

It's not all about temperature: breeding success also affects nest design

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ABSTRACT

There are numerous observational studies on intra-specific variation in avian nest building and a single experimental manipulation. The general consensus is that birds build nests in response to environmental conditions, but it is not clear whether such flexibility in nest building is reproductively advantageous. To test the relationship between building flexibility and reproductive success, we allowed captive zebra finches to build their first nest, using string, and to breed in temperature-controlled rooms held at 14°C or 30°C. Once the offspring had fledged, we returned half the pairs to breed at the same temperature while half the pairs were switched to the alternative temperature. We provided all pairs with string and left them to build and breed a second time. For their first nest, pairs that built at 14°C used more string than did pairs that built at 30°C, and pairs that bred successfully built a nest with more string than did unsuccessful pairs. When pairs built their second nest, however, temperature no longer explained the number of pieces of string they used; rather, irrespective of the ambient temperature, pairs that had successfully produced young from their first nest used the same amount of string for their second nest while those that had failed to reproduce with their first nest used more string. These latter pairs were then more likely to reproduce successfully. Ambient temperature, therefore, did affect the nest the pairs built, but only in the absence of reproductive experience.

Keywords: temperature, nest building, zebra finch, *Taeniopygia guttata*, plasticity, breeding success

INTRODUCTION

A nest is a structure that animals build by organising pieces of material into a certain spatial relationship. They are built by a wide variety of species, including paper wasps, *Polistes* spp (Downing and Jeanne 1987), three-spined sticklebacks, *Gasterosteus aculeates*, (Barber et al. 2001) and common chimpanzees, *Pan troglodytes*, (Goodall 1962), but perhaps the most familiar are the nests built by birds.

The most common and important purpose of an avian nest (and arguably nests built by other taxa) is to provide a suitable environment, for eggs and young to develop (e.g. Hansell 2005). All birds lay eggs that have a narrow thermal limit (36 – 40°C) for optimal embryonic development. Deviations up or down from this range for prolonged periods of time can result in abnormal development and/or death (Ardia et al. 2010; Beissinger et al. 2005; DuRant et al. 2013; Webb 1987). As this is a very narrow thermal window that can be readily affected by ambient temperature (e.g. Ardia 2013; Ardia et al. 2009; Ardia et al. 2006; Heenan et al. 2015), one way to mitigate the effect of ambient temperature on offspring development is to build a nest that is "suitable" for the local conditions. However, a suitable nest environment for one species may not be appropriate for another species, and therefore some of the *interspecific* variation in nest morphology might be explained by variation in environmental conditions, specifically the temperature range (Hansell 2000).

There is also *intraspecific* variation in nest morphology. The general assumption is that this variation occurs because birds experience different environmental conditions, particularly with regard to variation in ambient temperature (e.g. Deeming et al. 2012; Mainwaring et al. 2012). For example, as latitude and/or altitude increases and mean temperatures decrease, European blackbirds (*Turdus merula*), American robins (*Turdus migratorius*) and common 'amakihis (*Hemignathus virens virens*) build heavier nests with thicker walls, which retain more heat (Crossman et al. 2011; Kern and van Riper 1984; Mainwaring et al. 2014a; Rohwer and Law

2010). A range of other species, for example, yellow warblers (*Setophaga petechia*), great tits (*Parus major*), blue tits (*Cyanistes caeruleus*) and European robins (*Erithacus rubecula*) change which materials they put in their nest, and use better, and more, insulatory materials (for example, feathers, animal fur) in cooler environments at higher latitudes and altitudes (Cerezo and Deeming 2016; Crossman et al. 2011; Mainwaring et al. 2012). Nest-site selection may also vary between populations, for example, northern orioles (*Icterus galbula*) in cooler locations construct nests higher in the canopy than do orioles in warmer locations, perhaps because building a nest higher in the canopy exposes the nest to more solar radiation and, therefore, reduces cooling of eggs and chicks (Schaefer 1976).

Nest morphology can also vary within a population in response to ambient temperature. Birds that build earlier in the season, when temperatures are cooler, may construct thicker, heavier nests with more insulatory material than do conspecifics that construct a nest later in the season, when ambient temperature has increased. For example, as the breeding season progressed, and ambient temperature increased, new helmeted honeyeater (*Lichenostomus melanops cassidix*) nests had thinner nest walls and were less bulky (Franklin 1995). Additionally, nests with thinner walls and less lining were built by Eurasian skylarks (*Alauda arvensis*) and greater short-toed larks (*Calandrella brachydactyla*), the later in the season they began to build (Herranz et al. 2004). Indeed, there are multiple examples of such seasonal correlates to nest materials. Changes in the quantity and type of materials used in a nest is one of the most common ways nests vary within a population over the course of the breeding season. Chilean swallows (*Tachycineta meyeni*) include fewer feathers in their nest as daily temperature increases with breeding season, while they add feathers during the laying and incubation period and more feathers during cooler temperatures (Liljeström et al. 2009). Blue tits add more plant material to their nest as temperature during the seven days preceding clutch initiation decreases (Britt and Deeming 2011) while nests built later in the season have less cup lining (Mainwaring and Hartley 2008), and feather mass in long-tailed

tit (*Aegithalos caudatus*) nests also declines with the season (McGowan et al. 2004). The consistency of this effect of ambient temperature on nest morphology is typically interpreted as individual birds responding to the ambient conditions when building their nests.

Flexibility in response to temperature in nest building has been demonstrated experimentally in captive zebra finches (*Taeniopygia guttata*). Zebra finches that were housed at 18°C built heavier nests than when they were housed at 30°C (Campbell et al. 2018), irrespective of the order in which they experienced the two temperatures. The authors concluded that birds built heavier nests in the cooler temperature in an attempt to retain heat within the nest, which would in turn aid reproductive success. However, because the authors did not record within-nest temperature or reproductive success, it was not clear from this experiment whether heavier nests do increase the nest temperature significantly above that of the ambient, and thus provide a more suitable microclimate for offspring development (DuRant et al. 2013). Additionally, as an individual's experience of material and reproductive success can affect subsequent material choice (Muth and Healy 2011), it was not clear whether the reproductive performance – information all pairs would have had for their second nest – was related to the temperature in which they built, or whether there was any effect of experience of reproductive success on their nest morphology at the two temperatures.

In this study, we therefore investigated the relationship between individual flexibility in nest morphology and reproductive success. Based on the Campbell et al. (2018) experiment we expected birds to build bigger nests in cooler than in warmer ambient temperatures. Our experiments, however, differed in two key ways. In our experiments, we used captive zebra finches to determine 1) whether building a nest that consisted of more material did increase within-nest temperature, and if so, 2) whether this affected reproductive success, and 3) whether reproductive experience affected the morphology of the second nest. Zebra finches typically construct roofed nests, but will also build cup and platform nests (Zann 1996) and, in captivity,

they will readily construct nests using a variety of material (e.g. string - Bailey et al. 2014; paper - Bailey et al. 2015; coconut fibre - Muth and Healy 2011). Although both parents will engage in incubation and parental care, it is typically the male that builds the nest.

We considered zebra finch pairs to be reproductively successful if they hatched offspring, and unsuccessful, either if they laid no eggs or their eggs did not hatch over the course of the experiment. To investigate the relationship between temperature, nest morphology, nest temperature and reproductive success we ran two experiments each spanning 47 days. In Experiment 1, captive zebra finch pairs built a nest at an ambient temperature of either 14°C or 30°C, temperatures that are within the range at which zebra finches breed in the wild (Griffith et al. 2017). The choice of temperatures was based on those of Campbell et al. (2018), but because the temperature in the home cages of our zebra finches was approximately 22°C, we chose two experimental temperatures that differed from 22°C by the same number of degrees ($\pm 8^\circ\text{C}$).

We put temperature loggers into the nests to record nest temperature, and once offspring had hatched or the experiment had ended (Day 47), we collected the nests. In Experiment 1, we asked the following questions: 1a) Did pairs put more material into their nest when they built at the cooler temperature (confirming the Campbell et al. (2018) data)? 1b) Did pairs raise their nest temperature above the ambient? and 1c) If yes, did this affect a pair's reproductive success?

Once offspring had fledged, we ran Experiment 2. In Experiment 2, approximately six weeks after the completion of Experiment 1, the pairs from Experiment 1 were provided with the opportunity to build a second nest. For their second nest, half the birds were returned to the temperature they had experienced when they built their first nest, and we switched the remaining pairs to the alternate temperature, such that the birds that had built their first nest at 14°C built their second nest at 30°C, and *vice versa*. Pairs were left to build, and temperature loggers were again deployed. In Experiment 2, we asked the following questions: 2a) When the birds built their second nest, was it appropriate for the ambient temperature (for example, did a pair build a larger

nest at 14°C than they had at 30°C)? 2b) Did the birds' experience of temperature or reproductive success in Experiment 1 affect their nest design in Experiment 2?

If birds put more material into their nests at cooler temperatures, the birds that built at 14°C should have put more material into their nest than did the birds that built at 30°C. This extra material should also have raised the temperature in the nest and possibly have increased the birds' reproductive success. In Experiment 2, we expected that the birds that had experienced the alternate temperature to build their second nest appropriately for their new environment (e.g. birds that built at 14°C should have increased their nest size relative to the nest they had built at 30°C and *vice versa* as observed in Campbell et al. (2018)) whereas the birds that built at the same temperature should have built the same nest as they had built in Experiment 1.

METHODS

Subjects

The subjects were 36 adult male and 36 adult female zebra finches purchased from a local pet shop (London, Ontario). We had no knowledge of their breeding experiences, or of their age prior to purchase. Before the start of the experiment all birds were housed on a 14:10h light:dark schedule, in single-sex colony cages (4 – 6 individuals per cage) with full spectrum lighting at a temperature range of 19-22°C and humidity levels of 50-70%. Birds had *ad libitum* access to food (mixed seeds, cuttlebone and oyster shell grit), egg mix (boiled egg, corn meal and bread) and water.

All procedures were approved by the University of St Andrews Animal Welfare and Ethics Committee and the University of Western Ontario's Animal Care Committee (AUP: 2015-019).

Experimental Protocol

The experiments were conducted in two temperature- and humidity-controlled environmental chambers on the same light:dark schedule as that of the colony rooms and with an initial temperature of approximately 22°C. Each room contained 18 cages (46 x 46cm and 47cm high), with each cage separated by Bristol board, which resulted in pairs being visually, but not acoustically, isolated from each other. Each cage contained a food bowl, a grit cup, a water bottle, and a cuttlebone, plus a circular nest cup (diameter 89mm) that was placed 149mm from the cage ceiling. A nest cup was provided rather than a nestbox to allow the pairs to construct a nest as large or as small as they chose, rather than being restrained by a surrounding box. The cage floor was covered with wood shavings.

Experiment 1

Nest Building

Birds were randomly assigned to breeding pairs and each pair was placed into a cage in one of the two environmental rooms (each pair was housed in their own cage; 14°C = 18 pairs; 30°C = 18 pairs). Birds were left from three to seven days to form pair bonds. The temperature of each treatment room was then decreased/increased at a rate of 1.5°C every 12 hours, until one room reached 14°C and the other 30°C. On the morning after both rooms had reached the experimental temperature, three hours after the lights came on, each pair received 300 pieces of string with which to build a nest (Day 1, **Error! Reference source not found.**), material with which zebra finches readily construct a nest and from which they can successfully raise young (e.g. Bailey et al. 2014; Breen et al. 2019; Hall et al. 2015). The string (James Lever and Sons Ropes and Twines, UK) was unpolished off-white cotton (diameter 2.5mm) cut into 15cm lengths (Bailey et al. 2014), a length at which 300 pieces of string is sufficient (personal observation) to build a species-typical domed nest (Zann 1996).

On Day 1, the cages were checked one and three hours after a pair had received the string, and if a pair had fewer than 10 pieces of string remaining, we provided an additional 100 pieces. If the birds built a nest, but did not build in the nest cup, we removed the nest and put the string back on the cage floor to encourage the birds to build in the nest cup. A pair's attempt was demolished twice before we left them to build in a location other than the nest cup. Each time we destroyed a nest we provided no additional string, even if the birds had used all the string. We preferred birds to build in the nest cup so that we had a more equitable comparison of nest morphology for the two temperatures. If, for example, the birds built their nest in a food bowl, which was smaller than the nest cup and required fewer pieces, the variation in nest morphology expected in response to the different temperatures may have been obscured. From Days 2 to 4, birds' building attempts were checked three, four and seven hours after the lights came on. 100 pieces of string were provided when required, as before. At each check the nests were examined for eggs. Twenty-four hours after an egg had been laid, an iButton (model DS1925, accuracy $\pm 0.5^{\circ}\text{C}$) was placed in the nest, that was programmed to record temperature every five minutes. Nest temperature was then calculated from the iButton readings as the average temperature in the nest from the date on which an iButton was installed to either date of hatching or date on which the experiment ended for each pair. One male in the 14°C room died during this period and his female was removed and the pair was not replaced. From Day 4 the birds were left to breed (**Error! Reference source not found.**). Cages were checked daily and any eggs that were not in the nest were removed. We did not record the number of eggs that were laid and then discarded or incubated.

On Day 23 (**Error! Reference source not found.**), all the pairs that had an empty nest were removed, because they either had not yet laid an egg or had laid an egg but discarded it ($n = 11$ at 14°C ; $n = 10$ at 30°C). Pairs with eggs or hatchlings were left until Day 31. On Day 31, the pairs without hatchlings ($n = 1$ at 14°C ; $n = 3$ at 30°C) were removed (**Error! Reference source not found.**). All birds that were removed were returned to the single-sex colony rooms as we did

not want pairs to remain together without building material or young. The other birds remained in the environmental rooms till Day 47 when they were returned to the colony rooms ($n = 5$ at 14°C ; $n = 5$ at 30°C). Pairs with hatchlings were kept in family groups in separate cages in the colony rooms until the hatchlings had fledged. We did not count the number of hatchlings each pair produced and once the hatchlings had fledged the adults were moved to single-sex colony cages. By the end of Experiment 1, a further male and a female had died in the 14°C treatment, resulting in the loss of two pairs, which were not replaced. A male died in the 30°C treatment and was not replaced. The cause of death of these birds was unknown but did not appear to be related to experimental treatment.

Nest Collection

Once all birds had been returned to the colony room we collected the nests, pulled them apart and counted the number of pieces of string in each nest. In all but one nest (30°C), some of the string had been separated into individual threads, so each individual thread was also counted, and then divided by nine (the number of threads in a piece of string) to calculate the number of pieces of string used. In total, a sample of size of 35 (14°C , $n = 17$; 30°C , $n = 18$) nests were examined.

Experiment 2

Nest building

Between Experiments 1 and 2, birds were housed at a temperature of approximately 22°C for a period of up to six weeks. Pairs with chicks that had not yet fledged were kept in family cages until all offspring had fledged, which was when we separated pairs into single-sex colony cages. All birds spent at least two weeks in the single-sex colony cages before the start of Experiment 2.

For Experiment 2, we re-paired birds with their original partner, and we put half of the pairs that had built in the 14°C environmental room in the 30°C room ($n = 8$), and put half of the

pairs that had built in the 30°C room in the 14°C room ($n = 8$, see Supplementary Material). The other pairs were moved to the room of the temperature at which they had built in Experiment 1 (30°C, $n = 9$ and 14°C, $n = 7$). Due to a change in cage availability, nine pairs housed at 14°C and 11 pairs housed at 30°C were housed in slightly smaller cages (43 x 46 cm and 42 cm high) while the remaining pairs were housed in the original cages (46 x 46cm and 47cm high). The assignment of pairs to the smaller cages was done blind, except to ensure that we assigned pairs evenly with respect to their breeding success and treatment in Experiment 1.

Once all birds had been moved to the experimental rooms, the temperature of each room was changed, at the rate described above. On the morning after the rooms had reached the appropriate experimental temperature, each pair received 300 pieces of string with which to build a nest three hours after the lights came on (Day 1). String usage, eggs and hatchlings were checked as in Experiment 1, following the same timeline as before (**Error! Reference source not found.**). As for Experiment 1, we removed nests that were not built in the nest cup. All birds were returned to the colony and nests were processed as described for Experiment 1.

Statistical analysis

All statistical analysis was completed using R Studio (2012) with R Development Core Team (2016) using packages ‘*plotrix*’ (Lemon 2006) and ‘*dplyr*’ (Wickham et al. 2017). All graphs were created using ‘*ggplot2*’ (Wickham 2009) ‘*cowplot*’ (Wilke 2019), ‘*grid*’ (R Core Team 2019), ‘*gridExtra*’ (Baptiste 2017) and ‘*ggsignif*’ (Ahlmann-Eltze 2017) and InkScape (The Inkscape Team 2011). All General Linear Models (GLMs) were run with a Gaussian distribution, unless otherwise stated. Type II likelihood-ratio chi-square tests (‘*car*’ package (Fox and Weisberg 2011)) were performed on all finalized Linear Models (LMs), Linear Mixed Models (LMMS) and GLMs to assess the significance of the model. All means are shown with standard errors.

Experiment 1

To determine whether ambient temperature affected the nest a bird built, we ran a GLM, with total string as the dependent variable and treatment on two levels (14°C and 30°C).

As we expected more string to have been added to a nest to increase insulation and nest temperature, we ran a GLM with a two-way interaction (total string*treatment) and nest temperature as the dependent variable. To establish if nest temperature significantly differed from the ambient temperature in which the birds were housed, we used one-sample t-tests (two-tailed) to compare the nest temperature achieved by pairs that built at 30°C with a sample mean of 30, and to compare the nest temperature achieved by pairs that built at 14°C with a sample mean of 14. We then used two GLMs with Binomial distributions, *logit* link to identify the effect of treatment and total string on breeding success (successful or unsuccessful).

Experiment 2

In Experiment 1 all birds built in the nest cup, while in Experiment 2 nine birds built in the food bowl rather than in the nest cup (14°C, $n = 5$; 30°C, $n = 4$). Nest site location did not differ with room temperature (GLM, binomial distribution: $\chi^2_1 = 0.38$, $n = 31$, $p = 0.54$), but the birds that built in the food bowls used fewer pieces of string to build their nest (GLM: $\chi^2_1 = 434.18$, $n = 31$, $p < 0.001$; nest cup: 528 ± 46.40 ; food bowl: 354 ± 49.57). Data from pairs that built their nest in the food bowl were then excluded from all further analysis.

The analysis as described above were repeated, but Linear Mixed Models (LMMs) were used, with number of times a nest was removed and cage type (as some birds were housed in smaller cages in Experiment 2) as random factors, unless otherwise stated. Due to scaling issues in the model when determining whether breeding success was affected by treatment or total string, we log transformed the data for total string and used GLMMs with Binomial distributions.

Experiments 1 and 2

To determine whether total string in Experiment 2 was affected by experience of previous ambient temperature or the current ambient temperature we used an LMM, with the number of times a nest was removed in Experiment 2 as a random factor and whether or not pairs were housed in the same cage type as in Experiment 1 (Yes or No). Nest removal was included as a random factor as this may have affected the total string used in a completed nest. The difference in the total number of pieces of string used in the nest was calculated as Experiment 2 total – Experiment 1 total, and we used a LMM with difference as the dependent variable, the number of times a nest was removed in Experiment 2 as a random factor, and previous and current temperatures as the fixed effect (14°C/14°C; 14°C/30°C; 30°C/30°C; 30°C/14°C), to establish the effect of previous experience on the difference in total number of string pieces put into the nest.

To establish whether reproductive success in Experiment 1 affected the differences in total number of pieces of string in nests between Experiments 1 and 2, we ran an LMM with difference in total string as the dependent variable, breeding success on two levels (successful, n = 10; unsuccessful, n = 13) as the fixed effect. The number of times the nest was removed in Experiment 2 and if the pair were building in the same cage were entered as random effects. To determine whether pairs used different numbers of pieces of string in nests built in Experiment 2, based on breeding experience in Experiment 1, we used two one sample (two-tailed) t-tests to examine the data for successful and unsuccessful pairs, with a sample mean of 0.

If previously unsuccessful birds did increase the number of pieces of string used in their second nest, nest temperature should have increased with total amount of string and as nest temperature increased, previously unsuccessful birds should have been successful in Experiment 2. To test this, we used an LMM with nest temperature as the dependent variable, total string as the fixed effect and, as ambient temperature is known to affect nest temperature, ambient temperature of each treatment as a random effect (Ardia 2013; Ardia et al. 2006; Heenan et al.

2015). To test that higher nest temperatures did lead to previously unsuccessful birds being successful, we ran a GLM, Binomial distribution, with breeding success in Experiment 2 (successful vs unsuccessful) as the dependent variable and nest temperature as the fixed effect.

RESULTS

Experiment 1

Birds that built at 14°C used more pieces of string to build their nest than did birds that built at 30°C (GLM: $\chi^2_1 = 6.32$, $n = 35$, $p = 0.01$; 14° = 512 ± 42; 30° = 361 ± 43; **Error! Reference source not found.**). The temperature in the nest of birds that built at 14°C was lower than it was in the nests of birds that built at 30°C, but as the total number of pieces of string in the nest increased so did nest temperature (GLM, string*ambient temperature: $\chi^2_1 = 18.46$, $n = 26$, $p < 0.001$; string: $\chi^2_1 = 33.04$, $n = 26$, $p < 0.001$; ambient temperature: $\chi^2_1 = 48.05$, $n = 26$, $p < 0.001$; 14° = 25.99° ± 2.53°; 30° = 32.33° ± 0.67°; **Error! Reference source not found.**). Birds raised their nest temperature significantly above both the 14°C and 30°C ambient temperatures (14°C: $t_{1,10} = 4.74$, $p < 0.001$; 30°C: $t_{1,14} = 3.50$, $p = 0.004$; **Error! Reference source not found.**).

The probability of producing hatchlings increased with the total number of pieces of string put into the nest (GLM Binomial: $\chi^2_1 = 14.37$, $n = 35$, $p < 0.001$; **Error! Reference source not found.**) but the number of hatchlings did not differ between nests built at 14°C and 30°C (GLM Binomial: $\chi^2_1 = 0.23$, $n = 35$, $p = 0.63$).

Experiment 2

Unlike the outcome for Experiment 1, ambient temperature had no effect on the total number of pieces of string pairs used to build their nest (LMM: $\chi^2_1 = 0.29$, $n = 23$, $p = 0.59$; 14° = 461 ± 63; 30° = 495 ± 48; **Error! Reference source not found.****Error! Reference source not found.**).

Temperature was again higher in nests built at 30°C than it was in nests built at 14°C and in nests

that contained more string, but there was no interaction between ambient temperature and number of pieces of string (LMM, string: $\chi^2_1 = 21.95$, $n = 18$, $p < 0.001$; ambient temperature: $\chi^2_1 = 15.57$, $n = 18$, $p < 0.001$; string*ambient temperature: $\chi^2_1 = 1.02$, $n = 18$, $p = 0.31$; $14^\circ = 24.13^\circ \pm 2.37^\circ$; $30^\circ = 33.76^\circ \pm 0.62^\circ$; **Error! Reference source not found.**Figure). In Experiment 2, the temperature in all nests was significantly higher than the ambient temperature (14°C : $t_{1,10} = 5.70$, $p < 0.001$; 30°C : $t_{1,7} = 3.63$, $p = 0.008$; **Error! Reference source not found.**).

As in Experiment 1, in Experiment 2 the chance of reproductive success increased as the total number of pieces of string in the nest increased (GLMM Binomial log(string): $\chi^2_1 = 5.60$, $n = 23$, $p = 0.02$; **Error! Reference source not found.**), while ambient temperature had no effect on reproductive success (GLMM Binomial: $\chi^2_1 = 0.99$, $n = 23$, $p = 0.32$).

Experiments 1 and 2

The total number of pieces of string used in the nest in Experiment 2 was not affected by the previous or current temperature at which a pair built (GLM: $\chi^2_1 = 2.97$, $n = 23$, $p = 0.40$). The difference in the number of pieces of string used in nests built in Experiments 1 and 2 was also not affected by the previous or current temperatures at which the pairs built (GLM: $\chi^2_1 = 6.99$, $n = 23$, $p = 0.07$; $14^\circ\text{C}/14^\circ\text{C}$, $\beta = 110.74 \pm 95.6$; $14^\circ\text{C}/30^\circ\text{C}$, $\beta = -179.26 \pm 117$; $30^\circ\text{C}/14^\circ\text{C}$, $\beta = 1.87 \pm 135.8$; $30^\circ\text{C}/30^\circ\text{C}$, $\beta = 125.77 \pm 122.84$) a result that may differ with a larger sample size.

Previous reproductive success, however, did explain variation in the number of pieces of string in nests built in Experiment 2 (LMM: $\chi^2_1 = 4.02$, $n = 23$, $p = 0.04$; Successful = -8 ± 74 ; Unsuccessful = 147 ± 60). Pairs that had reproduced successfully in Experiment 1 used the same number of pieces of string in their nest in Experiment 2 ($t_{1,9} = -0.11$, $p = 0.91$; Figure 4), while pairs that had been unsuccessful in Experiment 1 increased the number of pieces of string used in their second nest ($t_{1,12} = 2.46$, $p = 0.03$; Figure 4).

By increasing the number of pieces of string used in their nest (by an average of 147 pieces), unsuccessful birds increased their nest temperature (LMM: $\chi^2_1 = 4.08$, $n = 13$, $p = 0.04$), and the higher the nest temperature the more likely that previously unsuccessful pairs reproduced successfully in Experiment 2 (GLM, Binomial: $\chi^2_1 = 5.19$, $n = 10$, $p = 0.02$).

DISCUSSION

In Experiment 1, zebra finches responded to ambient temperature when they built their nest. Birds that built at a cooler temperature (14°C) put more pieces of string into their nest than did birds that built at a warmer temperature (30°C). The more string they put in their nest, the more pairs increased their nest temperature above the ambient, which in turn increased their chances of reproductive success. But, when pairs built their second nest, in Experiment 2, ambient temperature no longer explained the number of pieces of string they used. Instead, pairs that had been reproductively successful with their first nest built a nest with a similar number of pieces of string, regardless of the change in ambient temperature. Pairs that had not produced young in Experiment 1, however, used more string to build their nest in Experiment 2. The more string a bird used to build a nest, the higher the in-nest temperature, regardless of ambient temperature and, subsequently, the probability of breeding successfully.

As expected, in Experiment 1, pairs that built at 14°C built a nest that contained more pieces of string, than did the nest of the pairs that built at 30°C. These data are consistent with those of Campbell et al. (2018), who found that zebra finches built larger nests at a cooler temperature (18°C vs 30°C), and from population studies whereby birds in cooler temperatures build larger nests (e.g. Crossman et al. 2011; Franklin 1995; Mainwaring et al. 2014a). In our study, as pairs put more string in their nest, nest temperature increased above the ambient: by a few degrees at 30°C, but by 10 or more degrees when they built at 14°C. By doing so, all birds raised their nest temperature closer to the optimum for passerine embryonic development (36 -

40°C, DuRant et al. 2013; Webb 1987), and were then more likely to reproduce successfully. These data show a clear relationship between the amount of nest material, nest microclimate and nest reproductive success (e.g. D'Alba et al. 2009). It is possible that birds built larger nests at 14°C because by attempting to keep warm they were more active. This seems unlikely, however, as temperature had no effect on the speed on nest building (see Supplementary material) and temperature does not affect the rate at which blue tits in the wild bring material to their nest (Tortora-Brayda 2020).

It is also possible that the higher number of pieces of string in nests built at 14°C, and in turn the increase in nest temperature, was due to adult quality, which affected reproductive success (Nur 1984; Perrins 1979; Perrins and McCleery 1989; Perrins and Moss 1975). Adult quality can influence nest structure whereby higher quality swallows built larger and heavier nests than did conspecifics in poorer condition (Møller et al. 1995; Soler et al. 2007). Perhaps the larger nests that our zebra finches built were the result of higher quality adults. We did not assess adult quality (for example, we did not quantify how many hatchlings were produced, but just recorded if pairs produced hatchlings at all) so we cannot confirm whether pairs that built larger nests also produced more offspring. It does seem that adult quality would be a sufficient explanation, however, as the first nests built at 14°C contained more string than did first the nests at 30°C, even though birds were randomly assigned to each treatment adult quality cannot account for all the variation in nest morphology.

In Experiment 2, when birds either built a new nest in the same temperature or were moved to the alternative temperature (e.g. from 30°C to 14°C), ambient temperature no longer affected the nest a pair built. This was a surprise, both because Campbell et al. (2018) reported a change in nest design with a change in temperature (e.g. from 30°C to 18°C) and because, the pairs in our study had already been flexible in their nest design in response to temperature. Instead, however, a pair's second nest was best explained by their previous reproductive experience. Previously

successful pairs put the same number of pieces of string in their second nest as in the first, even if that rendered the second nest “inappropriate” for their current environment (i.e. too few pieces for a nest built at 14°C). On the other hand, previously unsuccessful pairs increased the number of pieces of string they put into their second nest, again regardless of ambient temperature. These data are consistent with other evidence that birds change their building decisions once they have some experience of reproduction. In Muth and Healy (2011), for example, birds that produced young and built with material they did not prefer, subsequently changed their material preference. But the birds that built with their preferred material and produced young, stuck with their original building decision, just as did the birds in Experiment 2 (in our study). Why unsuccessful birds in this study were more likely to respond specifically by putting more pieces into their nest in Experiment 2 is not clear. One possibility may be that birds are able to monitor their nest temperature (e.g. Álvarez and Barba 2014; Walters et al. 2016). If so, then it seems plausible that builders can associate the nest temperature with reproductive outcome (just as they can associate nest colour with reproductive outcome: Muth and Healy 2011) . For those birds that did not build a warm enough nest to produce offspring the first time may have responded by attempting to build a warmer nest the second time. Further experimentation is required to test this possibility.

While effects of reproductive experience on subsequent nest building attempts have been observed in zebra finches (e.g. Muth and Healy 2011; Sargent 1965) and pinyon jays (*Gymnorhinus cyanocephalus*, Marzluff 1988), this flexibility was not in response to a changing environment. While variation in nest design is generally attributed to variation in climate (e.g. Mainwaring et al. 2014b) an effect we also saw in Experiment 1, it appears that the same birds will reuse a previously successfully nest design, regardless of climate. Further data are required to determine whether there is a point at which ambient temperature would override previous experience and lead to a change in nest design.

In both experiments, nests that contained more string were warmer, and warmer nests were more likely to produce chicks. Pairs may then have increased the number of pieces of string in their nest in an attempt to increase nest temperature, which in turn increases breeding success, but there may be other factors that caused pairs to increase the number of pieces of string used. For example, females may invest more resources when breeding with males that construct larger nests, as has been recorded in female magpies (*Pica pica*, de Neve and Soler 2002; Soler et al. 2001). Similarly, in zebra finches it is predominantly, but not exclusively, the male that constructs the nest (Zann 1996). The males may therefore have built larger nests in an attempt to increase female reproductive investment, which would increase his breeding success, and the increase in nest temperature may have occurred as a by-product. As we did not quantify breeding behavior (e.g. incubation bouts and duration), or record the number of eggs laid and number of chicks that fledged (we just recorded if eggs or hatchlings were present), we cannot confirm if males adjusted nest design in an attempt to change female behavior, which then increased his breeding success, or if males adjusted nest design in order to raise nest temperature, which then increased his breeding success. Further work is required to identify how breeding success alters other aspects of reproductive behavior, and how reproductive behavior differs with nest design.

This study provides some insight into how birds may adapt to the warming global climate (e.g. Charmantier and Gienapp 2014; Charmantier et al. 2008; Shutt et al. 2019; World Meteorological Organisation 2019). Our data, together with those of Campbell et al. (2018), show that birds can – and do – build different nests in different ambient temperatures. However, the overriding by experience of breeding success of the response to changes in ambient temperature suggests that experience may not always be beneficial. Specifically, if birds continue to use a previously successful nest but it does not match the current environmental conditions, which could result in a decline in breeding success.

In summary, our results show that birds can adjust their nest structure in response to local temperature, but that the effect may be limited to pairs building together, in a particular location, for the first time. Once birds have their own experience of building a nest, coupled with reproductive success (or lack thereof), it was this experience that explained their nest-building decisions. The role of breeding experience on nest design raises further questions as to the duration of such experience effects, for example, whether it persists from one breeding season to the next, or across a lifetime, and whether or not the effects of previous breeding success applies only when paired with the same mate. Our results also raise questions as to how birds estimate reproductive success and by what means this estimate affects their subsequent nest-building behavior. Ideally, all the questions would be addressed in wild birds, rather than birds building in the laboratory.

SUPPLEMENTARY MATERIAL

Supplementary data are available at Behavioral Ecology online.

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Analyses reported in this article can be reproduced using the data provided by Edwards et al. (2020).

REFERENCES

- Ahlmann-Eltze C. 2017. Ggsignif: Significance brackets for 'ggplot2'. R package version 040.
- Álvarez E, Barba E. 2014. Behavioural responses of great tits to experimental manipulation of nest temperature during incubation. *Ornis Fennica*. 91:220-230.
- Ardia DR. 2013. The effects of nestbox thermal environment on fledging success and haematocrit in tree swallows. *Avian Biology Research*. 6(2):99-103.
- Ardia DR, Perez JH, Chad EK, Voss MA, Clotfelter ED. 2009. Temperature and life history: Experimental heating leads female tree swallows to modulate egg temperature and incubation behaviour. *Journal of Animal Ecology*. 78(1):4-13.
- Ardia DR, Perez JH, Clotfelter ED. 2010. Experimental cooling during incubation leads to reduced innate immunity and body condition in nestling tree swallows. *Proceedings of the Royal Society B*. 277(1689):1881-1888.
- Ardia DR, Pérez JH, Clotfelter ED. 2006. Nest box orientation affects internal temperature and nest site selection by tree swallows. *Journal of Field Ornithology*. 77(3):339-344.
- Bailey IE, Morgan KV, Bertin M, Meddle SL, Healy SD. 2014. Physical cognition: Birds learn the structural efficacy of nest material. *Proc Roy Soc B*. 281(1784):20133225.
- Bailey IE, Muth F, Morgan K, Meddle SL, Healy SD. 2015. Birds build camouflaged nests. *The Auk*. 132(1):11-15.
- Baptiste A. 2017. Gridextra: Miscellaneous functions for "grid" graphics. R package version 23.
- Barber I, Nairn D, Huntingford FA. 2001. Nests as ornaments: Revealing construction by male sticklebacks. *Behavioral Ecology*. 12(4):390-396.
- Beissinger SR, Cook MI, Arendt WJ. 2005. The shelf life of bird eggs: Testing egg viability using a tropical climate gradient. *Ecology*. 86:2164-2175.
- Breen AJ, Bonneaud CC, Healy SD, Guillette LM. 2019. Social learning about construction behaviour via an artefact. *Animal Cognition*. 22(3):305-315.

- Britt J, Deeming DC. 2011. First-egg date and air temperature affect nest construction in blue tits *cyanistes caeruleus*, but not in great tits *parus major*. *Bird Study*. 58(1):78-89.
- Campbell BL, Hurley LL, Griffith SC. 2018. Behavioural plasticity under a changing climate; how an experimental local climate affects the nest construction of the zebra finch *taeniopygia guttata*. *Journal of Avian Biology*. 49(4):8.
- Cerezo AT, Deeming DC. 2016. Nest insulation in the european robin (*erithacus rubecula*): Effects of latitude and construction materials. *Avian Biology Research*. 9(2):96-102.
- Charmantier A, Gienapp P. 2014. Climate change and timing of avian breeding and migration: Evolutionary versus plastic changes. *Evol Appl*. 7(1):15-28.
- Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LE, Sheldon BC. 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*. 320(5877):800-803.
- Crossman CA, Rohwer VG, Martin PR. 2011. Variation in the structure of bird nests between northern manitoba and southeastern ontario. *PLoS One*. 6(4):e19086.
- D'Alba L, Monaghan P, Nager RG. 2009. Thermal benefits of nest shelter for incubating female eiders. *Journal of Thermal Biology*. 34(2):93-99.
- de Neve L, Soler JJ. 2002. Nest-building activity and laying date influence female reproductive investment in magpies: An experimental study. *Animal Behaviour*. 63:975-980.
- Deeming DC, Mainwaring MC, Hartley IR, Reynolds SJ. 2012. Local temperature and not latitude determines the design of blue tit and great tit nests. *Avian Biology Research*. 5(4):203-208.
- Downing HA, Jeanne RL. 1987. A comparison of nest construction behavior in 2 species of *polistes* paper wasps (insecta, hymenoptera, vespidae). *Journal of Ethology*. 5(1):53-66.
- DuRant SE, Hopkins WA, Hepp GR, Walters JR. 2013. Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biol Rev Camb Philos Soc*. 88(2):499-509.

- Edwards SC, Shoot TT, Martin RJ, Sherry DF, Healy SD. 2020. Data from: It's not all about temperature: Breeding success also affects nest design. Dryad Digital Repository. doi: 10.5061/dryad.4b8gtht92.
- Fox J, Weisberg S. 2011. An r companion to applied regression. Thousand Oaks, CA: Sage.
- Franklin DC. 1995. Helmeted honeyeaters build bulkier nests in cold weather. *The Auk*. 112(1):247-248.
- Goodall JM. 1962. Nest building behavior in the free ranging chimpanzee. *Annals of the New York Academy of Sciences*. 102(2):455-467.
- Griffith SC, Crino OL, Andrew SC. 2017. Commentary: A bird in the house: The challenge of being ecologically relevant in captivity. *Front Ecol Evol*. 5:21.
- Hall ZJ, Healy SD, Meddle SL. 2015. A role for nonapeptides and dopamine in nest-building behaviour. *Journal of Neuroendocrinology*. 27(2):158-165.
- Hansell MH. 2000. Bird nests and construction behaviour. Cambridge, UK: Cambridge University Press.
- Hansell MH. 2005. Animal architecture. Oxford, UK: Oxford University Press.
- Heenan CB, Goodman BA, White CR. 2015. The influence of climate on avian nest construction across large geographical gradients. *Global Ecol Biogeogr*. 24(11):1203-1211.
- Herranz J, Traba J, Morales MB, Suárez F. 2004. Nest size and structure variation in two ground nesting passerines, the skylark *alauda arvensis* and the short-toed lark *calandrella brachydactyla*. *Ardea*. 92(2):209-218.
- Kern MD, van Riper C, III. 1984. Altitudinal variations in the nests of the hawaiian honeycreeper *hemignathus virens virens*. *The Condor*. 86(4):443-454.
- Lemon J. 2006. Plotrix: A package in the red light district of r. *R-News*. 6(4):8-12.

- Liljeström M, Schiavini A, Reboreda JC. 2009. Chilean swallows (*tachycineta meyeni*) adjust the number of feathers added to the nest with time of breeding. *Journal of Ornithology*. 121:783-788.
- Mainwaring MC, Deeming DC, Jones CI, Hartley IR. 2014a. Adaptive latitudinal variation in common blackbird *turdus merula* nest characteristics. *Ecol Evol*. 4(6):841-851.
- Mainwaring MC, Hartley IR. 2008. Seasonal adjustments in nest cup lining in blue tits *cyanistes caeruleus*. *Ardea*. 96(2):278-282.
- Mainwaring MC, Hartley IR, Bearhop S, Brulez K, du Feu CR, Murphy G, Plummer KE, Webber SL, Reynolds SJ, Deeming DC. 2012. Latitudinal variation in blue tit and great tit nest characteristics indicates environmental adjustment. *J Biogeogr*. 39(9):1669-1677.
- Mainwaring MC, Hartley IR, Lambrechts MM, Deeming DC. 2014b. The design and function of birds' nests. *Ecology and Evolution*. 4(20):3909-3928.
- Marzluff JM. 1988. Do pinyon jays alter nest placement based on prior experience? *Animal Behaviour*. 36(1):1-10.
- McGowan A, Sharp SP, Hatchwell BJ. 2004. The structure and function of nests of long-tailed tits *aegithalos caudatus*. *Functional Ecology*. 18(4):578-583.
- Møller AP, Linden M, Soler JJ, Soler M, Moreno J. 1995. Morphological adaptations to an extreme sexual display, stone-carrying in the black wheatear, *oenanthe leucura*. *Behavioral Ecology*. 6(4):368-375.
- Muth F, Healy SD. 2011. The role of adult experience in nest building in the zebra finch, *taeniopygia guttata*. *Animal Behaviour*. 82(2):185-189.
- Nur N. 1984. The consequences of brood size for breeding blue tits. 1. Adult survival, weight change and the cost of reproduction. *Journal of Animal Ecology*. 53(2):479-496.
- Perrins CM. 1979. *British tits*. London: Collins.

- Perrins CM, McCleery RH. 1989. Laying dates and clutch size in the great tit. *Wilson Bull.* 101(2):236-253.
- Perrins CM, Moss D. 1975. Reproductive rates in great tits. *Journal of Animal Ecology.* 44(3):695-706.
- R Core Team. 2019. R: A language and environment for statistical computing. In: Computing RfFS, editor. Vienna, Austria.
- R Development Core Team. 2016. R: A language and environment for statistical computing. 3.3.1 ed. Vienna, Austria: R Foundation for Statistical Computing.
- R Studio. 2012. Integrated development environment for r. 1.0.138 ed. Boston, MA.
- Rohwer VG, Law JSY. 2010. Geographic variation in nests of yellow warblers breeding in churchill, manitoba, and elgin, ontario. *Condor.* 112(3):596-604.
- Sargent TD. 1965. The role of experience in the nest building of the zebra finch. *The Auk.* 82:48-61.
- Schaefer VH. 1976. Geographic variation in the placement and structure of oriole nests. *The Condor.* 78(4):443-448.
- Shutt JD, Cabello IB, Keogan K, Leech DI, Samplonius JM, Whittle L, Burgess MD, Phillimore AB. 2019. The environmental predictors of spatio-temporal variation in the breeding phenology of a passerine bird. *Proceedings of the Royal Society B-Biological Sciences.* 286(1908):9.
- Soler JJ, Martin-Vivaldi M, Haussy C, Møller AP. 2007. Intra- and interspecific relationships between nest size and immunity. *Behavioral Ecology.* 18(4):781-791.
- Soler JJ, Neve Ld, Martínez JG, Soler M. 2001. Nest size affects clutch size and the start of incubation in magpies: An experimental study. *Behavioral Ecology.* 12(3):301-307.
- The Inkscape Team. 2011. Inkscape. 0.92 ed.

- Tortora-Brayda IJM. 2020. Piecing together nest building: Blue tit nest building activity through time and the influence of female presence on male nest building in the southern masked weaver. University of St Andrews.
- Walters LA, Webber JA, Jones BA, Volker CL. 2016. Taking a break: The relationship between ambient temperature and nest attendance patterns of incubation carolina chickadees (*poecile carolinensis*). *Wilson J Ornithol.* 128(4):719-726.
- Webb DR. 1987. Thermal tolerance of avian embryos - a review. *Condor.* 89(4):874-898.
- Wickham H. 2009. *Ggplot2: Elegant graphics for data analysis.* Springer-Verlag New York.
- Wickham H, Francois R, Henry L, Müller K. 2017. *Dplyr: A grammer of data manipulation.* R package version 074.
- Wilke CO. 2019. *Cowplot: Streamlined plot theme and plot annotations for 'ggplot2'.* R package version 094.
- Wmo confirms past 4 years were warmest on record. 2019. [accessed 2019 02/07/2019].
<https://public.wmo.int/en/media/press-release/wmo-confirms-past-4-years-were-warmest-record>.
- Zann RA. 1996. *The zebra finch: A synthesis of field and laboratory studies.* Oxford: Oxford University Press.

FIGURES



Figure 1. Timeline of experiment. On Day 1 (yellow) pairs were provided with 300 pieces of string three hours after lights on, and then checked one hour and three hours later and provided with an additional 100 pieces if fewer than 10 pieces of string were remaining. Days 2 – 4 pairs were checked three, four and seven hours after lights on and provided with 100 pieces of string when required. Days 5 – 22 pairs were left to breed, and checked daily for eggs. Day 23 (blue) any pair without eggs in the nest cup were removed from the experiment and returned to single-sex colony cages. Days 24 – 30 pairs left to breed and checked daily for hatchlings. Day 31 any pair without hatchlings were removed and returned to single-sex colony cages. Day 32 – 46 pairs left to breed and on Day 47 all pairs removed from experiment. Pairs with hatchlings were kept in family cages in the colony room until offspring had fledged.

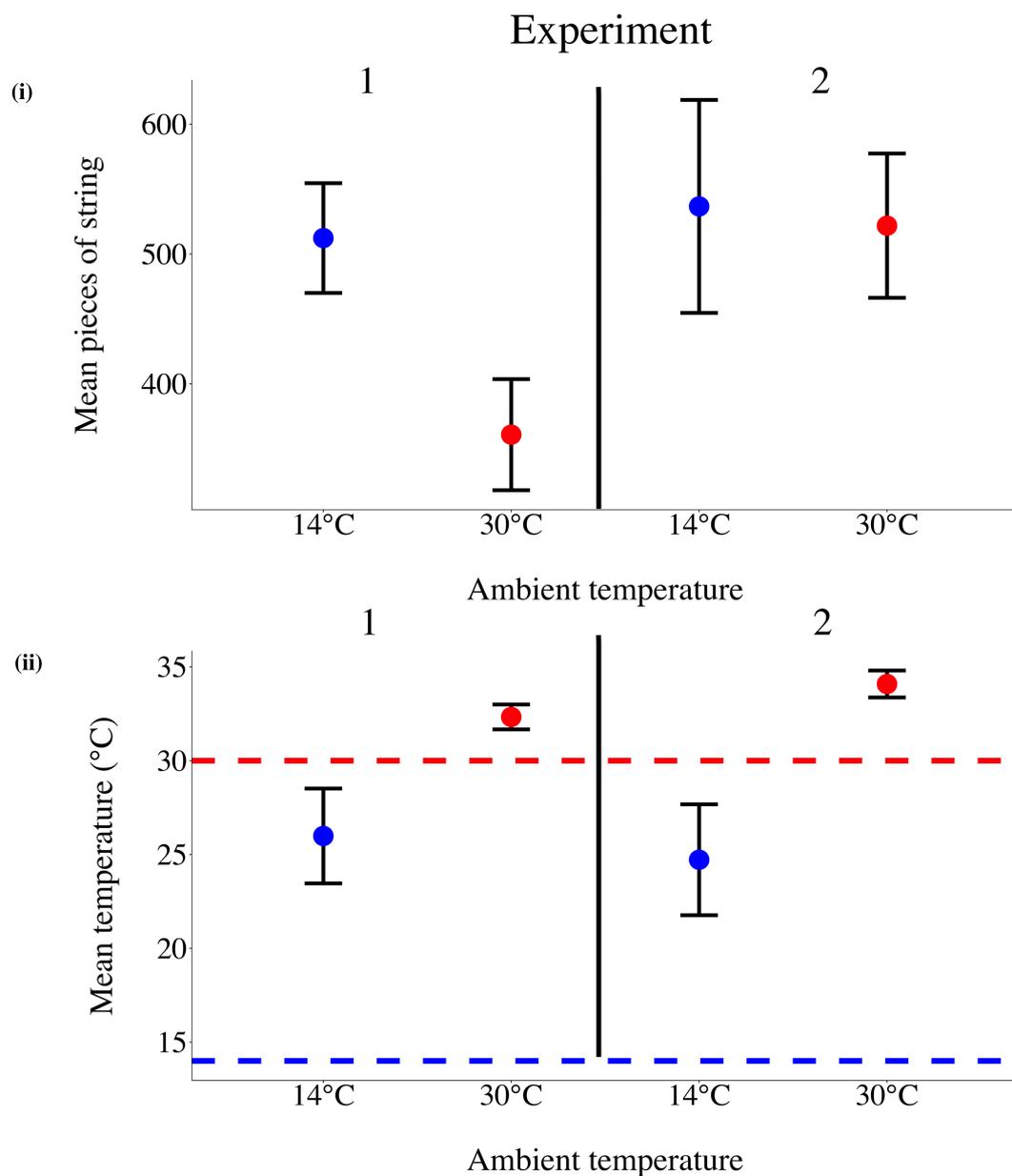


Figure 2. i) Mean number of pieces of string (\pm SE) used in the nest by Treatment and Experiment. ii) Mean nest temperature (\pm S.E.) for pairs that laid at least one egg in Experiment 1 (14°C n = 11; 30°C n = 15) and in Experiment 2 (14°C n = 8; 30°C n = 11). Dashed lines show ambient temperatures of 14°C (blue) and 30°C (red). Blue = 14°C, Red = 30°C; 1 = Experiment 1, 2 = Experiment 2. Only data from birds that built in the nest cup are shown.

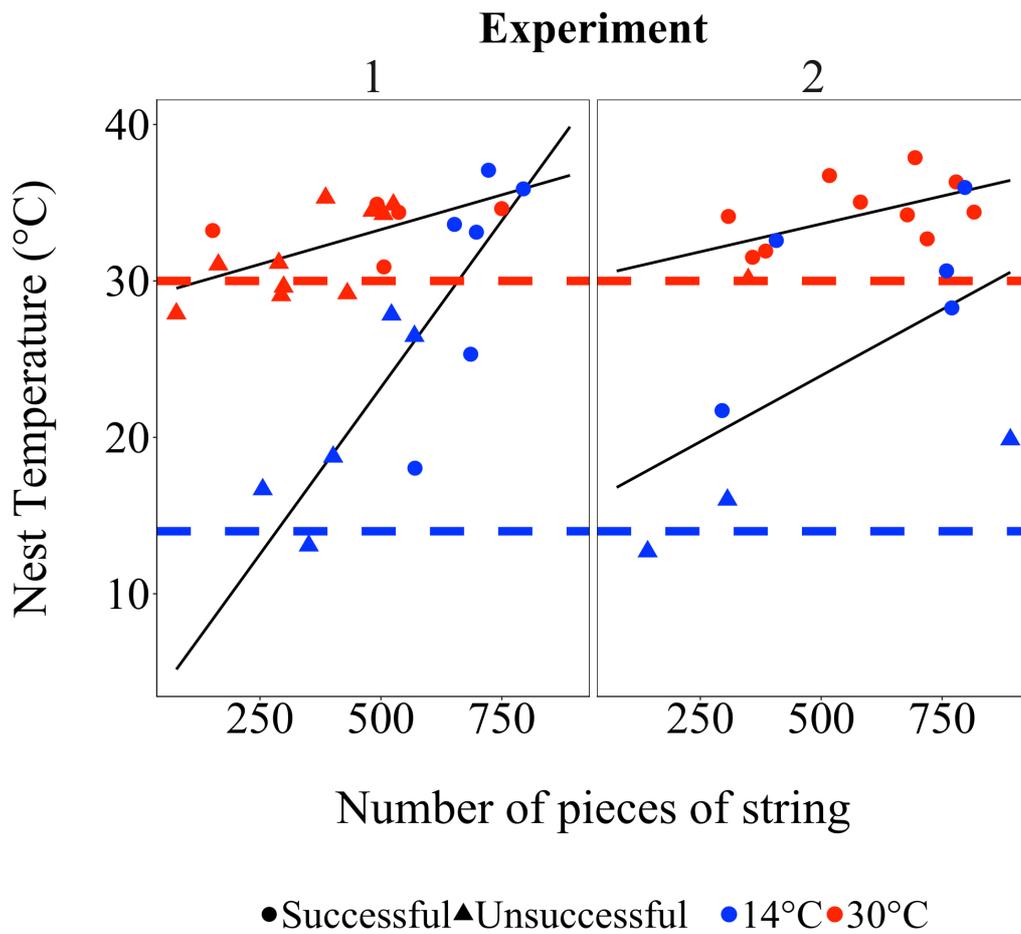


Figure 3. Correlations between the number of pieces of string in the nest and nest temperature. Nest temperature is the mean for the period from the day after the first egg was laid until hatching or until the pair was removed from the experiment. The black line represents the linear model (String~Nest temperature). Dashed lines show ambient temperatures of 14°C (blue) and 30°C (red). Blue = 14°C, Red = 30°C; 1 = Experiment 1, 2 = Experiment 2. Only data from birds that built in the nest cup are shown.

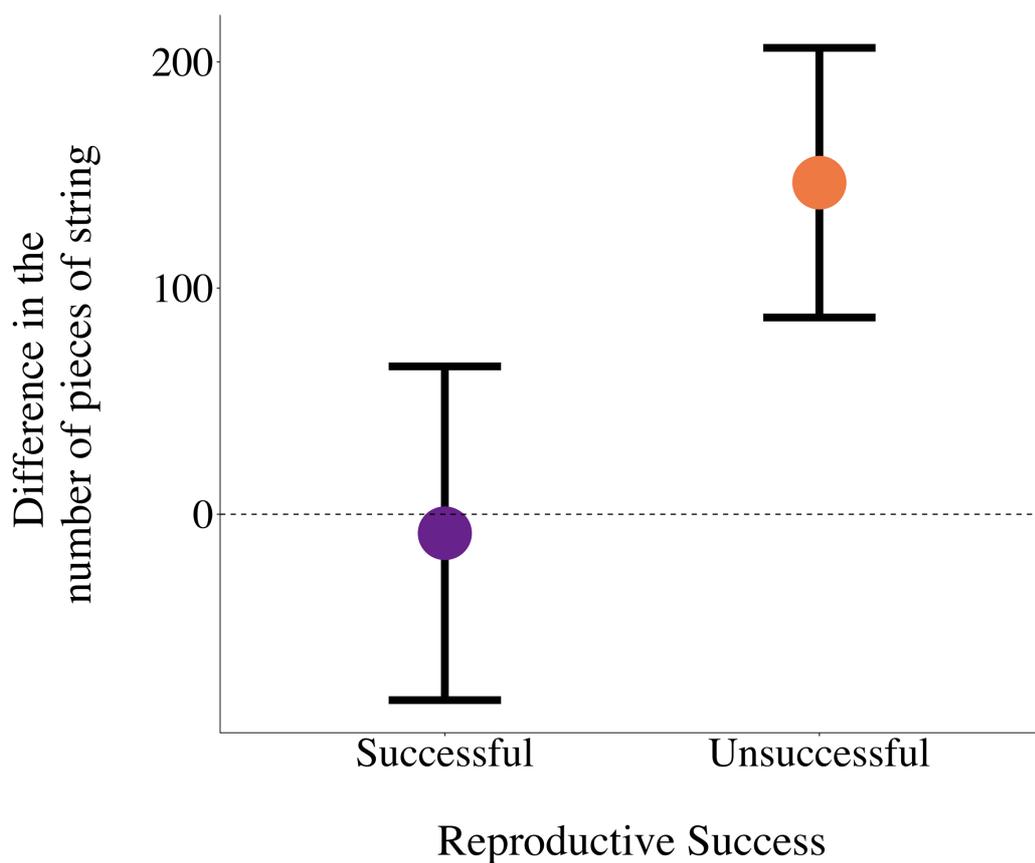


Figure 4. Mean difference in the number of pieces of string (\pm S.E.) used in Experiment 2 by birds that bred successfully (purple, $n = 11$) and unsuccessfully (orange, $n = 24$) in Experiment 1. The difference score was calculated as the Total String in Experiment 2 – Total String in Experiment 1. A positive number indicates that more pieces of string were used to build a nest in Experiment 2 than in Experiment 1. The dashed line represents no change in the total number of pieces of string used in Experiments 1 and 2.