From climate warming to accelerated cellular ageing: an experimental study in wild birds

Antoine Stier ^{a,b,*\$}, Bin-Yan Hsu ^{a\$}, Nina Cossin-Sevrin ^{a,c}, Natacha Garcin ^{a,b} & Suvi Ruuskanen ^{a,d}

^a Department of Biology, University of Turku, Turku, Finland

^b Univ Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 LEHNA, F-69622, Villeurbanne, France

^c Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France

^d Department of Biological and Environmental Science, University of Jyväskylä

\$: equal contribution

*Corresponding author: antoine.stier@gmail.com ; amstie@utu.fi

Antoine Stier: <https://orcid.org/0000-0002-5445-5524>

Bin-Yan Hsu: <https://orcid.org/0000-0002-3799-0509>

Nina Cossin-Sevrin: <https://orcid.org/0000-0002-8174-8357>

Suvi Ruuskanen: <https://orcid.org/0000-0001-5582-9455>

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Abstract

Climate change is increasing both the average ambient temperature and the frequency and severity of heat waves. While direct mortality induced by heat waves is increasingly reported, sub-lethal effects are also likely to impact wild populations. We hypothesized that accelerated ageing could be a cost of being exposed to higher ambient temperature, especially in early-life when thermoregulatory capacities are not fully developed. We tested this hypothesis in wild great tit (*Parus major*) by experimentally increasing nest box temperature by *ca.* 2°C during postnatal growth and measuring telomere length, a biomarker of cellular ageing predictive of survival prospects in many bird species. While increasing early-life temperature does not affect growth or survival to fledging, it accelerates telomere shortening and reduces medium-term survival from 34% to 19%. Heat-induced telomere shortening was not explained by oxidative stress, but more likely by an increase in energy demand (*i.e.* higher thyroid hormones levels, increased expression of glucocorticoid receptor, increased mitochondrial density) leading to a reduction in telomere maintenance mechanisms (*i.e.* decrease in the gene expression of telomerase and protective shelterin). Our results thus suggest that climate warming can affect ageing rate in wild birds, with potential impact on population dynamics and persistence.

Significance statement

Stressful environmental conditions are known to accelerate cellular ageing, especially when experienced early in life. One unexplored avenue through which climate warming might affect wild animal populations is accelerated ageing. Here we show that increasing nest temperature by *ca.* 2°C during postnatal growth in a wild bird species can impact numerous physiological pathways and medium-term survival. Notably, artificially warming nests accelerates the shortening of telomeres, which are the protective end-caps of chromosomes considered as a hallmark of ageing. We thus suggest that warm ambient temperatures might accelerate ageing in wild animals, which can potentially impact population dynamics and extinction risk in the face of climate change.

Introduction

Climate change is increasing both the average ambient temperature and the frequency and severity of heat waves (IPCC 2014). While direct mortality induced by heat waves is increasingly reported (*e.g.* [1]), sub-lethal effects are also likely to impact population dynamics and persistence [2]. Despite regulating their body temperature, endotherms can also be sensitive to (even small) changes in temperature, especially during early-life stages when their thermoregulation is not yet fully developed [3]. Given that early-life experiences are known to have long-lasting effects on health, reproduction and even longevity (*e.g.* [4,5]), changes in early-life thermal environment associated with climate change are predicted to impact offspring phenotype and survival. Accordingly, some studies demonstrated associations between pre- or postnatal temperatures and survival in wild endotherm populations (*e.g.* [6–10]), and even longevity in humans [11]. Yet, the underlying physiological mechanisms remain poorly understood.

The physiological mechanisms of heat stress have been rather well characterized in laboratory endotherms. (i) Acute heat stress is known to increase the levels of key thermoregulatory and metabolic hormones, thyroid hormones (THs) [12,13]. (ii) Heat stress influences mitochondria, the cell powerhouse, as it reduces its efficiency to convert nutrients to ATP and increases its number [14] and (iii) increases the production of reactive oxygen species [15] contributing to oxidative stress and cellular ageing [16]. Yet, extrapolating findings from laboratory studies to wild populations in the context of climate change is challenging at best, partly because the range of temperature manipulations often exceeds temperature changes experienced in natural populations. A few studies in wild populations have experimentally increased early-life postnatal temperatures in endotherms [10,17,18], and report alterations of growth and body temperature. However, the potential mid to long-term consequences of such effects have not been characterized.

A key challenge, especially in relatively long-lived animals and wild populations, is to quantify the potential long-term deleterious effects of early-life conditions. Using the length of telomeres (*i.e.* the protective structure located at the end of chromosomes that progressively shorten with age), a key hallmark of ageing, as a molecular biomarker may help to overcome this challenge. Indeed, telomere length has been shown to predict survival (*i.e.* meta-analysis in [19]), and even lifetime reproductive success [20], and most of the telomere shortening is known to occur early in life (*e.g.* [21]). Various early-life environmental stressors,

including high incubation temperature in birds (*e.g.* [21]), have been shown to accelerate telomere shortening [22]. Telomere shortening is accelerated by oxidative stress [23], mitochondrial dysfunction [24] and changes in metabolic demand [25], which are predicted to increase in response to thermal stress (see above). While thermal environment has been shown to affect telomere shortening in ectotherms (*e.g.* [26,27]), we critically lack data from endotherm species.

In this study, we comprehensively assessed the effects of an experimental increase in temperature during early postnatal development on growth, short and medium-term survival (*i.e.* to fledging and to the next autumn-winter), as well as on key physiological and ageing markers (thyroid hormones, mitochondrial density, oxidative stress, telomere length and gene expression) in a wild great tit (*Parus major*) population. The temperature elevation mimicked the predicted ~ 2°C temperature increase (IPCC), and was applied during a vulnerable period of postnatal growth, *i.e.* when offspring are not protected by their mother, but still not fully capable of thermoregulation. We predicted that increasing nest temperature would lead to (i) reduced growth due to possible energetic costs of heat dissipation and/or lowered mitochondrial efficiency, (ii) reduced survival prospects, (iii) increased THs, (iv) increased oxidative stress, (v) increased mitochondrial density and (vi) shorter telomeres due to either oxidative stress-induced shortening or a decrease in telomere maintenance mechanisms.

Material & methods

Experimental design

The experiment was conducted in a nest box population of great tits on the island of Ruissalo (60°26.055 N, 22°10.391 E) in Finland. Half of the nestlings of each nest (total N = 32 nests) were swapped between nests two days after hatching to account for the effects of genetic background and rearing environment. To increase nest box temperature during growth of ca. 2°C, one heating pad (Uniheat Shipping warmer®, USA) was installed under the ceiling in half of the nest boxes (N = 17) between d7 and d14 (hereafter, “heated” nests), *i.e.* second half of postnatal development. For the other half (N = 15), a control pad (identical to the heating pads but not producing heat) was installed (hereafter “control” nests). Heating pads were checked and replaced every second day, and control nests were also visited to standardize human disturbance between the experimental groups. The actual nest box temperature was recorded with a thermo-logger (iButton thermochron, measuring at 3min intervals, 0.0625°C accuracy) and an average temperature over the course of heating treatment was calculated for each nest box (see ESM).

Nestling body mass and tarsus length were measured prior to the treatment on d7 and post-treatment on d14 after hatching. Blood samples were collected from the brachial vein at d14 (ca. 70µl) using heparinized capillaries. Whole-blood samples for oxidative stress and DNA/RNA extraction were immediately snap-frozen in liquid nitrogen, and an aliquot of whole-blood was stored on ice pack until centrifugation in the laboratory at the end of the day to assess plasma thyroid hormone levels.

To study potential long-term and delayed effects of early-life heating treatment, we recaptured juvenile great tits during the following autumn-winter using mist-nets at seven feeding stations located across the field site (total of 126 hours of mist-netting). Mass and wing length of the juveniles were recorded and a blood sample (ca. 80µl) was collected and stored as explained above. Our method of recapture provides an estimate of post-fledging survival (*i.e.* apparent survival), but could be slightly biased by dispersal.

Plasma thyroid hormones and oxidative stress

Plasma thyroid hormones (T3 and T4, expressed as pg/µL) were measured from d14 nestlings with nano-LC-MS/MS following [28]. Total glutathione (tGSH), the most abundant intra-cellular antioxidant, was measured from whole-blood samples with the ThioStar®

Glutathione Fluorescent Detection Kit (K005-FI, Arbor Assays, USA; technical repeatability: $R = 0.97$ (95% C.I. [0.96-0.98])). As a measure of oxidative damage, we assessed blood lipid peroxidation (malonaldehyde, MDA) using the TBARS-assay following [29] (technical repeatability: $R = 0.92$ (95% C.I. [0.88-0.94])).

Mitochondrial density, telomere length and molecular sexing

Relative telomere length (rTL) and mitochondrial DNA copy number ($mtDNA_{cn}$, an index of mitochondrial density) were quantified on DNA extracted from blood cells using real-time quantitative PCR (qPCR) assays, following [30] (see details in ESM). This technique estimates relative telomere length by determining the ratio (T/S) of telomere repeat copy number (T) to a single copy gene (SCG), and the relative $mtDNA_{cn}$ as the ratio between one mitochondrial gene and the same single copy gene. Birds were molecularly sexed using a qPCR approach adapted from [31,32] (see details in ESM).

Gene expression analysis

We used RT-qPCR to quantify the relative expression levels of 6 genes of interest from RNA extracted from blood cells (see ESM for details on methods). We quantified the expression of genes related to (i) cellular stress response: the glucocorticoid receptor (GCR) *nr3c1*, two heat shock proteins of the HSP70 (*i.e.* HSPA2) and HSP90 (*i.e.* HSP90B1) families, as well as the nuclear factor erythroid 2-related factor 2 NRF2 (an oxidative-stress-induced regulator of several antioxidants and cellular protective genes); and to (ii) telomere maintenance processes: the telomeric repeat binding factor 2 TERF2 (a shelterin protein helping in protecting telomeres) and the telomerase reverse transcriptase TERT (catalytic subunit of the enzyme responsible for telomere elongation).

Statistical analysis

For each trait of interest (see Fig. 1), we sequentially fitted three generalized linear mixed models (GLMMs, R package *lme4*): Model I assessed the effects of heating treatment, Model II the association with actual nest box temperature, and Model III their interaction. Maximum sample size was $N = 32$ nests, $n = 98$ nestlings d14 and $n = 26$ juveniles, but the final sample size is smaller and varies between physiological parameters according to sample availability and success of laboratory analyses (see specific sample size in ESM tables 2-21 and Fig. 1). In all

models, the identity of nest of origin (*i.e.* where a nestling was born) and the nest of rearing (*i.e.* where a nestling grew up after cross-fostering at day 2) were treated as random factors. Other random factors and fixed-effect covariates are included when relevant for the trait in question (see details in ESM). We report standardized effect size for each trait as Cohen's *d* and 95% confidence interval using the *emmeans* package in *R*. For survival analyses, In Odds Ratios were transformed to Cohen's *d* using the formula: $d = \ln\text{OddsRatio} \times (\sqrt{3}/\pi)$ following [33]

Results

Fig. 1: Effects of nest heating treatment on key life-history and physiological traits in wild great tits. Effects are presented as standardized effect sizes (Cohen's *d*, or transformed to Cohen's *d*) and 95% confidence intervals. d14: nestlings being 14 days old, Juv: juveniles (*i.e.* captured during the autumn/winter following their fledging); surv: survival; T3 and T4: plasma thyroid hormones; MDA: biomarker of oxidative damage to lipids measured in blood, tGSH: total glutathione (*i.e.* intra-cellular antioxidant molecule) content in blood cells; mtDNAcn: mtDNA copy number, a proxy for mitochondrial density measured in blood cells; rTL: relative telomere length (*i.e.* a biomarker of ageing) of blood cells; GCR: gene expression of the glucocorticoid receptor, HSP70 and HSP90: gene expression of heat shock proteins, NRF2: gene expression of an oxidative-stress-induced regulator of several antioxidants and cellular protective genes, TERF2: gene expression of a shelterin protein helping in

protecting telomeres, TERT: gene expression of the catalytic subunit of the enzyme responsible for telomere elongation. Detailed information on statistics is available in ESM Tables S2-S21. Significant effects according to full statistical models are presented with * symbols, and large effects presented in bold. The standard Cohen's *d* scale for negligible <0.2, small <0.5, medium <0.8 or large >0.8 effect size is presented with a colour scale. Sample size for each trait is presented between brackets.

We confirmed that our heating treatment was effective in increasing average nest temperature by *ca.* 1.84°C (large effect size; $t_{27.5} = 2.66$, $p = 0.013$; Fig. 1) over the heating period (*i.e.* day 7 to day 14). Nestlings from heated nests were not significantly lighter or smaller than control ones at day 14 (negligible and small effect size respectively; $p = 0.748$ and 0.969 ; Fig. 1, Tables S2-S3). Yet, we found a significant quadratic effect of actual nest temperature on body mass at day 14 (nestlings being heaviest at mid-temperatures, $p = 0.050$; Fig. 2, Table S2), that might be driven by an interaction (although non-significant; Table S2) between heating treatment and nest temperature (Fig. 2). Juveniles from heated nests were non-significantly heavier (large effect size; $p = 0.078$) but not larger (small effect size; $p = 0.723$) than control ones when recaptured the following autumn/winter (Fig. 1, Tables S4-S5).

Fig. 2: Relationship between nestling (*i.e.* day 14) body mass, actual nest temperature and heating treatment. The quadratic relationship (yellow line $\pm$ 95% confidence interval) between nestbox temperature and nesting body mass is significant ($p = 0.050$), while the interaction between heating treatment and temperature is not ($p = 0.261$). See Table S1 for full details on statistical models.

Survival to fledging was very high overall and not significantly influenced by the heating treatment (control: 94% vs. heated: 98%; $p = 0.82$; Fig. 1, Table S6). Birds from heated nests tended (although not significantly so, $p = 0.223$) to be less likely to be recaptured as juveniles (control: 34% vs. heated: 19%; large effect size; Fig. 1, Table S7).

Nestlings from heated nests had higher circulating thyroid hormones levels than controls, significantly so for T3 (large effect size; $p = 0.032$) but not for T4 (large effect size; $p = 0.090$; Fig. 1, Tables S8-S9). Heating treatment did not significantly alter oxidative stress

levels (glutathione: small effect size, $p = 0.347$; oxidative damage to lipids: negligible effect size, $p = 0.682$; Fig. 1, Tables S10-11). However, total glutathione levels were positively related to actual nest temperature ($\beta = 0.061$, $p = 0.039$; Table S11).

Fig. 3: Effects of age and nest heating treatment on: (A) mtDNA copy number as a proxy of mitochondrial density and cellular energetics, and (B) relative telomere length as a biomarker of ageing. Means are plotted $\pm$ SE and full statistical models are presented in Tables S12-S15.

Mitochondrial density in blood cells decreased sharply during postnatal development (Fig. 3A), and nestlings from heated nests had a higher mitochondrial density at the end of the heating treatment (large effect size; $p = 0.002$; Fig. 1 & 3A, Table S10), but not when recaptured as juveniles (negligible effect size; $p = 0.554$; Fig. 1 & 3A, Table S11). Mitochondrial density at day 14 was also positively related to actual nest temperature ($\beta = 0.15$, $p = 0.050$, Table S10). Heating treatment did not significantly influence nestlings' telomere length (negligible effect size; $p = 0.580$; Fig. 1 & 3B, Table S12), but juveniles from heated nests had markedly shorter telomeres than controls (large effect size; $p = 0.033$; Fig. 1 & 3B, Table S13).

Gene expression was partly altered by nest heating treatment, with nestlings from heated nest showing significantly higher expression levels of the glucocorticoid receptor (GCR: large effect size; $p = 0.045$; Fig. 1, Table S16), and although non-significantly, lower expression levels of the telomere-related genes TERF2 (large effect size; $p = 0.088$; Fig. 1, Table S17) and TERT (large effect size; $p = 0.085$; Fig. 1, Table S18). There was however no clear effect on the expression of heat shock proteins (HSP70: medium effect size, $p = 0.298$; HSP90: negligible effect size, $p = 0.985$; Fig. 1, Tables S19-20) or the regulator of antioxidant protection NRF2 (small effect size, $p = 0.504$; Fig. 1, Table S21). Actual nest temperature was positively related to GCR expression ($\beta = 0.16$, $p = 0.074$, Table S20), and negatively to TERF2 ($\beta = -0.25$, $p = 0.010$, Table S20), TERT ($\beta = -0.27$, $p = 0.002$, Table S20), HSP70 ($\beta = -0.22$, $p = 0.020$, Table S20) and NRF2 ($\beta = -0.18$, $p = 0.032$, Table S21).

Discussion

By experimentally manipulating early postnatal temperature of *ca.* 2°C (in line with predictions of climate changes), we demonstrated that exposure to higher ambient temperature during early-life could affect several physiological pathways (*i.e.* thyroid hormones, mitochondrial biogenesis, glucocorticoid signalling) and ultimately lead to accelerated cellular ageing (*i.e.* shorter telomeres) through a potential deregulation of telomere maintenance processes (*i.e.* shelterin protein and telomerase). While immediate survival was not impacted by the experimental increase in nest temperature, birds from heated nests were less likely to be seen alive the following autumn-winter.

Growth rate is known to be influenced in a complex manner by weather conditions [34], and previous experimental studies increasing nest temperature have reported contrasted results (positive effect: [17]; negative effects: [10,18]). Our experimental manipulation did not affect body size or mass during postnatal growth, but our results suggest that intermediate nest temperature might be optimal in our great tit population (*i.e.* significant quadratic effect of actual nest temperature).

Apparent survival to autumn-winter was lower for birds originating from heated nests (19% vs. 34%, large effect size), but not significantly so. Obtaining large enough sample size to detect significant effects on survival in experimental studies on wild animals is unfortunately challenging. Yet, our results are in accordance with observational data from a 12-year monitoring study of great tit survival in Spain, showing a negative association between ambient temperature during postnatal growth and post-fledging survival [6].

While there is some evidence from laboratory acute heat stress studies that thyroid hormones could be up-regulated when facing increased ambient temperatures [12,13], we show here that even a small increase in early-life temperature can increase thyroid hormones levels. Importantly, high thyroid hormone levels have been linked to increased mortality risks in adult humans [35] and increased susceptibility to free radicals in birds [36]. Yet, we found no significant effect of nest heating on two oxidative stress biomarkers (glutathione and oxidative damage to lipids), nor on NRF2 gene expression (an oxidative-stress-induced regulator of several antioxidants). Similarly, the gene expression of two heat shock proteins (HSP70 and HSP90 families) was not clearly impacted by nest heating. This suggests that a *ca.* 2°C increase in temperature might be too low to induce oxidative stress or a heat shock response (compared to heat stress experiments in the lab that often use > +10°C; *e.g.* [37]). Yet, the

gene expression of the glucocorticoid receptor *nr3c1* was increased in heated nests, suggesting either a response to a stressful stimulus (*e.g.* [38]) or potentially an increase in metabolic demand [39].

Both the rise in thyroid hormones and the observed increase in mitochondrial density at the end of the heating period would support the hypothesis of an increase in metabolic demand. In line with our results on mitochondrial density, mild heat stress has been shown to increase mitochondrial biogenesis *in vitro* [14] and *in vivo* [40]. This could be a way to compensate the typical decrease in mitochondrial coupling efficiency observed at higher body temperature (*e.g.* [41]), but measuring both body temperature (*i.e.* [10] showed that nest heating induced hyperthermia) and mitochondrial coupling efficiency [42] would be needed here to test this hypothesis.

While telomeres were not immediately impacted by the heating treatment (*i.e.* no effect at the end of the heating period on day 14), we discovered some important delayed effect since juveniles from heated nests had markedly shorter telomeres than controls individuals. Sample size of juveniles was relatively limited, and the lower apparent post-fledging survival of birds from heated nests could lead to bias associated with selective disappearance. Yet, this is unlikely to bias our results since we would expect individuals with shorter telomeres to disappear earlier from the population (and not the opposite), as this has previously been shown in our model species [43]. Additionally, the trend towards a decreased expression of genes related to telomere maintenance (TERF2 and TERT) observed at day 14 in nestlings from heated nests support the results observed on telomere length in juveniles. Our results are in accordance with recent findings in humans showing a negative effect of warm temperature and a positive effect of cold temperature during gestation on newborn telomere length [44]. While oxidative stress-induced telomere shortening [23] is unlikely to explain our results (see above), the ‘metabolic telomere attrition hypothesis’ [25] could be a good candidate. Indeed, this theory stipulates that increased metabolic demand mediated by increased glucocorticoid signalling (*i.e.* increased *nr3c1* expression observed here) can decrease investment in telomere maintenance processes (*i.e.* decreased expression of TERF2 and TERT observed here) and be associated with mitochondrial dysfunction [45].

To conclude, our study provides the first evidence that a moderate increase in ambient temperature during early-life can accelerate cellular ageing in a wild endotherm species. While the exact consequences of such increase in early-life temperature on individual fitness

and population dynamics remain to be determined, previous evidence at the inter-population level has shown that shorter telomeres might precede population extinction and be used as an early warning sign [46].

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Data accessibility

All data will be archived publicly in FigShare upon acceptance of the manuscript.

Ethical permits

All procedures were approved by the Animal Experiment Committee of the State Provincial Office of Southern Finland (license number ESAVI/2902/2018) and by the Environmental Center of Southwestern Finland (license number VARELY549/2018) granted to SR.

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ESM of ‘From climate warming to accelerated cellular ageing: an experimental study in wild birds’

Antoine Stier ^{a,b,*§}, Bin-Yan Hsu ^{a§}, Nina Cossin-Sevrin ^{a,c}, Natacha Garcin ^{a,b} & Suvi Ruuskanen ^{a,d}

qPCR assays for telomere length, mtDNA copy number and molecular sexing

We extracted DNA from blood cells using a standard salt extraction alcohol precipitation method ([1]). Extracted DNA was diluted in elution buffer BE for DNA preservation. DNA concentration and purity (260/280 > 1.80 and 260/230 > 2.00) were checked with a ND-1000-Spectrophotometer (NanoDrop Technologies, Wilmington, USA). DNA integrity was verified in 24 samples chosen randomly using gel electrophoresis (50 ng of DNA, 0.8 % agarose gel at 100 mV for 60 min) and DNA staining with Midori Green. Each sample was then diluted to a concentration of 1.2 ng.μL⁻¹ for subsequent qPCR analysis.

Relative telomere length (*rTL*) and mitochondrial DNA copy number (*mtDNAcn*, an index of mitochondrial density) were quantified using qPCR. Here, we used recombination activating gene 1 RAG1 as a single copy gene (verified as single copy using a BLAST analysis on the great tit genome) and cytochrome oxidase subunit 1 (COI1) as a mitochondrial gene (verified as non-duplicated in the nuclear genome using a BLAST analysis). The qPCR reactions were performed on a 384-QuantStudio™ 12K Flex Real-Time PCR System (Thermo Fisher), in a total volume of 12μL including 6ng of DNA, primers at a final concentration of 300nM and 6μL of SensiFAST™ SYBR lo-ROX (Bioline). Telomere, RAG1 and COI2 reactions were performed in triplicates on the same plates (10 plates in total); the qPCR conditions were: 3min at 95°C, followed by 35 cycles of 10 s at 95°C, 15 s at 58°C and 10s at 72°C. A DNA sample being a pool of DNA from 10 adult individuals was used as a reference sample and was included in triplicate on every plate. The efficiency of each amplicon was estimated from a standard curve of the reference sample ranging from 1.5 to 24ng. The primer sequences, as well as qPCR efficiencies and technical precision estimates (coefficient of variation and technical repeatability) are provided in Table S1. The relative telomere length and *mtDNAcn* of each sample were calculated as $(1+E_{f_{Tel\ or\ COI2}})^{\Delta Cq_{Tel\ or\ COI2}}$

$COI2 / (1 + Ef_{RAG1})^{\Delta Cq_{RAG1}}$; Ef being the amplicon efficiency, and ΔCq the difference in Cq -values between the reference sample and the focal sample.

The use of mtDNA_{cn} as an index of mitochondrial density has been questioned in human [2], but we have previously shown good correlations between mtDNA_{cn} and mitochondrial respiration rates in pied flycatcher [3] and great tit (Cossin-Sevrin et al. *in revision*). Great tits have quite peculiar telomeres, characterized notably by some ultra-long telomeres that do not seem to shorten with age in adults [4]. Since qPCR only provides an estimate of overall telomere length, it could be suboptimal for this study species. Yet, relative telomere length (*i.e.* measured using qPCR) in this species has been shown to shorten during the nestling stage [5,6], to respond to environmental factors (e.g. hatching asynchrony: [5]; altitude: [6]; urbanization: [7]) and to predict adult survival [8]. Within-individual repeatability of telomere length has recently been suggested to be an important factor to evaluate the pertinence of telomere length data in a given study/species [9], and the biological repeatability in our dataset was $R = 0.44$ [0.25-0.60], which is close to the average reported by qPCR studies (*i.e.* $R = 0.47$), and well within the range of what has been reported for great tits [9].

Birds were molecularly sexed using a qPCR approach adapted from [10,11]. Forward and reverse sexing primers were 5'- CACTACAGGGAAAACTGTAC-3' (2987F) and 5'- CCCCTTCAGGTTCTTTAAAA -3' (3112R), respectively. qPCR reactions were performed in a total volume of 12μL including 6ng of DNA, primers at a final concentration of 800nM and 6μL of SensiFAST™ SYBR® Lo-ROX Kit (Bioline). qPCR conditions were: 3 min at 95°C, followed by 40 cycles of 45 s at 95°C, 60 s at 52°C and 60s at 72°C, then followed by a melting curve analysis (95°C 60s, 45°C 50s, increase to 95°C at 0.1°C/s, 95°C 30s). Samples were run in duplicates in a single plate and 6 adults of known sex were included as positive controls. Sex was determined by looking at the dissociation curve, with two peaks indicating the presence of a Z and W chromosome (female), and one peak indicating the presence of only the Z chromosomes (male).

RT-qPCR assays for evaluating gene expression

We used RT-qPCR to quantify the expression levels of 6 genes of interest in d14 nestlings (Table S1). First, RNA was extracted from 10 μ L of blood (immediately after the first thawing following -80°C storage) using Nucleospin RNA Plus extraction kit (Macherey-Nagel) following manufacturer instructions. Second, RNA concentration and purity were quantified using optical density. Samples not meeting quality criteria (i.e. RNA concentration > 25 ng/ μ L, 260/280 and 260/230 > 1.80) were excluded for further analysis. RNA integrity was checked using E-Gel 2% electrophoresis system (Invitrogen), and the ribosomal RNA 18S vs. 28S bands intensity, and deemed satisfactory. Samples were stored at -80°C for 2 weeks before cDNA synthesis. 600ng of RNA were used for cDNA synthesis using the SensiFAST™ cDNA Synthesis kit (Bioline) following manufacturer instructions. cDNA was diluted at a final concentration of 1.2 ng/ μ L for qPCR analysis. No-RT control samples were prepared following the same protocol, but without reverse transcriptase enzyme.

We assessed the expression genes related to 1) cellular stress response: the glucocorticoid receptor (GCR) nr3c1, two heat shock proteins of the HSP70 (i.e. HSPA2) and HSP90 (i.e. HSP90B1) families, as well as the nuclear factor erythroid 2-related factor 2 NRF2 (an oxidative-stress-induced regulator of several antioxidants and cellular protective genes); and 2) telomere maintenance processes: the telomeric repeat binding factor 2 TERF2 (a shelterin protein helping in protecting telomeres) and the telomerase reverse transcriptase TERT (catalytic subunit of the enzyme responsible for telomere elongation). qPCR was performed in a total volume of 12 μ L containing 5 μ L of each diluted cDNA sample (i.e. 1.2ng/ μ L) and 7 μ L of reaction mix containing primers (forward and reverse) at a final concentration of 300nM and Sensifast SYBR®No-ROX Mix (Bioline). The succinate dehydrogenase complex subunit A (SDHA; [12]) and the ribosomal protein L13 (RPL13, [13]) were used as reference genes. SDHA and RPL13 were identified as the most stable reference genes using geNorm software (geNorm M < 0.7, geNorm V < 0.15) and the geometric mean of these two genes was thus used as our reference gene. Primers (Table S1) have been designed whenever possible on exon-exon junction using NCBI primer designing tool using the *Parus major* reference genome. Specificity has

been checked using BLAST analysis and confirmed by a single narrow peak in melting curve analyses and the presence of a single PCR product of the expected size on agarose gel. Amplification in no-RT controls never occurred before at least 5 cycles after the lower Cq sample (> 8 Cq for all genes but TERT), and thus contamination by genomic DNA could not interfere with our results.

We ran gene-specific qPCR plates and each sample was analyzed in duplicate. Experimental groups were always balanced within each plate. We used a cDNA reference samples (*i.e.* ratio = 1) being a pool of 5 different individuals on every plate. One inter-plate standard sample was also run on every plate. qPCR assays were performed on a Mic qPCR instrument (Bio Molecular Systems) and included a two-step cycling with the following conditions: 2 minutes at 95°C; then 40 cycles of 5s at 95°C followed by 20s at 60°C (fluorescence reading) for all reactions. The expression of each gene was calculated as $(1+E_{\text{Target}})^{\Delta Cq(\text{Target})} / \text{geometric mean } [(1+E_{\text{SDHA}})^{\Delta Cq_{\text{SDHA}}}; (1+E_{\text{RPL13}})^{\Delta Cq_{\text{RPL13}}}]$, Ef being the amplification's efficiency and ΔCq being the difference between the Cq-values of the reference sample and the sample of interest.

Nest box temperature recording and data process

On day 7 upon the installation of the heating pad, a thermo-logger (iButton thermochron) was installed on the backboard inside the nest box, approximately 5 cm above the nest rim. Each iButton was programmed to record temperature data at a 3-min interval to the accuracy of 0.0625 °C and the time stamp was synced by the 1-Wire software (Maxim integrated) to one computer at the University of Turku. After the retrieval of iButtons on day 14, temperature data were exported. We calculated the daily (00:00 to 23:59) average temperature for each nest box, and then averaged the daily temperatures across the heating duration as the nest box temperature we used in the data analyses.

Statistics: Model specification

For each trait of interest, we sequentially fitted three generalized linear mixed models (GLMMs, R package *lme4* [15]): Model I assessed the effects of heating treatment, Model II the association with actual nest box temperature, and Model III their interaction (*i.e.* to test for potential accentuated effects of heating already warmer nests). In Model II, nest box temperature was first centered to the grand mean and both linear and quadratic effects of nest box temperature were tested. When the quadratic effect of nest box temperature was not significant, we dropped the quadratic term and performed the significance test on the linear effect of nest box temperature. In Model III, nest box temperature was mean-centered within each heating treatment to avoid collinearity, to properly test the interaction between nest temperature and the heating treatment. We assumed Gaussian error distribution for all traits, except for nestling and juvenile survival. Physiological markers (tGSH, MDA, plasma T3 and T4 concentrations and mtDNAcn), telomere length and gene expression data were all natural-log transformed before model fitting. Visual inspection on the residuals of all models confirmed no clear violation of such assumption. Because rTL, mtDNAcn and gene expression levels are relative values, we performed z-transformation to allow across-study comparison on the results (Verhulst 2020). Kenward-Roger approximation (package *pbkrtest* [16]) was used for significance testing. For nestling and juvenile survival, we fitted binomial GLMMs with Laplace

approximation. Nestlings that died before day 7 (*i.e.* before the heating treatment started, $n=20$) or day 9 ($n=5$) were all excluded. Sex-specific effects of heating treatment (Model I) and nest temperature (Model II) were tested but always came back non-significant (see Table S2-S21). Results of the main factors and covariates in Model I and II were therefore reported from the models without interactions.

The identities of nest of origin (*i.e.* where a nestling was born) and nest or rearing (*i.e.* where a nestling was reared after cross-fostering) were always considered random factors. However, in many cases, we observed very low estimate on the variance of the random factor in question. In such cases, we are able to exclude the common reasons that can cause singularity because our random factors contain more than 5 levels and in most cases the sample size per group is ≥ 2 . Secondly, we observed that the singular fit occurred on different random factors (the nest of origin or the nest of rearing) for different traits. This suggests that the natal condition such as genetics or prenatal maternal effects explain the variation better for some traits, while the postnatal rearing condition accounts for more variation in other traits. Therefore, the singularity in our models more likely reflects very low but true between-nest variance and should not be considered model failure. In support of the argument, in many cases where singularity occurred, we still observed that the variance for the random factor in question could be estimated, only it was extremely low (to the magnitude of 10^{-5} and often even lower). This again indicates that the singular fit reflects very low between-group variance and high within-group variance. Therefore, we believe that our data points are effectively independent and should not inflate type I error. Nevertheless, to fully reveal the information for readers' own judgement, we marked all the low random-effect variance in red to highlight singularity in the supplementary tables.

Due to missing data, sample sizes varied by traits and models, and are specified along with Table S2-S15.

Table S2: Results of linear mixed models for nestling body mass

Day-14 body mass (n=96)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.3767	0.6138			
Nest of origin (n=21)	Intercept	0.2964	0.5444			
Residual		0.5562	0.7458			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		1314.750	516.246	2.547		
Heating (No-heat)		-0.100	0.302	-0.332	1, 17.95	0.106
Brood size		-0.272	0.098	-2.772	1, 26.90	6.984
Day-7 body mass		0.394	0.067	5.870	1, 81.40	32.251
Date		-0.073	0.029	-2.518	1, 36.22	6.252
Sex (male)		0.436	0.190	2.299	1, 74.67	5.083
Heating × sex		-0.495	0.374	-1.321	1, 74.76	1.667
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.2533	0.5033			
Nest of origin (n=21)	Intercept	0.3115	0.5581			
Residual		0.5661	0.7524			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		1267.548	482.647	2.626		
Brood size		-0.277	0.087	-3.163		
Day-7 body mass		0.386	0.066	5.814		
Date		-0.071	0.027	-2.595		
Sex (male)		0.401	0.189	2.118		
Nest temperature		-0.047	0.079	-0.604		
(Nest temperature) ²		-0.073	0.034	-2.158	1, 22.65	4.292
Sex × (nest temperature) ²		0.043	0.041	1.055	1, 77.53	1.058
Model IIIa. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.4020	0.6340			
Nest of origin (n=21)	Intercept	0.2715	0.5210			
Residual		0.5591	0.7477			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		1114.376	547.111	2.037		
Heating (No-heat)		-0.021	0.316	-0.068		
Brood size		-0.249	0.103	-2.417		
Day-7 body mass		0.393	0.067	5.848		
Date		-0.062	0.031	-2.010		
Sex (male)		0.414	0.192	2.162		
Nest temperature						
(mean-centered within treatment)		-0.146	0.140	-1.046		
Heating × temperature		0.204	0.171	1.193	1, 18.33	1.348

Table S3: Results of linear mixed models for nestling body size

Day-14 body size (tarsus length, n=96)						
Model Ib. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	<0.0001	<0.0001			
Nest of origin (n=21)	Intercept	0.1960	0.4428			
Residual		0.2155	0.4643			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		231.400	304.800	0.759		
Heating (No-heat)		0.005	0.123	0.041	1, 15.09	0.002
Brood size		<0.001	0.044	0.012	1, 14.68	0.001
Day-7 tarsus length		0.216	0.053	4.067	1, 83.42	15.594
Date		-0.012	0.017	-0.699	1, 34.07	0.480
Sex (male)		0.623	0.112	5.585	1, 75.39	29.349
Measurer B		0.149	0.179	0.832	1, 25.89	0.962
Measurer C		0.272	0.185	1.474		
Heating × sex		-0.431	0.225	-1.920	1, 79.02	3.452
Model IIb. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	<0.0001	<0.0001			
Nest of origin (n=21)	Intercept	0.2012	0.4485			
Residual		0.2139	0.4625			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		236.415	302.383	0.782		
Brood size		-0.003	0.043	-0.070		
Day-7 tarsus length		0.224	0.052	4.295		
Date		-0.012	0.017	-0.722		
Sex (male)		0.621	0.113	5.515		
Measurer B		0.149	0.177	0.839		
Measurer C		0.322	0.188	1.716		
Nest temperature		0.013	0.045	0.283	1, 26.57	0.458
(Nest temperature) ²		-0.014	0.017	-0.824	1, 22.39	0.619
Sex × (nest temperature) ²		-0.039	0.024	-1.602	1, 76.45	2.396
Model IIIb. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.4023	0.6342			
Nest of origin (n=21)	Intercept	0.5150	0.7176			
Residual		0.6524	0.8077			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		1340.607	648.956	2.066		
Heating (No-heat)		0.050	0.334	0.149		
Brood size		-0.320	0.116	-2.754		
Day-7 tarsus length		0.385	0.096	4.030		
Date		-0.075	0.037	-2.046		
Sex (male)		0.489	0.208	2.347		
Measurer B		0.102	0.447	0.229		
Measurer C		0.145	0.406	0.357		
Nest temperature						
(mean-centered within treatment)		-0.161	0.158	-1.022		
Heating × temperature		0.240	0.187	1.286	1, 18.33	1.556

Table S4: Results of linear mixed models for juvenile body mass

Juvenile body mass (n=25)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=16)	Intercept	0.0125	0.1118			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.8796	0.9379			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		13.911	3.985	3.491		
Heating (No-heat)		-1.057	0.418	-2.532	1, 8.44	4.013
Brood size		0.141	0.134	1.051	1, 6.45	0.751
Sex (male)		0.408	0.419	0.973	1, 15.55	0.650
Day-14 body mass		0.225	0.204	1.104	1, 18.46	0.853
Heating × sex		0.051	0.956	0.053	1, 11.32	0.002
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=16)	Intercept	0.2858	0.5346			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.9387	0.9689			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		12.630	4.584	2.755		
Brood size		0.080	0.163	0.493		
Sex (male)		0.312	0.497	0.629		
Day-14 body mass		0.275	0.234	1.176		
Nest temperature		0.079	0.108	0.729	1, 9.47	0.426
(Nest temperature) ²		<0.001	0.061	0.007	1, 8.97	<0.001
Sex × (nest temperature) ²		0.119	0.145	0.822	1, 11.33	0.526
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=16)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.9371	0.9680			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		13.422	4.150	3.234		
Heating (No-heat)		-1.050	0.428	-2.456		
Brood size		0.131	0.141	0.927		
Sex (male)		0.570	0.457	1.246		
Day-14 body mass		0.248	0.213	1.162		
Nest temperature (mean-centered within treatment)		0.116	0.220	0.527		
Heating × temperature		-0.218	0.251	-0.870	1, 6.36	0.450

Table S5: Results of linear mixed models for juvenile body size

Juvenile body size (wing length, n=26)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=17)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		2.9310	1.7120			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	80.931	7.178	11.275			
Heating (No-heat)	0.315	0.726	0.433	1, 8.86	0.134	0.723
Brood size	0.314	0.241	1.307	1, 7.23	1.261	0.297
Sex (male)	1.664	0.735	2.264	1, 18.76	3.488	0.078
Day-14 body mass	-0.365	0.366	-0.999	1, 17.95	0.720	0.407
Heating × sex	0.169	1.625	0.104	1, 12.86	0.008	0.932
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=17)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	0.1316	0.3628			
Residual		2.7276	1.6516			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	80.937	7.012	11.543			
Brood size	0.352	0.240	1.468			
Sex (male)	1.612	0.735	2.192			
Day-14 body mass	-0.336	0.358	-0.940			
Nest temperature	0.116	0.154	0.752	1, 9.99	0.455	0.516
(Nest temperature) ²	-0.099	0.087	-1.145	1, 8.24	0.883	0.374
Sex × (nest temperature) ²	-0.178	0.211	-0.847	1, 12.80	0.545	0.474
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=16)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		2.7970	1.6720			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	82.177	7.140	11.509			
Heating (No-heat)	0.346	0.711	0.486			
Brood size	0.311	0.242	1.285			
Sex (male)	1.233	0.761	1.622			
Day-14 body mass	-0.417	0.366	-1.141			
Nest temperature						
(mean-centered within treatment)	-0.190	0.379	-0.503			
Heating × temperature	0.530	0.431	1.230	1, 9.44	1.034	0.335

Table S6: Results of generalized linear mixed models for nestling survival

Nestling survival from day 7 to fledging (n=101)				
Model I. Heating treatment				
Random effects:		Variance	Std. Dev.	
Nest of rearing (n=34)	Intercept	397.8000	19.9500	
Nest of origin (n=21)	Intercept	<0.0001	<0.0001	
Fixed effects:		Estimate	Std. Error	z
Intercept		12.574	6.237	2.016
Heating (No-heat)		-1.399	6.175	-0.227
				0.821
Model II. Nest box temperature				
Random effects:		Variance	Std. Dev.	
Nest of rearing (n=34)	Intercept	372.0000	19.2900	
Nest of origin (n=21)	Intercept	<0.0001	<0.0001	
Fixed effects:		Estimate	Std. Error	z
Intercept		12.207	5.032	2.426
Nest temperature		0.364	1.485	0.245
(Nest temperature) ²		-0.066	0.591	-0.112
				0.911
Model III. Heating treatment × Nest box temperature				
Random effects:		Variance	Std. Dev.	
Nest of rearing (n=34)	Intercept	366.9000	19.1600	
Nest of origin (n=21)	Intercept	<0.0001	<0.0001	
Fixed effects:		Estimate	Std. Error	z
Intercept		12.426	6.050	2.054
Heating (No-heat)		-0.945	6.675	-0.142
Nest temperature				
(mean-centered within treatment)		0.027	3.055	0.009
				0.993
Heating × temperature		0.503	3.613	0.139
				0.889

For survival, binomial GLMMs were fitted with maximum likelihood by Laplace approximation. As there were only 4 day-7 nestlings that failed to fledge, other covariates (sex and day-7 body mass) were excluded in these models.

Table S7: Results of generalized linear mixed models for juvenile survival

Post-fledging survival (n=97)				
Model I. Heating treatment				
Random effects:		Variance	Std. Dev.	
Nest of rearing (n=32)	Intercept	1.0590	1.0293	
Nest of origin (n=21)	Intercept	<0.0001	0.0014	
Fixed effects:	Estimate	Std. Error	z	p
Intercept	-9.411	4.840	-1.944	
Heating (No-heat)	0.837	0.687	1.218	0.223
Day-14 body mass	0.399	0.257	1.553	0.120
Sex (male)	0.812	0.629	1.291	0.197
Heating × sex	-0.174	1.157	-0.151	0.880
Model II. Nest box temperature				
Random effects:		Variance	Std. Dev.	
Nest of rearing (n=32)	Intercept	1.0576	1.0284	
Nest of origin (n=21)	Intercept	0.1757	0.4191	
Fixed effects:	Estimate	Std. Error	z	p
Intercept	-10.867	5.282	-2.058	
Day-14 body mass	0.471	0.273	1.724	0.085
Nest temperature	-0.001	0.167	-0.009	0.993
(Nest temperature) ²	0.107	0.091	1.177	0.239
Sex (male)	0.922	0.650	1.419	0.156
Sex × (nest temperature) ²	0.005	0.167	0.030	0.976
Model III. Heating treatment × Nest box temperature				
Random effects:		Variance	Std. Dev.	
Nest of rearing (n=32)	Intercept	0.4695	0.6852	
Nest of origin (n=21)	Intercept	0.3719	0.6098	
Fixed effects:	Estimate	Std. Error	z	p
Intercept	-11.861	5.038	-2.354	
Heating (No-heat)	0.709	0.641	1.107	0.268
Day-14 body mass	0.534	0.267	2.000	0.046
Nest temperature				
(mean-centered within treatment)	0.485	0.316	1.532	0.126
Sex (male)	0.856	0.630	1.359	0.174
Heating × temperature	-0.661	0.387	-1.708	0.088

Table S8: Results of linear mixed models for nestling plasma T3 levels

Day-14 nestling blood T3 level (n=24)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	0.0942	0.3069			
Residual		0.0952	0.3085			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	1.458	1.294	1.127			
Heating (No-heat)	-0.484	0.151	-3.204	1, 6.19	7.559	0.032
Sex (male)	-0.078	0.157	-0.497	1, 10.80	0.140	0.716
Day-14 body mass	-0.070	0.071	-0.987	1, 13.41	0.660	0.431
Heating × sex	0.631	0.336	1.880	1, 18.81	2.564	0.126
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	0.0111	0.1052			
Nest of origin (n=13)	Intercept	0.0228	0.1509			
Residual		0.1477	0.3843			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-0.138	1.549	-0.089			
Sex (male)	0.086	0.192	0.448			
Day-14 body mass	0.008	0.083	0.093			
Nest temperature	0.124	0.058	2.138	1, 17.32	2.649	0.122
(Nest temperature) ²	-0.043	0.026	-1.649	1, 10.51	1.947	0.192
Sex × (nest temperature) ²	0.042	0.074	0.568	1, 12.09	0.189	0.671
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	0.0893	0.2989			
Residual		0.1091	0.3303			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	1.114	1.514	0.736			
Heating (No-heat)	-0.478	0.161	-2.965			
Sex (male)	-0.025	0.183	-0.139			
Day-14 body mass	-0.053	0.082	-0.648			
Nest temperature (mean-centered within treatment)	0.035	0.100	0.346			
Heating × temperature	0.023	0.111	0.209	1, 13.02	0.034	0.857

Table S9: Results of linear mixed models for nestling plasma T4 levels

Day-14 nestling blood T4 level (n=24)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	0.0934	0.3056			
Residual		0.0525	0.2291			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	2.423	1.004	2.413			
Heating (No-heat)	-0.283	0.118	-2.403	1, 5.23	4.314	0.090
Sex (male)	0.233	0.122	1.905	1, 8.99	1.998	0.191
Day-14 body mass	-0.033	0.055	-0.599	1, 11.027	0.243	0.632
Heating × sex	0.293	0.288	1.019	1, 17.02	0.763	0.395
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	0.0032	0.0566			
Nest of origin (n=13)	Intercept	0.0827	0.2876			
Residual		0.0753	0.2744			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	1.928	1.232	1.565			
Sex (male)	0.223	0.155	-0.142			
Day-14 body mass	-0.009	0.066	-0.142			
Nest temperature	0.040	0.052	0.767	1, 12.31	0.523	0.483
(Nest temperature) ²	-0.019	0.022	-0.898	1, 8.66	0.644	0.444
Sex × (nest temperature) ²	0.085	0.065	1.308	1, 9.94	1.165	0.306
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=19)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	0.1076	0.3280			
Residual		0.0489	0.2210			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	2.961	1.089	2.719			
Heating (No-heat)	-0.246	0.120	-2.051			
Sex (male)	0.180	0.135	1.328			
Day-14 body mass	-0.061	0.059	-1.045			
Nest temperature (mean-centered within treatment)	-0.094	0.085	-1.109			
Heating × temperature	0.116	0.090	1.282	1, 12.91	1.211	0.291

T4 and T3 were natural-log transformed.

Table S10: Results of linear mixed models for nestling blood oxidative damage to lipids (MDA) levels

Day-14 nestling blood MDA level (n=57)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=26)	Intercept	0.0005	0.0220			
Nest of origin (n=18)	Intercept	<0.0001	<0.0001			
Batch of TBARS (n=6)	Intercept	0.0994	0.3152			
Residual		0.0519	0.2278			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		-2.991	0.530	-5.642		
Heating (No-heat)		0.033	0.074	0.447	1, 15.72	0.174
Sex (male)		0.017	0.071	0.240	1, 47.59	0.050
Day-14 body mass		-0.018	0.028	-0.629	1, 37.22	0.327
Heating × sex		0.027	0.146	0.183	1, 45.71	0.028
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=26)	Intercept	<0.0001	<0.0001			
Nest of origin (n=18)	Intercept	<0.0001	<0.0001			
Batch of TBARS (n=6)	Intercept	0.1126	0.3356			
Residual		0.0502	0.2241			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		-3.069	0.525	-5.847		
Sex (male)		0.017	0.069	0.239		
Day-14 body mass		-0.010	0.028	-0.350		
Nest temperature		-0.005	0.019	-0.284	1, 11.51	0.017
(Nest temperature) ²		-0.015	0.009	-1.608	1, 11.62	1.715
Sex × (nest temperature) ²		-0.032	0.017	-1.905	1, 33.96	2.634
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=26)	Intercept	0.0006	0.0241			
Nest of origin (n=18)	Intercept	<0.0001	<0.0001			
Batch of TBARS (n=6)	Intercept	0.0890	0.2984			
Residual		0.0521	0.2282			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		-2.930	0.533	-5.501		
Heating (No-heat)		0.050	0.074	0.672		
Sex (male)		0.003	0.073	0.042		
Day-14 body mass		-0.021	0.028	-0.742		
Nest temperature						
(mean-centered within treatment)		-0.027	0.030	-0.892		
Heating × temperature		0.053	0.036	1.488	1, 10.96	1.835

Table S11: Results of linear mixed models for nestling blood total glutathione (tGSH) levels

Day-14 nestling blood tGSH level (n=56)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=27)	Intercept	<0.0001	<0.0001			
Nest of origin (n=18)	Intercept	0.0237	0.1539			
Batch of GSH (n=2)	Intercept	0.0207	0.1438			
Residual		0.0936	0.3060			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		-0.226	0.773	-0.292		
Heating (No-heat)		-0.098	0.095	-1.030	1, 14.62	0.944
Sex (male)		-0.007	0.097	-0.067	1, 49.43	0.004
Day-14 body mass		-0.056	0.042	-1.351	1, 47.08	1.558
Heating × sex		0.247	0.194	1.277	1, 44.97	1.360
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=27)	Intercept	0.0063	0.0791			
Nest of origin (n=18)	Intercept	0.0058	0.0761			
Batch of GSH (n=2)	Intercept	0.0148	0.1217			
Residual		0.0949	0.3080			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		-0.485	0.751	-0.646		
Sex (male)		-0.000	0.091	-0.001		
Day-14 body mass		-0.046	0.040	-1.154		
Nest temperature		0.061	0.025	2.438	1, 15.48	5.099
(Nest temperature) ²		0.006	0.013	0.491	1, 17.84	0.184
Sex × nest temperature		0.013	0.045	0.296	1, 48.24	0.072
Sex × (nest temperature) ²		0.008	0.024	0.307	1, 40.72	0.073
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=27)	Intercept	0.0122	0.1105			
Nest of origin (n=18)	Intercept	<0.0001	<0.0001			
Batch of GSH (n=2)	Intercept	0.0064	0.0797			
Residual		0.0927	0.3044			
Fixed effects:		Estimate	Std. Error	t	df	F
Intercept		-0.389	0.736	-0.529		
Heating (No-heat)		-0.095	0.099	-0.964		
Sex (male)		-0.018	0.095	-0.184		
Day-14 body mass		-0.047	0.040	-1.185		
Nest temperature						
(mean-centered within treatment)		0.009	0.039	0.226		
Heating × temperature		0.084	0.051	1.640	1, 13.84	2.180

Table S12: Results of linear mixed models for nestling blood cell mtDNA copy number

Day-14 nestling mtDNA copy number (n=90)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.1105	0.3224			
Nest of origin (n=21)	Intercept	0.3423	0.5851			
Residual		0.5128	0.7161			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	0.028	1.431	0.019			
Heating (No-heat)	-0.821	0.220	-3.735	1, 16.85	13.112	0.002
Sex (male)	0.127	0.193	0.655	1, 79.06	0.400	0.529
Day-14 body mass	0.018	0.078	0.224	1, 84.38	0.046	0.831
Heating × sex	-0.174	0.374	-0.466	1, 77.93	0.203	0.653
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.3526	0.5938			
Nest of origin (n=21)	Intercept	0.1323	0.3637			
Residual		0.5271	0.7261			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-0.204	1.551	-0.132			
Sex (male)	0.102	0.200	0.511			
Day-14 body mass	0.013	0.083	0.157			
Nest temperature	0.153	0.077	1.983	1, 29.95	4.163	0.050
(Nest temperature) ²	-0.004	0.036	-0.103	1, 28.97	0.010	0.922
Sex × nest temperature	0.014	0.094	0.146	1, 79.05	0.020	0.888
Sex × (nest temperature) ²	0.024	0.053	0.452	1, 83.26	0.186	0.667
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.1909	0.4369			
Nest of origin (n=21)	Intercept	0.2422	0.4921			
Residual		0.5170	0.7191			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	0.173	1.483	0.117			
Heating (No-heat)	-0.786	0.245	-3.207			
Sex (male)	0.143	0.197	0.728			
Day-14 body mass	0.009	0.081	0.114			
Nest temperature (mean-centered within treatment)	0.051	0.113	0.452			
Heating × temperature	0.036	0.136	0.266	1, 18.85	0.065	0.802

Table S13: Results of linear mixed models for juvenile blood cell mtDNA copy number

Juvenile mtDNA copy number (n=23)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.9976	0.9988			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-7.514	4.240	-1.772			
Heating (No-heat)	0.404	0.515	0.784	1, 7.97	0.382	0.554
Sex (male)	-0.237	0.441	-0.538	1, 17.13	0.212	0.651
Juvenile body mass	0.404	0.224	1.803	1, 15.60	1.979	0.179
Heating × sex	0.833	0.936	0.891	1, 15.37	0.493	0.493
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	<0.0001	<0.0001			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.9743	0.9871			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-6.209	3.641	-1.705			
Sex (male)	-0.139	0.438	-0.318			
Juvenile body mass	0.325	0.200	1.627			
Nest temperature	-0.057	0.096	-0.589	1, 8.02	0.123	0.735
(Nest temperature) ²	0.070	0.052	1.359	1, 6.91	1.435	0.270
Sex × (nest temperature) ²	-0.107	0.109	-0.982	1, 12.42	0.663	0.431
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	0.0158	0.1258			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		1.049	1.0244			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-6.805	4.475	-1.521			
Heating (No-heat)	0.494	0.553	0.894			
Sex (male)	-0.200	0.490	-0.409			
Juvenile body mass	0.359	0.238	1.505			
Nest temperature (mean-centered within treatment)	0.281	0.332	0.847			
Heating × temperature	-0.327	0.361	-0.906	1, 10.73	0.508	0.491

Table S14: Results of linear mixed models for nestling blood cell relative telomere length

Day-14 nestling telomere length (n=90)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.3542	0.5951			
Nest of origin (n=21)	Intercept	0.0614	0.2477			
Residual		0.6041	0.7773			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-1.977	1.534	-1.288			
Heating (No-heat)	0.161	0.280	0.574	1, 21.12	0.315	0.580
Sex (male)	-0.212	0.210	-1.011	1, 81.19	0.959	0.331
Day-14 body mass	0.110	0.084	1.317	1, 84.06	1.589	0.211
Heating × sex	-0.264	0.399	-0.663	1, 77.89	0.406	0.526
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.2174	0.4662			
Nest of origin (n=21)	Intercept	0.2136	0.4621			
Residual		0.6089	0.7803			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-1.999	1.604	-1.246			
Sex (male)	-0.211	0.208	-1.014			
Day-14 body mass	0.108	0.086	1.251			
Nest temperature	-0.043	0.077	-0.557	1, 29.11	0.512	0.480
(Nest temperature) ²	0.030	0.035	0.857	1, 27.41	0.662	0.423
Sex × (nest temperature) ²	-0.008	0.055	-0.154	1, 82.35	0.021	0.884
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=32)	Intercept	0.1579	0.3973			
Nest of origin (n=21)	Intercept	0.2808	0.5299			
Residual		0.6093	0.7805			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-2.149	1.575	-1.364			
Heating (No-heat)	0.040	0.247	0.162			
Sex (male)	-0.197	0.209	-0.941			
Day-14 body mass	0.121	0.086	1.397			
Nest temperature (mean-centered within treatment)	0.069	0.116	0.596			
Heating × temperature	-0.229	0.138	-1.663	1, 18.36	2.516	0.130

Table S15: Results of linear mixed models for juvenile blood cell relative telomere length

Juvenile telomere length (n=24)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	<0.0001	0.0011			
Nest of origin (n=13)	Intercept	0.1108	0.3329			
Residual		0.4093	0.6398			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	1.648	2.887	0.571			
Heating (No-heat)	1.475	0.373	3.952	1, 5.90	7.716	0.033
Sex (male)	0.260	0.313	0.833	1, 17.38	0.473	0.501
Juvenile body mass	-0.153	0.153	-0.999	1, 13.25	0.575	0.462
Heating × sex	0.088	0.683	0.129	1, 16.87	0.011	0.917
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	0.9899	0.9949			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.3033	0.5507			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	4.777	2.939	1.625			
Sex (male)	-0.037	0.365	-0.101			
Juvenile body mass	-0.250	0.158	-1.582			
Nest temperature	-0.072	0.134	-0.536	1, 10.61	0.295	0.598
(Nest temperature) ²	-0.033	0.073	-0.457	1, 8.93	0.159	0.699
Sex × (nest temperature) ²	0.113	0.123	0.913	1, 14.34	0.604	0.450
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=14)	Intercept	0.2534	0.5034			
Nest of origin (n=13)	Intercept	<0.0001	<0.0001			
Residual		0.2857	0.5345			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	1.164	2.714	0.429			
Heating (No-heat)	1.530	0.412	3.712			
Sex (male)	-0.045	0.314	-0.143			
Juvenile body mass	-0.118	0.144	-0.823			
Nest temperature						
(mean-centered within treatment)	-0.087	0.251	-0.349			
Heating × temperature	0.296	0.275	1.075	1, 9.18	0.709	0.421

Table S16: Results of linear mixed models for nr3c1 expression levels

Day-14 nestling nr3c1 expression (n=40)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.6435	0.8022			
Nest of origin (n=20)	Intercept	<0.0001	0.0004			
Residual		0.2912	0.5396			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	2.460	2.414	1.019			
Heating (No-heat)	-0.756	0.340	-2.226	1, 17.16	4.667	0.045
Sex (male)	0.032	0.274	0.117	1, 25.80	0.011	0.917
Day-14 body mass	-0.103	0.129	-0.799	1, 34.87	0.534	0.470
Plate	-0.444	0.260	-1.705	1, 22.84	2.380	0.137
Heating × sex	0.651	0.563	1.157	1, 28.28	1.027	0.320
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.6502	0.8063			
Nest of origin (n=20)	Intercept	<0.0001	0.0009			
Residual		0.3231	0.5684			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	0.888	2.689	0.330			
Sex (male)	0.033	0.282	0.117			
Day-14 body mass	-0.036	0.141	-0.252			
Plate	-0.418	0.272	-1.536			
Nest temperature	0.163	0.087	1.868	1, 23.59	3.506	0.074
(Nest temperature) ²	-0.016	0.043	-0.379	1, 25.80	0.125	0.727
Sex × (nest temperature) ²	0.023	0.072	0.315	1, 27.42	0.079	0.780
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.6244	0.7902			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.3194	0.5652			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	1.402	2.626	0.534			
Heating (No-heat)	-0.780	0.342	-2.279			
Sex (male)	0.051	0.291	0.176			
Day-14 body mass	-0.046	0.141	-0.329			
Plate	-0.429	0.281	-1.530			
Nest temperature (mean-centered within treatment)	0.110	0.155	0.711			
Heating × temperature	-0.011	0.202	-0.056	1, 21.87	0.003	0.958

Table S17: Results of linear mixed models for TERF2 expression levels

Day-14 nestling TERF2 expression (n=40)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.6949	0.8336			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.2067	0.4546			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-2.937	2.292	-1.281			
Heating (No-heat)	0.625	0.336	1.860	1, 17.27	3.264	0.088
Sex (male)	-0.149	0.250	-0.595	1, 21.63	0.288	0.597
Day-14 body mass	0.138	0.122	1.126	1, 33.70	1.046	0.314
Plate	0.184	0.236	0.781	1, 19.22	0.501	0.488
Heating × sex	0.340	0.517	0.657	1, 22.66	0.329	0.572
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.5250	0.7246			
Nest of origin (n=20)	Intercept	0.0857	0.2927			
Residual		0.1931	0.4395			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-0.397	2.334	-0.170			
Sex (male)	-0.163	0.238	-0.685			
Day-14 body mass	0.025	0.123	0.201			
Plate	0.178	0.226	0.786			
Nest temperature	-0.245	0.081	-3.031	1, 25.35	7.765	0.010
(Nest temperature) ²	-0.027	0.039	-0.673	1, 26.67	0.395	0.535
Sex × nest temperature	0.061	0.120	0.508	1, 24.87	0.208	0.652
Sex × (nest temperature) ²	-0.015	0.060	-0.248	1, 21.81	0.049	0.826
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.4607	0.6787			
Nest of origin (n=20)	Intercept	0.1587	0.3983			
Residual		0.1880	0.4335			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-1.891	2.297	-0.823			
Heating (No-heat)	0.619	0.298	2.077			
Sex (male)	-0.059	0.245	-0.242			
Day-14 body mass	0.081	0.123	0.661			
Plate	0.065	0.231	0.283			
Nest temperature (mean-centered within treatment)	-0.073	0.143	-0.513			
Heating × temperature	-0.225	0.178	-1.260	1, 20.77	1.394	0.251

Table S18: Results of linear mixed models for TERT expression levels

Day-14 nestling TERT expression (n=40)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	<0.0001	<0.0001			
Nest of origin (n=20)	Intercept	0.4313	0.6567			
Residual		0.5662	0.7524			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-1.172	2.426	-0.483			
Heating (No-heat)	0.537	0.274	1.959	1, 13.88	3.452	0.085
Sex (male)	-0.116	0.293	-0.397	1, 29.48	0.126	0.726
Day-14 body mass	0.051	0.131	0.389	1, 32.00	0.126	0.725
Plate	0.039	0.278	0.141	1, 26.65	0.017	0.899
Heating × sex	0.070	0.621	0.113	1, 32.50	0.010	0.921
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	<0.0001	<0.0001			
Nest of origin (n=20)	Intercept	0.1894	0.4352			
Residual		0.5335	0.7304			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	0.309	2.324	0.133			
Sex (male)	-0.107	0.266	-0.403			
Day-14 body mass	-0.019	0.123	-0.153			
Plate	-0.081	0.263	-0.308			
Nest temperature	-0.268	0.073	-3.672	1, 23.37	12.876	0.002
(Nest temperature) ²	0.035	0.035	0.991	1, 24.90	0.819	0.374
Sex × nest temperature	0.120	0.128	0.938	1, 29.54	0.681	0.416
Sex × (nest temperature) ²	-0.099	0.065	-1.523	1, 32.70	1.803	0.189
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	<0.0001	<0.0001			
Nest of origin (n=20)	Intercept	0.2481	0.4981			
Residual		0.4797	0.6926			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-0.235	2.273	-0.103			
Heating (No-heat)	0.612	0.249	2.457			
Sex (male)	0.004	0.271	0.015			
Day-14 body mass	0.002	0.123	0.015			
Plate	-0.190	0.266	-0.716			
Nest temperature (mean-centered within treatment)	-0.103	0.128	-0.805			
Heating × temperature	-0.265	0.154	-1.714	1, 15.78	2.448	0.138

Table S19: Results of linear mixed models for HSP70 expression levels

Day-14 nestling HSP70 expression (n=40)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.7109	0.8431			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.2268	0.4762			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-2.537	2.353	-1.078			
Heating (No-heat)	0.378	0.342	1.104	1, 17.25	1.150	0.298
Sex (male)	-0.008	0.259	-0.033	1, 22.34	0.001	0.977
Day-14 body mass	0.119	0.126	0.950	1, 34.02	0.746	0.394
Plate	0.183	0.244	0.141	1, 19.82	0.461	0.505
Heating × sex	0.022	0.539	0.040	1, 23.71	0.001	0.972
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.5995	0.7743			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.2111	0.4959			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-0.419	2.388	-0.175			
Sex (male)	-0.035	0.245	-0.143			
Day-14 body mass	0.022	0.125	0.176			
Plate	0.172	0.234	0.736			
Nest temperature	-0.216	0.080	-2.710	1, 23.95	6.164	0.020
(Nest temperature) ²	-0.023	0.040	-0.577	1, 26.15	0.290	0.595
Sex × nest temperature	0.045	0.123	0.366	1, 25.68	0.108	0.745
Sex × (nest temperature) ²	0.007	0.062	0.113	1, 22.86	0.010	0.920
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.6003	0.7748			
Nest of origin (n=20)	Intercept	<0.0001	0.0005			
Residual		0.2077	0.4558			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-1.613	2.359	-0.684			
Heating (No-heat)	0.394	0.319	1.236			
Sex (male)	0.058	0.253	0.229			
Day-14 body mass	0.070	0.126	0.555			
Plate	0.068	0.242	0.280			
Nest temperature (mean-centered within treatment)	-0.073	0.143	-0.507			
Heating × temperature	-0.231	0.187	-1.235	1, 22.41	1.356	0.256

Table S20: Results of linear mixed models for HSP90 expression levels

Day-14 nestling HSP90 expression (n=40)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.4735	0.6881			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.3562	0.5968			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-7.956	2.331	-3.414			
Heating (No-heat)	-0.006	0.317	-0.019	1, 17.07	<0.001	0.985
Sex (male)	-0.153	0.275	-0.558	1, 30.07	0.254	0.618
Day-14 body mass	0.419	0.125	3.360	1, 34.57	9.541	0.004
Plate	0.308	0.263	1.170	1, 26.80	1.122	0.299
Heating × sex	0.189	0.557	0.338	1, 28.92	0.088	0.769
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.4259	0.6526			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.3544	0.5953			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-6.470	2.469	-2.621			
Sex (male)	-0.177	0.269	-0.659			
Day-14 body mass	0.346	0.130	2.661			
Plate	0.287	0.261	1.098			
Nest temperature	-0.130	0.077	-1.677	1, 23.17	2.268	0.146
(Nest temperature) ²	-0.020	0.038	-0.530	1, 25.24	0.243	0.627
Sex × (nest temperature) ²	0.030	0.067	0.453	1, 30.00	0.164	0.689
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.3964	0.6296			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.3644	0.6037			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-6.564	2.364	-2.777			
Heating (No-heat)	0.026	0.302	0.085			
Sex (male)	-0.185	0.268	-0.691			
Day-14 body mass	0.345	0.127	2.724			
Plate	0.278	0.258	1.076			
Nest temperature (mean-centered within treatment)	-0.160	0.085	-1.874			
Heating × temperature	-0.372	0.173	-2.151	1, 20.54	4.054	0.057

Table S21: Results of linear mixed models for NRF2 expression levels

Day-14 nestling NRF2 expression (n=40)						
Model I. Heating treatment						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.4850	0.6964			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.4215	0.6492			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-6.242	2.446	-2.552			
Heating (No-heat)	0.233	0.330	0.705	1, 17.05	0.465	0.504
Sex (male)	0.294	0.291	1.011	1, 31.01	0.834	0.368
Day-14 body mass	0.308	0.131	2.354	1, 34.32	4.694	0.037
Plate	0.316	0.279	1.131	1, 27.74	1.048	0.315
Heating × sex	-0.047	0.591	-0.080	1, 29.52	0.005	0.945
Model II. Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.3795	0.6160			
Nest of origin (n=20)	Intercept	<0.0001	<0.0001			
Residual		0.4109	0.6410			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-4.624	2.501	-1.848			
Sex (male)	0.266	0.276	0.961			
Day-14 body mass	0.228	0.132	1.730			
Plate	0.256	0.269	0.951			
Nest temperature	-0.182	0.077	-2.355	1, 22.89	5.194	0.032
(Nest temperature) ²	0.011	0.038	0.285	1, 24.93	0.070	0.794
Sex × nest temperature	0.164	0.132	1.241	1, 32.20	1.226	0.277
Sex × (nest temperature) ²	0.007	0.069	0.097	1, 31.17	0.008	0.932
Model III. Heating treatment × Nest box temperature						
Random effects:		Variance	Std. Dev.			
Nest of rearing (n=31)	Intercept	0.2992	0.5470			
Nest of origin (n=20)	Intercept	0.0394	0.1985			
Residual		0.3334	0.5774			
Fixed effects:	Estimate	Std. Error	t	df	F	p
Intercept	-5.598	2.266	-2.470			
Heating (No-heat)	0.234	0.279	0.840			
Sex (male)	0.486	0.263	1.848			
Day-14 body mass	0.276	0.122	2.268			
Plate	0.045	0.256	0.174			
Nest temperature (mean-centered within treatment)	0.070	0.129	0.543			
Heating × temperature	-0.461	0.167	-2.758	1, 20.35	6.649	0.018

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