RESEARCH ARTICLE

Low incubation temperature slows the development of cold tolerance in a precocial bird

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ABSTRACT

Incubating birds trade off self-maintenance for keeping eggs warm. This causes lower incubation temperature in more challenging conditions, with consequences for a range of offspring traits. It is not yet clear how low developmental temperature affects cold tolerance early in life. This is ecologically important because before full thermoregulatory capacity is attained, precocial chicks must switch between foraging and being brooded when their body temperature declines. Hence, we studied how cold tolerance during conditions similar to a feeding bout in the wild was affected by incubation temperature in Japanese quail (*Coturnix japonica*). Cold-incubated (35.5°C) chicks took the longest to develop, hatched at a smaller size, and remained smaller during their first week of life compared with chicks incubated at higher temperatures (37.0 and 38.5°C). This was reflected in increased cooling rate and reduced homeothermy, probably on account of reductions in both heat-producing capacity and insulation. Lower cold tolerance could exacerbate other temperature-linked phenotypic effects and, hence, also the trade-off between future and current reproduction from the perspective of the incubating parent.

KEY WORDS: Body temperature, Development, Endothermy, Heterothermy, Life history, Poultry, Thermoregulation

INTRODUCTION

The demands of avian incubation often parallel, or even exceed, those during nestling feeding (Nord and Williams, 2015). Thus, incubation is a trade-off between parental self-maintenance and investment in keeping the eggs at a temperature that is conducive for growth and maturation (Monaghan and Nager, 1997; Reid et al., 2002). In line with this trade-off, parents incubating under strenuous conditions, such as when food availability is low, when it is cold, or when clutches are large, reduce the amount of heat passed to the eggs through reduced incubation temperatures and/or via changes in the amount of time spent incubating (Ardia et al., 2010; MacDonald et al., 2013; Nord and Nilsson, 2012; Nord et al., 2010). By analogy, parents invest more in keeping eggs warm when the demands of incubation are relieved (e.g. by experimental heating of the nest or clutch; Ardia et al., 2009; Reid et al., 2000). It follows that average incubation temperature in the wild varies depending on both environmental factors and parental conditions.

Even slight variation in egg temperature may have far-reaching effects on offspring phenotype (DuRant et al., 2013b).

Accordingly, chicks from eggs that develop at low average temperature hatch with reduced energy reserves (DuRant et al., 2011b; Hepp et al., 2006; Olson et al., 2006), have a lower growth rate (DuRant et al., 2010), are smaller at independence (Nord and Nilsson, 2011), and show reduced locomotor performance (Hopkins et al., 2011), lower immunocompetence (DuRant et al., 2011a) and increased stress sensitivity (DuRant et al., 2010) compared with chicks from eggs that develop at high temperatures. These effects can be large enough to reduce long-term survival (Berntsen and Bech, 2016; Hepp and Kennamer, 2012; but see Nord and Nilsson, 2016).

Low incubation temperature can increase the metabolic rate of chicks (e.g. Nord and Nilsson, 2011). This has been interpreted as a maternal effect that prepares chicks for life in a cold world, on the premise that high metabolic rate is indicative of improved thermogenic capacity (Nichelmann and Tzschentke, 2002). Pre-natal temperature conditioning in this manner is exploited by the poultry industry, where short-duration ($\leq$24 h) thermal stimuli from the second trimester (when the hypothalamus–thyroid–pituitary–adrenal axis starts to develop) improve post-hatching cold or heat tolerance depending on the direction of the manipulation (Shinder et al., 2011; Yahav, 2009). However, the ecological relevance of these studies is unclear, as free-ranging birds are unlikely to experience such precisely timed and dosed temperature variation (Nord and Giroud, 2020). Temperature variation in nature is likely to be more constant, e.g. for the duration of a developmental stage, in line with consistent differences in parental investment. The phenotypic consequences of this long-term exposure are often markedly different from those following short-term manipulation in poultry studies. For example, continuous mildly hypothermic incubation reduces chicks' tolerance of long-term cold exposure, whereas continuous mildly hyperthermic incubation increases it (DuRant et al., 2013a, 2012). However, we are still missing studies that assess how cold- and warm-incubated chicks deal with low temperature during shorter time periods, such as when precocial offspring alternate short foraging bouts with brooding by parents. This is of great ecological interest, because low body temperature (T_b) will limit feeding bout duration (Pedersen and Steen, 1979) and, hence, body mass gain (Jørgensen and Blix, 1985).

We investigated whether variation in incubation temperature alters thermoregulation over short time periods, similar to feeding bouts in the wild. We incubated Japanese quail (*Coturnix japonica*) eggs at three biologically relevant temperatures and then measured chicks' capacity to resist cooling in a common garden setting during the first week of life. If low incubation temperature is conducive for subsequent cold tolerance (as in poultry studies), we predicted that cold-incubated chicks would show increased cold tolerance. If, in contrast, continuously sub-normal incubation temperature constrains growth and maturation (as in studies of wild birds), we predicted that cold tolerance would be higher in warm- than in cold-incubated chicks.

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MATERIALS AND METHODS

Incubation and housing

Japanese quail, *Coturnix japonica* Temminck and Schlegel 1849, eggs (Stjärnås Djur och Fjäder, Lessebo, Sweden) of unknown sex were incubated in three incubators (Ruvmax, Ödskölt, Sweden) maintained at low (35.5°C; 19 eggs), normal (37.0°C; 20 eggs) or high (38.5°C; 20 eggs) temperature. The incubators were set up next to each other in a room with constant photic and thermal conditions. Our previous work on another model, but with the same incubators, has shown that the incubator per se does not influence the phenotypic effects of variation in developmental temperature observed in chicks (Nord and Nilsson, 2011). We measured temperature (±0.0625°C) immediately adjacent to the eggs in 24 h cycles, using a temperature data logger (iButton DS1922-L, Maxim, Sunnyvale, CA, USA; accuracy ±0.5°C), to ensure that the eggs were at target temperature. Relative humidity in the incubators was maintained at 65–70% until pipping, and was then increased to 80%.

At hatching, we weighed the chicks to the nearest 0.1 g and marked them uniquely on the tarsi using non-toxic felt-tipped pens. They were then transferred to a holding pen (1.5×2.0 m) with a bedding of *Populus* wood shavings and *ad libitum* access to food (poultry chick feed; Lantmännen, Malmö, Sweden), water and a heat lamp (38.5°C at floor level). Air temperature in the facility ranged from 15 to 17°C.

All procedures were approved by the Malmö/Lund Animal Ethics Committee (permit no. M 238-07).

Cooling challenge and growth monitoring

The cooling experiment was designed to simulate chicks alternating between short foraging bouts and parental brooding in the wild. Thus, starting one day after hatching (henceforth day 1), we collected chicks from the pen and immediately (within 10 s of capture) measured T_b by inserting a type K thermocouple (diameter: 0.9 mm) connected to a Testo 925 thermometer (Testo AG, Lenzkirch, Germany) 15 mm through the cloaca (insertion to further depths did not alter the readings). Less than 30 s later, the chicks were put in open 1 l plastic vials inside a climate chamber (BK600, Vötsch Industrietechnik, Balingen, Germany) kept at 20°C for 10 min, after which we measured T_b again, weighed the chick, and returned it to the pen. This was repeated every other day until day 7, when it was assumed that the chicks had reached homeothermy (as defined by Ricklefs, 1987; Visser, 1998).

Data analyses

Hatching success varied between treatments, being highest at 37.5°C (70%; 14 of 20 eggs), lower at 35.5°C (53%; 10 of 19 eggs), and lower still at 38.5°C (40%; 8 of 20 eggs). There were no visible signs of development in the eggs that did not hatch. We did not record any mortality in the two higher temperature treatments, but four chicks in the 35.5°C group died between day 1 and day 3, and one died between day 3 and day 5. Hence, sample sizes differed with age in this treatment.

We calculated the relative rate of change in T_b, $R_{\Delta T_b}$, as the natural logarithm of T_b change over time, following Andreasson et al. (2016):

$$R_{\Delta T_b} = \frac{\log(T_{b_2} - T_a) - \log(T_{b_1} - T_a)}{t} / m_b^{0.67}, \qquad (1)$$

where T_{b_1} and T_{b_2} were T_b before and after cooling, T_a was ambient temperature in the climate chamber, and t was the length of cooling (i.e. 10 min). We divided the resultant T_b change by body mass, m_b, to the power of 0.67, to account for a higher surface area to volume

ratio in smaller birds. The absolute value of $R_{\Delta T_b}$ was square root transformed before analysis.

We then calculated a homeothermy index, H, following (Ricklefs, 1987):

$$H = \frac{T_{b_2} - T_a}{T_{b_1} - T_a}, \qquad (2)$$

according to which a chick with $H=1$ is completely homeothermic and a chick with $H=0$ is completely poikilothermic.

We used R 3.6.1 (http://www.R-project.org/) for the statistics. We compared the length of the incubation period and body mass at hatching using linear models (lm function in R base) with treatment as a factor. Body mass, $R_{\Delta T_b}$ and H during the experiment were compared using linear mixed effects models (lmer function in lme4; Bates et al., 2015) with treatment, day and treatment×day as factors and chick identity as a random intercept. The interaction was removed when non-significant, but all main effects were retained. P-values for the mixed models were inferred from likelihood ratio tests. Parameter estimates and their s.e.m. were calculated using the emmeans package (Lenth, 2016). When the interaction was significant ($P<0.05$), *post hoc* tests were performed between treatments within days. Data in the text are predicted means±s.e.m.

RESULTS

The length of the incubation period was temperature dependent, being shortest at 38.5°C (16.0±0.1 days), longer at 37.0°C (17.3± 0.1 days) and markedly prolonged at 35.5°C (19.7±0.1 days) (Fig. 1A, Table 1). At hatching, chicks incubated at 38.5°C (8.59± 0.20 g) and 37.0°C (8.15±0.15 g) weighed significantly more than chicks incubated at 35.5°C (7.46±0.18 g) (Fig. 1B, Table 1).

The body mass gain during the first week of life differed between the treatments (treatment×age: $P<0.001$) (Fig. 2A, Table 1). High-temperature chicks were heavier than chicks from the two other groups on day 1. Chicks incubated at 37.0°C subsequently recovered, such that body mass was similar to that in the 38.5°C group for the remainder of the experiment, and well above the body mass of 35.5°C chicks (Fig. 2A).

Chicks from the 35.5°C group suffered a larger reduction in T_b during the cooling challenge than chicks from the two other groups
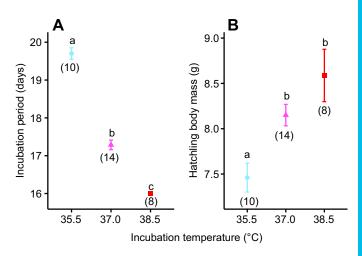

Fig. 1. Effects of incubation temperature on incubation period and hatchling body mass. The figure shows mean±s.e.m. (A) incubation period and (B) body mass at hatching in Japanese quail that were incubated at low (35.5°C), normal (37.0°C) or high (38.5°C) temperature. Different lowercase letters indicate statistically significant differences, and numbers within parentheses denote sample sizes.

Table 1. Output of the statistical analyses

Model	Estimate±s.e.m.	F/χ^2	d.f.	P
Incubation period (days)				
Treatment		191.6	2, 29	<0.001
35.5°C [A]	19.7±0.1			
37.0°C [B]	17.3±0.1			
38.5°C [C]	16.0±0.1			
Hatchling body mass (g)				
Treatment		9.1	2, 29	0.001
35.5°C [A]	7.46±0.18			
37.0°C [B]	8.15±0.15			
38.5°C [B]	8.59±0.20			
Body mass day 1–day 7 (g)				
Treatment				
35.5°C [A]	11.30±0.39			
37.0°C [B]	14.30±0.27			
38.5°C [C]	15.50±0.36			
Age				
1 day [A]	8.13±0.26			
3 days [B]	11.47±0.28			
5 days [C]	15.95±0.29			
7 days [D]	19.17±0.29			
Treatment×age		24.1	6	<0.001
1 day				
35.5°C [A]	6.68±0.45			
37.0°C [A]	7.84±0.37			
38.5°C [B]	9.87±0.50			
3 days				
35.5°C [A]	8.44±0.56			
37.0°C [B]	12.47±0.37			
38.5°C [B]	13.51±0.48			
5 days				
35.5°C [A]	12.92±0.61			
37.0°C [B]	16.78±0.37			
38.5°C [B]	18.15±0.50			
7 days				
35.5°C [A]	17.20±0.61			
37.0°C [B]	20.00±0.37			
38.5°C [B]	20.32±0.50			
T_b change slope ($R_{\Delta T_b}$)				
Treatment		22.8	2	<0.001
35.5°C [A]	0.0706±0.0037			
37.0°C [B]	0.0485±0.0026			
38.5°C [B]	0.0479±0.0035			
Age		266.5	3	<0.001
1 day [A]	0.1126±0.0024			
3 days [B]	0.0559±0.0026			
5 days [C]	0.0334±0.0026			
7 days [D]	0.0206±0.0026			
Treatment×age		7.6	6	0.270
Homeothermy index (H)				
Treatment		14.6	2	0.001
35.5°C [A]	0.795±0.016			
37.0°C [B]	0.865±0.011			
38.5°C [B]	0.867±0.015			
Age		240.4	3	<0.001
1 day [A]	0.606±0.011			
3 days [B]	0.850±0.012			
5 days [C]	0.937±0.012			
7 days [D]	0.975±0.012			
Treatment×age		7.1	6	0.309

The table shows test statistics and degrees of freedom, and parameter estimates for final terms, for models describing the effect of incubation temperature on incubation period, hatchling body mass, body mass gain and development of cold tolerance during the first week after hatching in Japanese quail. The test statistic was F for the incubation period and hatchling body mass models, and χ^2 for all other models. We did not report test statistics for main effects when the interaction between age and treatment was significant, because such effects are difficult to compute and interpret when higher order terms are included in the model. We provide estimates and *post hoc* tests for these main effects, but note that these may be misleading because of the involvement in the interaction. Different letters within brackets denote statistically significant *post hoc* comparisons.
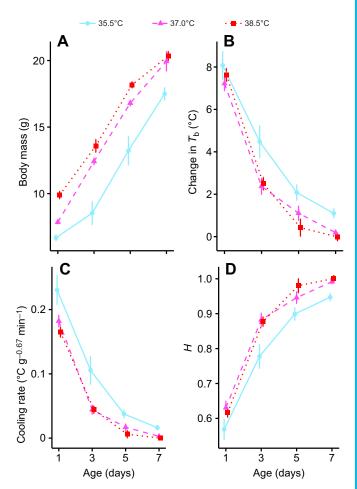

Fig. 2. Effects of incubation temperature on body mass gain and thermoregulatory performance in the first week of life. The figure shows mean±s.e.m. (A) body mass, (B) the change in body temperature (T_b) during the cooling challenge, (C) surface area-adjusted change in T_b and (D) homeothermy index (H), during the first week after hatching in Japanese quail that were incubated at low (35.5°C), normal (37.0°C) or high (38.5°C) temperature. Note that no model was fitted to the data presented in B, and that we calculated logarithms of the data in C and ran the analysis on the square root-transformed values of those logarithms according to Eqn 1. Significance of multiple comparisons is reported in Table 1, and sample sizes are reported in the Materials and Methods.

throughout the experiment (Fig. 2B). When this was expressed as $R_{\Delta T_b}$ (Eqn 1), we found that cold tolerance improved with age at a similar rate in all groups (Fig. 2C, Table 1). However, chicks that had been incubated at the two highest temperatures had lower cooling rates than those incubated at 35.5°C throughout the study (Fig. 2C, Table 1).

H (Eqn 2) improved with age by a similar magnitude in all groups (Fig. 2D, Table 1). Yet, cold-incubated chicks were less homeothermic than chicks from the other two treatments during all measurements (Fig. 2D, Table 1).

DISCUSSION

We found that low incubation temperature slowed development both before (Fig. 1) and after hatching (Fig. 2A), and that these effects were large enough to affect the capacity to withstand cooling representative of that during a typical foraging bout of a precocial chick (Fig. 2B–D). This corroborates research on wild precocial birds where the cooling challenge was substantially longer and stronger than in this study (DuRant et al., 2013a, 2012). It is interesting to note

that developmental trajectories for high- and mid-temperature chicks were rather similar, in line with results obtained for a range of traits in other bird studies (e.g. DuRant et al., 2010, 2011a,b; Hopkins et al., 2011; Nord and Nilsson, 2011). This highlights that effects of incubation temperature on phenotype are non-linear. Here, this non-linearity was already evident at the embryonic stage: a 1.5°C temperature increase advanced hatching by 1 day with no change in hatchling size, but a corresponding decrease prolonged incubation by 3 days and resulted in smaller hatchlings.

Because we did not measure any thermogenic responses to cooling, i.e. metabolic heat production and shivering, we do not know whether reduced cold tolerance in chicks from the 35.5°C group was a reflection of increased dry heat transfer on account of a larger surface area to volume ratio, a dampened or delayed thermogenic response to cooling, or both. It is unlikely that the response was caused by size-related differences in heat loss alone, because precocial chicks produce heat in response to cold a day after hatching (Marjoniemi and Hohtola, 1999), with a transition from a weak to a pronounced thermogenic response a few days later when skeletal muscles grow and mature (Aulie, 1976). It has been suggested that the purpose of this early life facultative heat production is not to maintain T_b per se – probably a futile endeavour in light of the low thermal mass of young chicks – but rather to reduce the rate of body cooling to make feeding time less dependent on environmental temperature (Jørgensen and Blix, 1988). In view of this, it is tempting to speculate that the observed body cooling was not a reflection of low thermogenic capacity but rather an adaptive response to conserve energy whilst maintaining some foraging capacity. Even so, it seems likely that chicks incubated at higher temperature would be able to extend foraging time in early life more than chicks incubated at lower temperature, because their larger size should contribute directly to both thermogenesis (as there is more heat-producing tissue) and insulation (as thermal properties are more conducive for heat retention in a larger body). This is well in line with the observation that it took chicks from the 35.5°C treatment 2 days longer (i.e. until body mass was comparable) to reach the cooling rate and degree of homeothermy that mid- and high-temperature chicks had already attained by day 3 (Fig. 2).

In the wild, the observed physiological and biophysical effects could exacerbate other consequences of low incubation temperature, e.g. smaller size, higher energy turnover rate and reduced locomotor performance (DuRant et al., 2010, 2011b; Hepp et al., 2006; Nord and Nilsson, 2011), because precocial chicks with reduced capacity to withstand cooling probably must reduce foraging bout length to avoid costs of hypothermia (Carr and Lima, 2013; Pedersen and Steen, 1979). Shorter bout length, together with any reduction in foraging efficiency from hypothermia-related cognitive impairment (Rashotte et al., 1998), could hamper total food intake with downstream consequences for growth and survival (Jørgensen and Blix, 1985). Thus, growth in cold-incubated chicks might suffer not only from shorter foraging bouts but also by reduced energy acquisition rate during that time. From the perspective of the female, it is interesting to speculate that investment in self-maintenance by reduced incubation effort (cf. Nord and Williams, 2015) could result in a cost later during the same breeding event, if a parent to cooling-intolerant chicks has to divert time from self-feeding to brooding.

We have demonstrated that even slight changes in the thermal environment that eggs experience can have sufficiently large effects on growth and maturation to impact how well chicks withstand cooling. Future studies should elucidate the proximate nature of this observation, and also assess how thermoregulatory consequences of low incubation temperature impact the foraging behaviour of young chicks, and the brooding behaviour of their parents, in wild models of precocial birds.

Data availability
Data are available from figshare: https://doi.org/10.6084/m9.figshare.12933191.v1

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