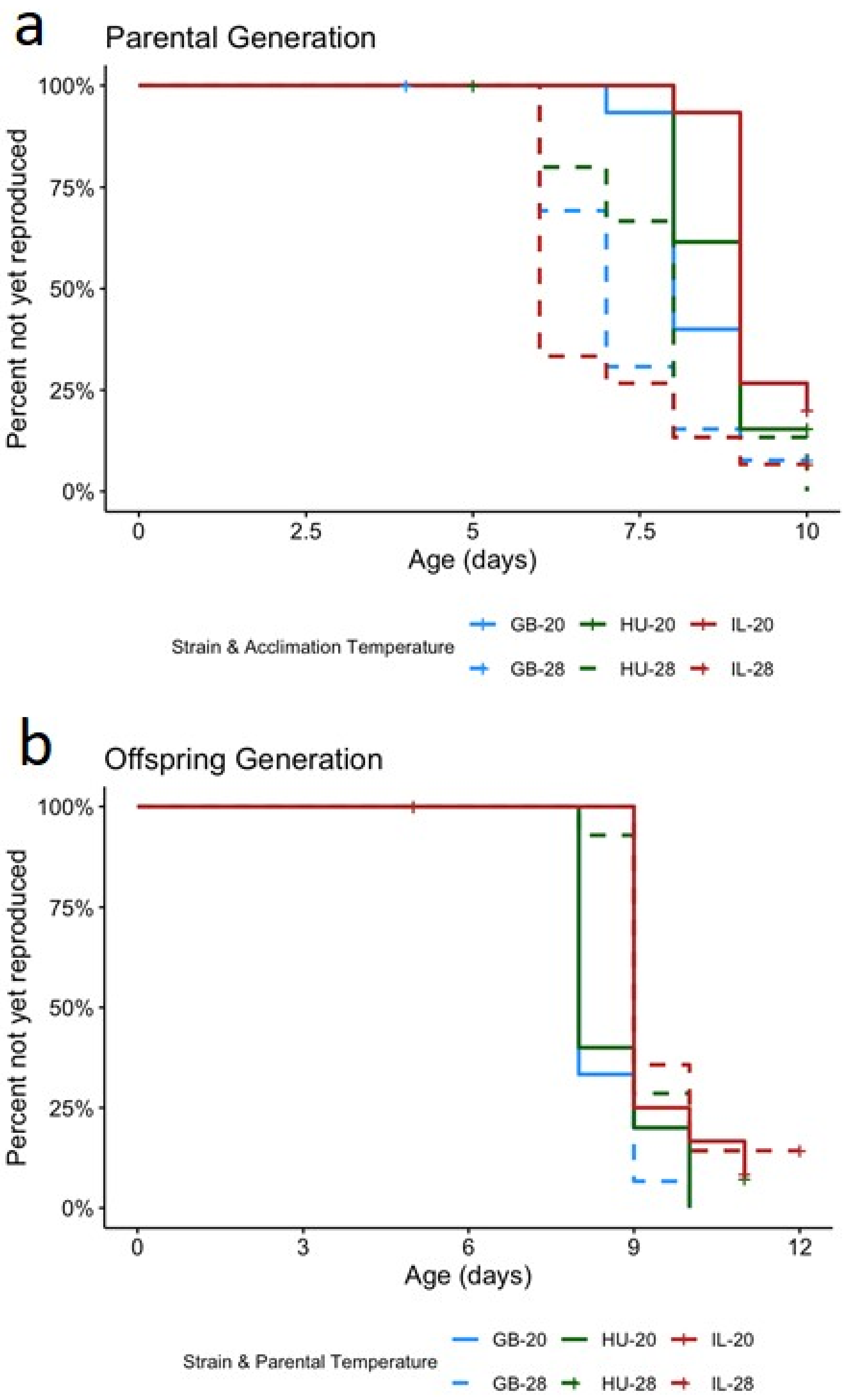

Figure 5. Effects of parental thermal acclimation on reproductive timing in *Daphnia magna* across two generations. (a) Age at first reproduction of the parental generation

reared at either 20°C (control) or 28°C (heat-acclimated). (b) Age at first reproduction of the offspring generation, all reared at 20°C but whose parents were acclimated to either 20°C (control) or 28°C (heat-acclimated). To assess reproductive timing, the age (in days) at which each individual first reproduced was recorded. Kaplan-Meier survival curves show the proportion of individuals that had not yet produced over time. Censored individuals (those that did not reproduce during the observation period) are marked with crosses. Strains are color coded: GB (blue), HU (green) and IL (red), with dashed lines indicating the 28°C group.