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# Is embryonic hypothermia tolerance common in birds?

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Avian incubation temperatures oscillate within narrow limits to ensure proper embryonic development. However, field observations and experimental studies have found that some species can tolerate very low incubation temperatures, either regularly or occasionally. We artificially incubated eggs from five domestic species, which represent a range of egg sizes, to examine whether a diversity of avian species could exhibit an unusual hypothermia tolerance, as observed in the field. We found that eggs of the chicken (*Gallus gallus domesticus*), pigeon (*Columba livia domestica*), Japanese quail (*Coturnix japonica*) and budgerigar (*Melopsittacus undulatus*) survived the incubation period and hatched after experiencing 10°C hypothermia for 6 h each day. However, embryos of white-rumped munia (*Lonchura striata*) died after 10 days of hypothermia. Our results showed that unusual hypothermia tolerance occurs in several avian species. This phenomenon might have been selected through the evolutionary history of birds. Future research should identify the importance of phylogeny, egg size and embryonic stage in tolerance to hypothermia.

## 1. Introduction

Incubation behaviour is an adaptive trait that is important for our understanding of avian evolution [1]. Most avian eggs are maintained within narrow temperature limits during incubation to ensure proper embryological development [2]; 25°C is considered to be a physical zero temperature (PZT) for most species, because embryonic development is thought to cease below this temperature. Therefore, wild birds have various strategies to prevent egg temperatures from falling below PZT during incubation, including selection of nesting material, nest-type and position, and incubation behaviour [2,3].

However, the embryos of some wild birds show extremely high hypothermia tolerance during incubation. For example, the embryos of some procellariiform and alcidae species can endure extraordinary low temperatures for several days, due to egg neglect during early embryonic development [4]. Boersma & Wheelwright [5] considered that embryonic tolerance to chilling has evolved to a greater degree in the Procellariiformes than in other orders. However, field studies of a passeriform species, the superb lyrebird (*Menura superba*), and a galliform species, the blood pheasant (*Ithaginis cruentus*), have revealed that the embryos of both species hatched successfully after enduring several hours of low temperature (10°C) each day during incubation [6,7]. Earlier experimental studies of domestic common pheasants (*Phasianus colchicus*) and chickens (*Gallus gallus domesticus*) also suggested that their embryos can survive low temperatures that their closely related wild species seldom encounter for periodic or irregular periods and still hatch [8,9]. These results suggest that avian embryonic hypothermia tolerance might be more widespread in avian

**Table 1.** Egg sizes and sample sizes of five bird species used in the artificial hypothermia incubation experiments.

| species | group (sample size) | weight (g) | length (mm) | breadth (mm) |
|---|---|---|---|---|
| chicken | control (10) | 48.9 ± 1.6 | 53.9 ± 0.7 | 40.8 ± 0.5 |
| | experiment (10) | 51.6 ± 1.6 | 54.7 ± 0.5 | 41.2 ± 0.4 |
| pigeon | control (26) | 22.8 ± 0.3 | 43.2 ± 0.3 | 31.0 ± 0.2 |
| | experiment (25) | 22.7 ± 0.3 | 43.4 ± 0.3 | 30.9 ± 0.2 |
| Japanese quail | control (15) | 10.9 ± 0.3 | 31.8 ± 0.5 | 25.1 ± 0.2 |
| | experiment (15) | 10.4 ± 0.3 | 31.6 ± 0.4 | 24.8 ± 0.2 |
| budgerigar | control (40) | 2.6 ± 0.05 | 19.29 ± 0.11 | 15.38 ± 0.10 |
| | experiment (43) | 2.6 ± 0.04 | 19.11 ± 0.11 | 15.40 ± 0.07 |
| white-rumped munia | control (17) | 1.08 ± 0.04 | 15.89 ± 0.10 | 11.56 ± 0.07 |
| | experiment (20) | 1.07 ± 0.03 | 15.85 ± 0.11 | 11.53 ± 0.07 |

species than traditionally thought. Although answers to these questions are important for our understanding of avian evolution, no such studies have been conducted on a wide range of avian species.

In this study, we used eggs of five bird species of different sizes to determine whether their embryos could tolerate 10°C for 6 h daily during incubation and hatch successfully. We chose 10°C for 6 h daily because we wanted to mimic what blood pheasant eggs experience in the field and it represents a considerably lesser hypothermia than most embryos encounter in the wild [7]. We hypothesized that tolerance to such hypothermia (10°C for 6 h daily) was a common trait in bird embryos. Because periodic cooling increases the incubation period and results in greater consumption of protein and lipid reserves during embryonic development [10,11], larger embryos should consume relatively less energy to maintain metabolic rate due to smaller surface area to volume ratio [12]. Thus, we suggested that cold tolerance could be inferior in smaller species compared with larger ones. As effects of hypothermia on incubation are more significant in later incubation periods compared with early incubation periods [13], we predicted that deaths of embryos would be more likely to occur in later incubation periods if they fail to hatch. We also predicted that embryos subjected to hypothermia would have more prolonged incubation periods than eggs incubated at normal temperatures.

## 2. Material and methods

We tested the eggs of five domestic avian species, which varied in size from 1 to 50 g (table 1) and were relatively easy to obtain. Eggs of the chicken, pigeon (*Columba livia domestica*) and Japanese quail (*Coturnix japonica*) were obtained from local suppliers and eggs of the budgerigar (*Melopsittacus undulatus*) and white-rumped munia (*Lonchura striata*) from aviculturists.

We used 20 chicken, 30 quail, 51 pigeon, 83 budgerigar and 37 munia eggs in our incubation experiment. We divided the eggs into two groups (control and experimental) for each species. Each egg was marked with a marker pen and measured (weight, length and breadth) with an analytical balance and digital caliper (table 1). Eggs in control groups were incubated continually in the incubator (model: P-800, Grumbach, Germany) with motor reverse every 2 h and 55–65% relative humidity at optimum incubation temperature for each species (chicken and quail: 37.5°C, pigeon: 38.2°C, budgerigar and munia: 38.0°C). Eggs in experimental groups were incubated in the same incubator under the same incubation conditions as the control groups throughout the incubation period, except when they were moved to another incubator (model: KB240, Binder, Germany) for the low-temperature treatment (10°C from 10.00 to 16.00 each day from the second day of incubation until the eggs started piping or hatching). The overall incubation temperatures for each species in the experimental group were: chicken and quail, 28.13°C; pigeon, 28.65°C; budgerigar and munia, 28.5°C.

Egg development was determined by candling every 4 days until the eggs hatched or the embryos died during incubation. Eggs with no sign of development were considered to be infertile and removed from the incubator at the first candling.

We used survival analysis to simultaneously test the effect of hypothermia on both hatching period and hatching success in SAS 9.1. Results are presented as mean ± s.e.

## 3. Results

We found no significant differences in fertilization success between control and experimental groups for each species ($\chi^2$-test, all $p > 0.05$, figure 1*a*).

The survival probabilities were not different between treatment and control groups for embryos of chicken, pigeon, quail and budgerigar. However, hypothermia treatment significantly reduced survival probabilities of munia embryos ($p = 0.014$, table 2).

Further analysis suggested that the experiment significantly prolonged the incubation period in the following four species (Wilcoxon rank test: chicken 20.6 ± 0.2 versus 27.0 ± 0.0 days, $p < 0.001$; pigeon 18.5 ± 0.1 versus 23.6 ± 0.2 days, $p < 0.001$; quail 17.0 versus 22.0 days, $p < 0.001$; budgerigar 16.9 ± 0.4 versus 21.3 ± 0.2 days, $p < 0.001$; figure 1*b*). However, experimental hypothermia did not influence their hatching success ($\chi^2$-test: chicken $\chi^2 = 0.600$, $p = 1.000$; pigeon $\chi^2 = 0.256$, $p = 0.284$; quail $\chi^2 = 0.124$, $p = 0.222$; budgerigar $\chi^2 = 0.765$, $p = 0.382$; figure 1*c*). Hatching success was significantly reduced in the munia experimental group (control 5/6, experimental 0/10, $\chi^2 = 0.000$, $p = 0.001$); all embryos died after 10 days of incubation in the experimental group (13.6 ± 0.7 days).

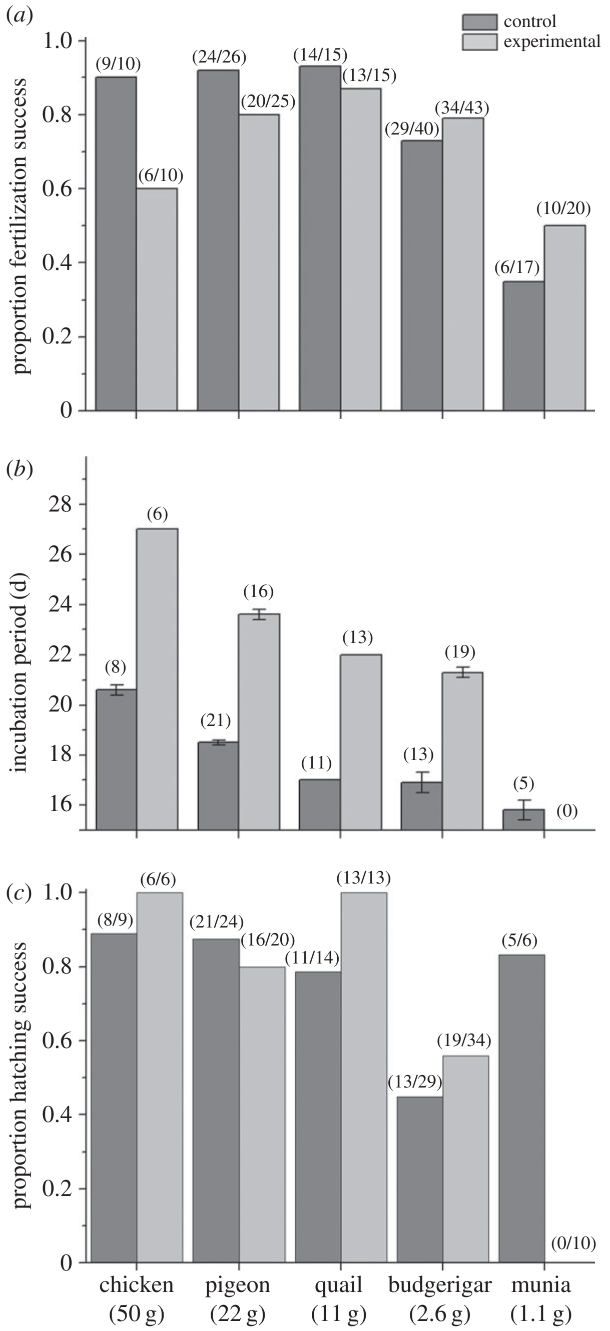


**Figure 1.** Comparison of fertilization success (*a*), incubation period (*b*), and hatching success (*c*) of five bird species between control and experimental hypothermia incubation groups. Sample sizes are given above histograms, and egg weights are shown in parentheses below the *x*-axis. Means ± s.e. are shown in (*b*).

## 4. Discussion

To the best of our knowledge, this is the first study to artificially incubate different-sized eggs to determine the effect of unusual hypothermia during incubation on hatchability, although this has been documented in the wild for some avian species [7]. Our results showed that there were no differences in embryo survival probabilities in four of the five species (egg weight 2.6–50 g) after a treatment of 6 h at 10°C each day during incubation compared with normal incubation temperatures. This suggests that hypothermia tolerance is more widespread in avian species than previously thought. Earlier researchers found that eggs of poultry species in experimental conditions and some wild species

**Table 2.** Differences of survival probabilities of eggs from normal and hypothermia treatment temperatures of five bird species used in the experiments.

| species | $\chi^2$ | d.f. | *p*-value |
|---|---|---|---|
| chicken | 0.667 | 1 | 0.414 |
| pigeon | 0.404 | 1 | 0.525 |
| Japanese quail | 1.931 | 1 | 0.165 |
| budgerigar | 4.234 | 1 | 0.525 |
| white-rumped munia | 6.035 | 1 | 0.014 |

incubating under acute weather situations or when experiencing unpredictable or low-quality food sources could endure extreme incubational hypothermia and hatch successfully [7,9]. However, our results indicated this trait was shared by a wide range of birds, including some small species.

It was interesting to note that most bird eggs showed the ability to endure hypothermia, although rarely reported in the wild. This might be a primitive trait, also shared by birds' relative reptiles [1], or it evolved through avian evolutionary history. For example, embryonic tolerance to hypothermia might be a result of adaptations to the cold periods experienced during the several ice ages that occurred in the Pleistocene [14]. However, the budgerigar in Australia did not experience this, which argues against this idea. Another possibility is that the Chicxulub meteoritic impact at the Cretaceous–Tertiary boundary caused long periods with low temperatures and short supplies of food resources [15], which might have caused the extinction of most avian species. Surviving bird species may have taken long off-nest bouts to look for food, selecting for species whose embryos could survive low temperatures during the absences. Extant bird species have descended from these surviving species and may have retained this potential to different degrees. However, whether there might be detrimental effects to the phenotype of offspring that have endured hypothermia during incubation needs to be addressed in future studies [16].

All embryos of white-rumped munia died after 10 days of incubation in the experimental groups. Embryos in these small eggs consumed relatively more energy, owing to greater surface area to volume ratio [12], and these small altricial eggs have relatively less yolk content and more water content than precocial ones [17], which might not support the greater energy consumption during the longer incubation period caused by lower temperature [18]. This result also supported the hypothesis that avian embryos are less tolerant to hypothermia in the late incubation period than earlier [13], owing to the greater energy demands of the more complex late-stage embryonic development [11].

Lower incubation temperature prolongs the incubation period, owing to reduced embryonic growth [11]. Our results confirmed that hypothermia treatment prolonged the incubation period in experimental groups in the chicken, pigeon, quail and budgerigar. The growth and development of embryos might have been suspended at 10°C and resumed when normal incubation temperatures were restored.

Sample sizes were small in this study, which restricted our hypothesis from obtaining very rigorous support. However, this study could be used as a basis for further study into this topic. More studies covering a variety of species with large sample sizes are needed to confirm the effects of hypothermia and identify the importance of phylogeny, egg size and embryonic stage in tolerance to hypothermia.

Ethics. The purchase, transportation and incubation of all eggs were supervised by the Animal Care and Use Committee of the Institute of Zoology, the Chinese Academy of Sciences (permit no. IOZ 2013-012).

Data accessibility. The data can be accessed at http://dx.doi.org/10.5061/dryad.6nn23 [19].

Authors' contributions. All authors conceived and designed the study. J.-M.Z. conducted the experiments, analysed the data, and wrote the paper. All authors contributed to manuscript revisions. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

Competing interests. We have no competing interests.

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