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'Green incubation': avian offspring benefit from aromatic nest herbs through improved parental incubation behaviour

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Development of avian embryos requires thermal energy, usually from parents. Parents may, however, trade off catering for embryonic requirements against their own need to forage through intermittent incubation. This dynamically adjusted behaviour can be affected by properties of the nest. Here, we experimentally show a novel mechanism by which parents, through incorporation of aromatic herbs into nests, effectively modify their incubation behaviour to the benefit of their offspring. Our study species, the European starling, includes in its nest aromatic herbs which promote offspring fitness. We provided wild starlings with artificial nests including or excluding the typically selected fresh herbs and found strong support for our prediction of facilitated incubation. Herb effects were not explained by thermal changes of the nests *per se*, but by modified parental behaviours. Egg temperatures and nest attendance were higher in herb than herbless nests, egg temperatures dropped less frequently below critical thresholds and parents started their active day earlier. These effects were dynamic over time and particularly strong during early incubation. Incubation period was shorter in herb nests, and nestlings were heavier one week after hatching. Aromatic herbs hence influenced incubation in beneficial ways for offspring, possibly through pharmacological effects on incubating parents.

1. Introduction

For an avian embryo, development requires external thermal energy supply, mainly from parental incubation [1,2]. Within the thermal range tolerated by the embryo, low incubation temperatures slow development and are detrimental over longer intervals [3,4], whereas high temperatures accelerate and enhance embryonic development [3,5,6]. For example, experimentally reduced incubation temperature increased hatching failure and decreased fledgling size in blue tits (*Cyanistes caeruleus*) and wood ducks (*Aix sponsa*) [5,7], whereas higher temperatures increased body mass in nestling tree swallows (*Tachycineta bicolor*) [8]. Thus, the incubation temperature an embryo experiences can contribute substantially to fitness [5,9]. However, although an embryo would benefit from a steady supply of body heat from its incubating parents, for the parents incubation is costly and resources such as time and energy are usually constrained [6,10,11].

This conflict is exacerbated in species where one sex contributes disproportionately to incubation (predominantly uniparental incubation [10,12]). Uniparental incubators intermittently leave the nest, for example for foraging, and thus alternate (i.e. 'shuttle') [13] between phases spent on the nest (i.e. on-bouts) and phases away from the nest (i.e. off-bouts) [13]. Off-bouts are usually restricted

to the bird's active day, and thus embedded in their daily rhythm [14,15]. Specific patterns of shuttling have energetic implications because of the thermodynamics of egg cooling and rewarming. For example, shifts from few, long off-bouts to many, short off-bouts in rapid succession are advantageous to incubating parents because of reduced investment required to rewarm unattended eggs [4,13]. Parents resolve conflicts between their own interests and those of their embryonic offspring by adjustment of incubation behaviour depending on their energy state. If incubators have surplus energy, they commonly invest more into incubation. In different songbird species, females in experimentally heated nests increased nest attendance (i.e. proportion of day spent on the nest), but reduced it when nests were cooled [11,16–18]. Parental behaviours are thus dynamically modified during the incubation period to serve their needs, for example, if resources [19] are depleted or in response to parasite infestation [20], but also to cater for embryonic needs [13,14,21]. The sensitivity of the developing embryo to suboptimal temperatures typically increases with age [3] and the thermal properties of eggs also change [13,22]. In many species, parents accordingly raise temperatures with increasing embryo age [21] and may fully avoid drops in egg temperatures below thresholds that halt embryonic development ('physiological zero', arguably 27°C [3,4]). The composition and construction of a nest can play an important role in balancing trade-offs between the parent and the embryo by shaping the micro-environment to which breeding adults and offspring are exposed [1,10,23]. Nest properties can shape the abiotic environment within the nest, such as ambient temperature and humidity, and affect biotic processes, such as interactions with conspecifics, parasites and predators. In particular, the choice of building materials can provide specific benefits for parents and offspring, for example, reduced embryonic heat loss and parental energy savings from insulating materials [6,23]. Popular materials that are selectively used in nests include fresh herbs that release volatile chemical components (electronic supplementary material, figure S1). In several species, for example European starlings (Sturnus vulgaris), inclusion of such herbs before clutch initiation benefitted the offspring, improving their growth rate, blood parameters, fledging mass or subsequent return rate from the winter quarters [24–26].

The ways that aromatic herbs benefit offspring are not entirely clear, but could be partly mediated by the parents. Effects could include energetic benefits, for example through improved insulation [27] or from heat produced by decomposing fresh plant material, which is used for incubation by Megapodiidae [23]. Conceivably, decomposing herbs could slow the cooling of eggs while parents are absent and accelerate warming after their return. Another (non-exclusive) explanation is a pharmacological role of aromatic herbs, for example through parasite repelling, immuno-enhancement or hormone changes [24,28,29]. Reduced parasite loads, supported by nest counts of mosquitos and bacteria [30–32], could benefit nestlings directly [30–32] or through improved condition of their parents. For example, female great tits (Parus major) in experimentally flea-reduced nests slept longer than those in flea-infested nests [24]. Finally, because herbs are also used as social and sexual signals [33] (see electronic supplementary material, movie), they may affect offspring through modified parental physiology or behaviour [29,33].

Here, we hypothesize that inclusion of green herbs benefits offspring already before they hatch through efficient parental incubation. Parents could pass on thermal or parasite-repelling benefits obtained from herbs to their offspring by improving incubation performance; they could modify their behaviour in response to signals conveyed by herbs or respond directly to pharmacological effects [28,29,33]. To the best of our knowledge, no study has investigated whether herb inclusion modifies incubation behaviour, thereby explaining fitness consequences for offspring and incubating birds.

We used an experimental approach to study effects of fresh herbs on incubation patterns of European starlings (hereafter referred to as 'starlings'). In starlings the primary incubators are females, but because males sometimes contribute, we conservatively refer to it as 'parental' incubation [19,34]. We substituted natural nests with artificial ones which either did or did not contain the fresh aromatic plants that starlings typically collect [24,35]. By inserting a dummy egg that continuously recorded temperature, we estimated egg temperature patterns in both nest types. We investigated thermal effects of herbs using the starlings' tendency of longer absences during midday to compare egg cooling in the absence of the parents and rewarming after their return. We then compared between nest types the means and variances of egg temperatures, and the durations of phases of low egg temperatures and of stable heat transfer during static incubation phases. Incubation behaviour was inferred by assigning every consecutive temperature measurement to either on-bout or off-bout, indicating when, and for how long, the incubating starlings left their eggs. Finally, to approximate fitness consequences, we estimated embryonic development time and measured hatching success and nestling body mass a week after hatching.

Specifically, our hypotheses were as follows:

I. Herbs lead to warmer egg temperature.
II. Herb-induced increase in egg temperature could be due to:
   (a) Improved thermal properties of the nest, either through insulation or heat production from herbal decomposition. We expected to detect these effects during the parents' midday outings by slower cooling and faster rewarming in herb nests. We also predicted thermal benefits to change over the incubation period as herbs continue to decompose.
   (b) Lower parasite load, inducing parents to extend their night rest on herb nests.
   (c) Improved parental incubation performance due to direct effects of herbs, measured as lower egg temperature variance and higher attendance.
III. Higher egg temperatures in nests provisioned with herbs accelerate embryonic development and improve nestling condition.

2. Material and methods

(a) Starling study system

The study took place in 2006 and 2007 in a starling colony overlooking wet grassland habitat close to Lake Ammersee, Germany (48° N, 11° E, 700 m.a.s.l.). On trees along the edge of a riparian forest we fixed 53 nest boxes (6 m apart and 3 m above the ground). Male starlings collect most of the nest material, including soft twigs and dry grass [36], and interweave fresh, volatile herbs (electronic supplementary material, figure S1) [35,37].

In our colony, the amount of herbs males include in nest boxes is variable (mass range of 148 nests: 0–350 g [24]) but almost all contain at least some herbs [29,35]. Herb collection is part of male courtship behaviour (see electronic supplementary material, movie), starting at least 14 days before females lay, peaking during pair formation (approx. 5 days before laying) and stopping with clutch initiation [29]. At our site, starlings typically breed twice a year between April and July [24,35].

(b) Experimental procedure

We checked the nest boxes every 5 days to assess onset of laying and incubation. Because clutch size in our colony is 5–6 eggs, this visitation interval enabled us to back-calculate the laying date of the first egg and predict incubation onset. As nests throughout the study population reached incubation onset, we substituted experimental nests for natural nests in an alternating fashion. Nest exchange after the start of incubation, our established procedure over 15 study years, causes no detectable brood desertion [24,29]. We replaced 36 natural nests by artificial nests, retaining the dry nest bowl woven by the female to provide a familiar sight and sensation for the incubating bird at its return. Artificial nests contained either only dry grass (18 'herbless nests': 80 g of dry grass) or grass and fresh herbs (18 'herb nests': 40 g of dry grass and 40 g of herbs). Herb nests contained volatile plants collected nearby of species preferred as green nest material by starlings of our population: milfoil (*Achillea millefolium*), hogweed (*Heracleum spondyleum*), cow parsley (*Anthriscus silvestris*), black elder (*Sambucus niger*), goutweed (*Aegopodium podagraria*) and willow (*Salix alba*) [24,35]. These artificially added components defined the herbal nest environment because males add no further herbs after laying [35]. Clutch size did not differ between herb and herbless nests (GLMM, Poisson: $p = 0.72$). In 2006, we studied second breeding attempts, whereas in 2007 we studied first and second breeding attempts. Therefore, the onset of incubation differed between years (2006 (mean ± s.d.): 9 May ± 1.0 d; 2007: 24 April ± 12.7 d), but not between nest treatments (difference between nest-types (±s.e.): -0.22 ± 3.21; $t_{32} = -0.069$, $p = 0.945$). The mean incubation onsets for herb and herbless nests were 8 and 9 May in 2006, and 24 and 23 April in 2007.

(c) Offspring development and breeding success

Incubation in our colony usually takes 13 days following completion of the clutch (range: 12–14 d). Hence, to estimate variation in incubation period, on incubation day 13 at noon we checked for hatching and early postnatal development of hatchlings. We scored each egg or hatchling as follows: egg = score 0 (assumed to hatch on the following day; retained only if eggs later hatched); wet hatchling = score 1 (assumed to have hatched on this same day); dry hatchling = score 2 (assumed to have hatched the preceding day). These data formed the basis for a combined developmental score per nest, where higher scores indicated shorter incubation. If only eggs were present on day 13, we returned on the following day to ascertain that the clutch had hatched. Hatching success was calculated as the number of hatched nestlings relative to the number of eggs at the onset of incubation. Nestlings were weighed 7 days after we first found hatchlings in a nest with an electronic balance (Sartorius, Germany) to the nearest 0.1 g. The mean age of a brood at this time was inferred from its developmental score during the first control plus 6 days.

(d) Egg temperature measurements

Egg temperature was assessed with Micro T-loggers ('i-buttons'; F. W. Parrett, London, UK). Loggers were placed in plastic dummy eggs which in form and colour simulated starling eggs. These dummy eggs were placed in the nests in the morning of day 2 of incubation in 2006, and of days 1–3 in 2007. We placed a single dummy egg in each nest, which in each case replaced a natural egg that we removed at the margin of the clutch. All birds retained the dummy eggs and therefore incubated their natural clutch sizes. Temperature data were collected in 5 min intervals until hatching. Owing to limited storage capacity, data needed to be downloaded after 7 days into an institute-based PC, causing a brief recording gap (less than 1 h) around mid-incubation time.

The series of equally spaced, continuous temperature logger data (see electronic supplementary material, figure S1 for an example) were analysed using the software environment R [38] to calculate egg temperature metrics and infer incubation behaviour [39]. For most analyses we delineated day and night, either astronomically (by twilight, for egg temperatures) or behaviourally (by first morning departure from the nest and last evening return; see the electronic supplementary material, methods).

(e) Inferred incubation behaviour

We used consecutive logger readings of egg temperature to derive times when parents were likely to be present (on-bouts) or absent (off-bouts) from their nests (see the electronic supplementary material, methods). From the series of on-bouts and off-bouts, we inferred further metrics of incubation behaviour. First, we calculated attendance of parents on the nest (proportion of on-bout time of the 24 h day). For each day, we counted the times when parents sat statically on the nest by extracting sequences of on-bouts with unchanging temperature that lasted at least 15 min (i.e. for three subsequent recordings). We also counted off-bouts and calculated their mean duration. To understand better the implications of the parents' absences, we calculated the duration when eggs cooled below 32°C, and for reference also below 27°C (h). To explore thermal properties of nests, we examined changes in temperature during the birds' longest outings of a given day, which are typically taken at midday. For daily longest outings that occurred between 11.00 and 13.00 we estimated durations from the sequence of inferred off-bouts, noted temperature at the beginning and end, and calculated the cooling during the outing and the time taken to rewarm the eggs to 35°C.

(f) Statistical procedures

Egg temperatures were used until the morning of hatching for a total of 29 boxes (herb nests: 184 observation days; herbless: 160 days; for details, see the electronic supplementary material, methods). For every modelled response variable, data analysis started with a global model including all predictors assumed to be biologically important. Then, starting from the most complex model, stepwise simplification was applied using likelihood-ratio tests (LRTs) of fully nested models until a minimal adequate model was found (electronic supplementary material, table S1). Whenever interactive terms were used, lower-level terms were also included. Statistical significance for each term was calculated by assessing the reduction in explanatory power after dropping the term or order from the model. Likewise, whenever the quadratic term of incubation day was present, the linear term was also included. As we were primarily concerned with the effects of herbs, nest type (two-level factor for 'herb' and 'herbless' nests) was always retained in the final models. Two random factors, 'nest box' and 'year' (nest box nested in year), were always retained. Visual inspection of residuals suggested that assumptions of normality were rarely violated; if they were, transformations to natural logarithms were used (electronic supplementary material, table S1). Most models assumed Gaussian distributions, but where Poisson and binomial distributions were modelled we checked for overdispersion by

Table 1. Effects of herbs on egg temperatures in herb and herbless nests of starlings. Estimated coefficients and test statistics for the minimal adequate models selected using likelihood-ratio tests (LRT) (electronic supplementary material, table S1). Linear mixed models (LMMs) were used to estimate coefficients and test statistics for all terms contained in the final models. Nest type was always included in final models. Model output shows coefficients, standard errors (s.e.), degrees of freedom (d.f.), t-values and p-values. In every model, year and nest-box ID within year were included as random factors. 'Day' refers to incubation day. Nest type (herbs) was set as the reference level; accordingly, the coefficient for nest type (herbless) represents the difference between the two nest types.

predictor	coefficient	s.e.	d.f.	t-value	p-value
mean daytime egg temperature					
intercept	35.26	0.13	304	278.21	<0.001
day	5.03	0.90	304	5.57	<0.001
day^2	−9.74	0.66	304	−14.78	<0.001
nest type	−0.52	0.19	34	−2.78	0.009
day × nest type	2.79	1.32	304	2.12	0.035
variance in daytime egg temperature (log-transformed)					
intercept	1.37	0.13	305	10.75	<0.001
day	−2.33	0.58	305	−4.01	<0.001
day^2	4.88	0.58	305	8.38	<0.001
nest-type	0.29	0.12	34	2.39	0.023
mean night-time egg temperature					
intercept	35.93	0.13	304	267.80	<0.001
day	5.82	0.63	304	9.21	<0.001
day^2	−5.03	0.46	304	−10.85	<0.001
nest type	−0.11	0.17	34	−0.63	0.532
day × nest-type	2.18	0.93	304	2.35	0.020
variance in night-time egg temperature (log-transformed)					
intercept	−0.48	0.06	304	−7.32	<0.001
day	−2.22	0.53	304	−4.20	<0.001
day^2	1.56	0.53	304	2.97	0.003
nest-type	−0.10	0.09	34	−1.07	0.291

comparing residual deviance against residual degrees of freedom. Statistical analyses used packages nlme [40] and lme4 [41] in R v. 3.3.1 [38]. Following model selection, we summarized minimal adequate models and estimated coefficients for all contributing factors (tables 1–4).

Linear mixed models (LMMs) were employed to analyse day-to-day values of means and variances of daytime and night-time egg temperatures, nest attendance, duration of daytime off-bouts (min), duration of daytime temperature drops less than 32°C (h), and times of onset and end of the birds' active day. We also used LMMs for analysing the parents' midday outings. The numbers of static phases and off-bouts were modelled using generalized linear mixed models (GLMMs) with Poisson error distribution. In every model, incubation day (days after incubation onset) as a linear and a quadratic term, and nest type and its interactions with linear and quadratic incubation day, were initially included as explanatory terms. For analysis of midday outings, temperatures at the

Table 2. Effects of herbs on nest thermal properties during midday outings in herb and herbless nests of starlings. Details as in table 1.

predictor	coefficient	s.e.	d.f.	t-value	p-value
temperature upon return					
intercept	−8.69	5.47	144	−1.59	0.114
day	−4.75	2.47	144	−1.92	0.057
nest type	0.05	0.44	33	0.10	0.918
duration of midday outing	−0.11	0.01	144	−7.26	<0.001
temperature at start	1.08	0.15	144	7.87	<0.001
cooling rate (log-transformed)					
intercept	−1.12	0.05	147	−21.64	<0.001
nest type	−0.11	0.08	33	−1.40	0.171
time to rewarm (h)					
intercept	63.20	5.23	145	12.08	<0.001
day	−33.89	7.93	145	−4.27	<0.001
nest type	−1.88	1.32	33	−1.43	0.163
temperature upon return	−1.38	0.19	145	−7.42	<0.001

beginning and end were additionally included as covariates in initial models of cooling rate and warm-up time, respectively. Hatching success and developmental score were analysed using (G)LMMs with binomial and Gaussian error distributions, respectively. In these models, year was included as a random intercept. We also assessed effects of nest type on body mass of nestlings, using LMMs that included year and nest box as random factors, and nestling age as a fixed factor.

3. Results

(a) Effects of herbs on egg temperatures

All measures of overall egg temperatures showed clear variation over the course of incubation (figure 1 and table 1). Mean egg temperatures increased during the early phase of incubation at daytime and night-time. Daytime temperatures were consistently higher in herb nests than in herbless nests ($p = 0.009$; figure 1a) and were significantly affected by linear and quadratic terms of incubation day (for both, $p < 0.0001$). In addition, daytime egg temperatures increased more rapidly during incubation in herb compared to herbless nests (interaction of nest type with incubation day; $p = 0.035$). Similarly, the variance in daytime temperatures (figure 1c) differed significantly over the course of incubation, reaching minimal levels around mid-incubation. Daytime temperatures varied significantly less in herb nests than in herbless nests ($p = 0.023$). Night-time mean egg temperatures (figure 1b) also increased during early incubation and levelled off towards the end, expressed by significant linear and quadratic temporal terms. Night temperatures did not consistently differ between nest types but were initially higher in herb nests (interaction: $p = 0.020$). Variation in night-time temperature (figure 1d) of both nest types decreased significantly over the incubation period.

Table 3. Effects of herbs on inferred incubation behaviour in herb and herbless nests of starlings. Details as in table 1.

predictor	coefficient	s.e.	d.f.	t-value	p-value
attendance (proportion of time on nest over 24 h day)					
intercept	0.86	0.01	277	105.22	<0.001
day	−0.14	0.06	277	−2.17	0.031
day^2	−0.21	0.05	277	−4.75	<0.001
nest type	−0.01	0.01	34	−1.14	0.263
day × nest type	0.24	0.09	277	2.61	0.009

predictor	coefficient	s.e.	Z-value	p-value
number of daytime static phases; Poisson				
intercept	2.14	0.08	27.98	<0.001
day	−0.41	0.50	−0.50	0.414
day^2	−1.85	0.39	−4.79	<0.001
nest type	−0.13	0.08	−1.69	0.090
day × nest type	1.46	0.76	1.92	0.055

predictor	coefficient	s.e.	d.f.	t-value	p-value
duration of daytime egg temperature drops < 32°C (h)					
intercept	0.68	0.05	306	12.48	<0.001
day	−2.09	0.47	306	−4.43	<0.001
day^2	2.97	0.48	306	6.22	<0.001
nest type	0.26	0.08	34	3.18	0.003
day × nest type	−0.93	0.69	306	−1.34	0.180
day^2 × nest type	1.68	0.69	306	2.44	0.015

predictor	coefficient	s.e.	Z-value	p-value
number of daytime off-bouts; Poisson				
intercept	2.99	0.06	50.46	<0.001
day	1.31	0.33	3.99	<0.001
nest type	0.05	0.09	0.63	0.531
day × nest type	−1.24	0.46	−2.71	0.007

predictor	coefficient	s.e.	d.f.	t-value	p-value
duration of daytime off-bouts (min)					
intercept	10.05	0.26	276	38.10	<0.001
day	−2.76	2.94	276	−0.94	0.368
day^2	10.68	2.94	276	3.62	<0.001
nest type	0.56	0.38	34	1.44	0.157
day × nest type	−8.39	4.18	276	−2.01	0.046
day^2 × nest type	−9.12	4.16	276	2.19	0.029
first morning departure (h)					
intercept	−0.66	0.12	322	−5.67	<0.001
day	−0.27	1.19	322	−0.23	0.818
day^2	−0.06	1.19	322	−0.05	0.962
nest type	−0.39	0.13	34	−3.08	0.004
day × nest type	0.65	1.72	322	0.38	0.703
day^2 × nest type	4.33	1.71	322	2.53	0.012
last evening return (h)					
intercept	0.81	0.14	296	5.71	<0.001
day	2.04	0.57	296	3.61	<0.001
nest type	0.06	0.08	34	0.77	0.458

Table 4. Effects of herbs on hatching success and offspring development in herb and herbless nests of starlings. Details as in table 1.

predictor	coefficient	s.e.	Z-value	p-value
hatching success; binominal				
intercept	0.77	0.21	278.76	<0.001
nest type	−0.21	0.31	−0.69	0.494

predictor	coefficient	s.e.	d.f.	t-value	p-value
developmental score					
intercept	1.26	0.26	31	4.76	<0.001
nest type	−0.53	0.19	31	−2.80	0.009
nestling weight (all chicks)					
intercept	48.72	2.03	56	23.97	<0.001
nest type	−7.63	2.75	22	−2.78	0.011

(b) Effects of herbs on nest thermal properties during midday outings

Midday outings of starlings lasted between 10 and 70 min (mean ± s.e.: 31 ± 1 min). Their length varied with incubation day but not between herb and herbless nests ($p = 0.196$; electronic supplementary material, figure S2). Temperatures upon return depended only on starting temperature and duration of an outing, not on nest type (table 2). Cooling rates were steady and did not differ by nest type (herb nests: 0.35 ± 0.002°C min^{-1}; herbless nests: 0.32 ± 0.002°C min^{-1}). Similarly, the time taken for eggs to regain 35°C depended on the temperature upon return, but not on nest type. Rewarming took 24.9 and 24.8 min in herb and herbless nests, respectively, resulting in warming rates of 0.39°C and 0.43°C min^{-1}. We also found no significant interactions between nest type and incubation day on cooling or rewarming, counter to expectations for effects of progressively decomposing herbs.

(c) Effects of herbs on inferred incubation behaviour

Several inferred behaviours contributed to the higher egg temperatures in herb nests, but were modified during the course of incubation (figure 2 and table 3). For nest attendance (figure 2a), which increased until mid-incubation and thereafter declined, we found no overall differences between herb and herbless nests. However, attendance was initially higher in herb nests (interaction of nest type with day; $p = 0.009$). Correspondingly, changes in duration of off-bouts across incubation showed a U-shaped pattern over time with a trough around mid-incubation (figure 2d), although the starlings steadily increased the number of these outings (figure 2c). For both characteristics of off-bouts, effects of nest type depended on incubation day (table 3), peaking during early incubation. Parents on herb nests initially undertook fewer outings (interaction of nest type with day; $p = 0.007$), and these were of shorter duration than those of parents on herb nests (interaction of nest type with day: linear: $p = 0.046$; quadratic: $p = 0.029$), but differences were absent after mid-incubation.

The number of static phases tended to be higher in herb than herbless nests and increased over the course of incubation (electronic supplementary material, figure S3; $p = 0.090$). Conversely, eggs in herb nests cooled below 32°C for
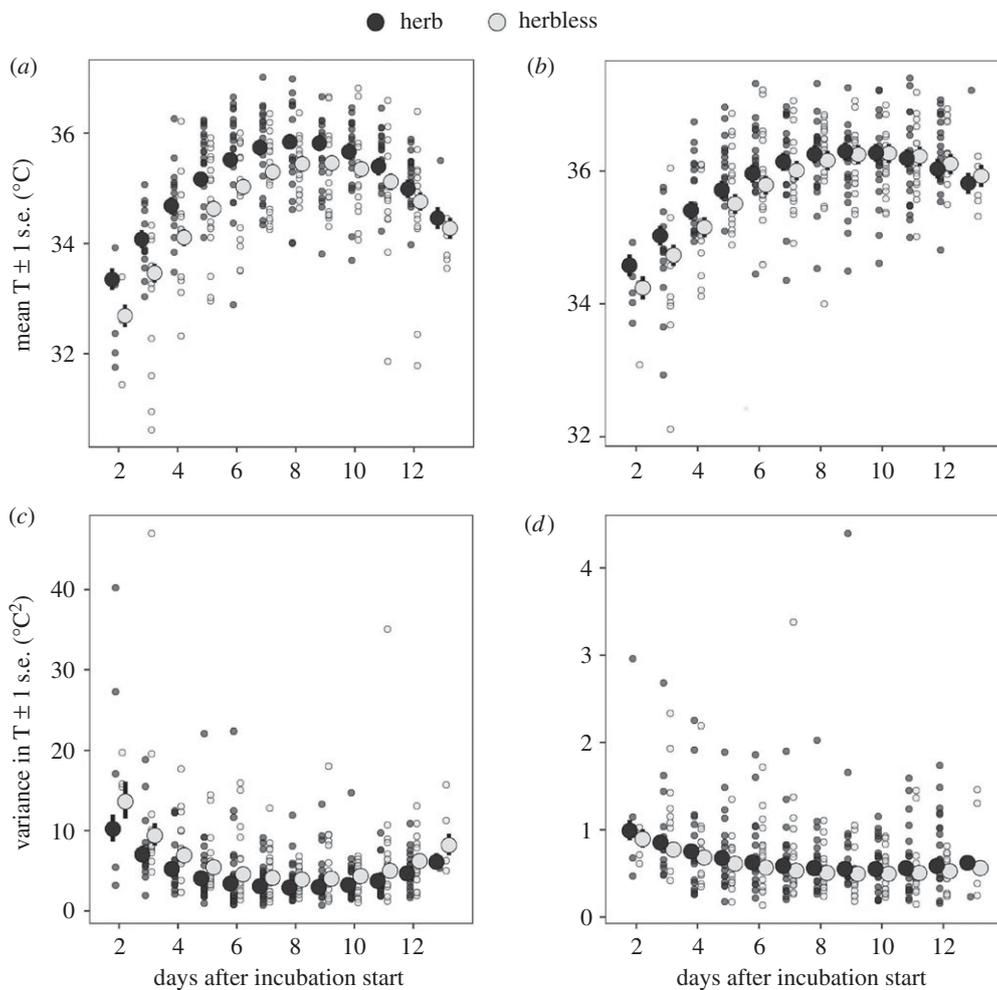
Figure 1. Egg temperatures (T) of herb and herbless nests over the course of incubation. (a) Mean daytime and (b) mean night-time temperatures; (c) mean daytime and (d) mean night-time variance; shown are raw data points (small dots) and model predictions $\pm$ 1 s.e. (large dots) based on minimal adequate models (tables 1–4). Points are slightly offset within days to aid visibility.

significantly shorter times (figure 2b; $p = 0.003$; for temperatures below 27°C see electronic supplementary material, figure S4). Differences between nest types were greatest during the first week of incubation. For both nest types, the occurrence of low egg temperatures was highest in the early incubation phase and rose again slightly prior to hatching.

Incubating starlings left their nests within roughly 1 h after civil twilight began, and returned to their nest boxes within 1 h before it ended (electronic supplementary material, figure S5). Parents incubating in herb nests started their day earlier than those in herbless nests (LMM; $p = 0.004$; table 3). The difference was on average 23 min, but peaked at over 1 h around mid-incubation (interaction of nest type with quadratic term of day; $p = 0.012$). Starlings closed their day progressively earlier across incubation, but no differences were evident between nest types (LMM; $p = 0.458$).

(d) Effects of herbs on hatching success and offspring development

Hatching success of nests was not influenced by inclusion of herbs (GLMM; $p = 0.494$; table 4), nor by other factors in the model. However, the developmental score of offspring on incubation day 13 was significantly higher for herb compared with herbless nests (figure 3a), indicating that embryonic development was accelerated by inclusion of herbs (LMM; $p = 0.009$). Furthermore, herbs were associated with

additional post-hatching benefits, as nestlings in herb nests were more successful in gaining body mass (figure 3b). After their first post-hatching week, they were heavier on average by nearly 8 g (16% of body mass) than nestlings from herbless nests (LMM; $p = 0.011$). These results were not affected by removal of the outlying values of four very light nestlings (electronic supplementary material, table S2 and figure S6).

4. Discussion

In agreement with our first hypothesis, experimental provisioning of nests with green herbs raised egg temperatures. Among the alternative responsible mechanisms proposed in the second hypothesis, our findings from midday outings did not support different thermal properties of the nest types. Parasite-mediated effects on attendance were also poorly supported because the parents' night rest was no longer in herb than in herbless nests. Instead, differences in egg temperature were mainly associated with the birds' alternation between on-bouts and off-bouts, and thus with improved incubation behaviour. Our findings also confirm the third hypothesis of accelerated embryonic development and improved offspring condition in herb-provisioned nests.

The starlings achieved higher egg temperatures in herb nests by modifying a suite of behaviours which jointly we describe as 'steady incubation'. Parents sat statically for at least 15 min more often on herb than on herbless nests.
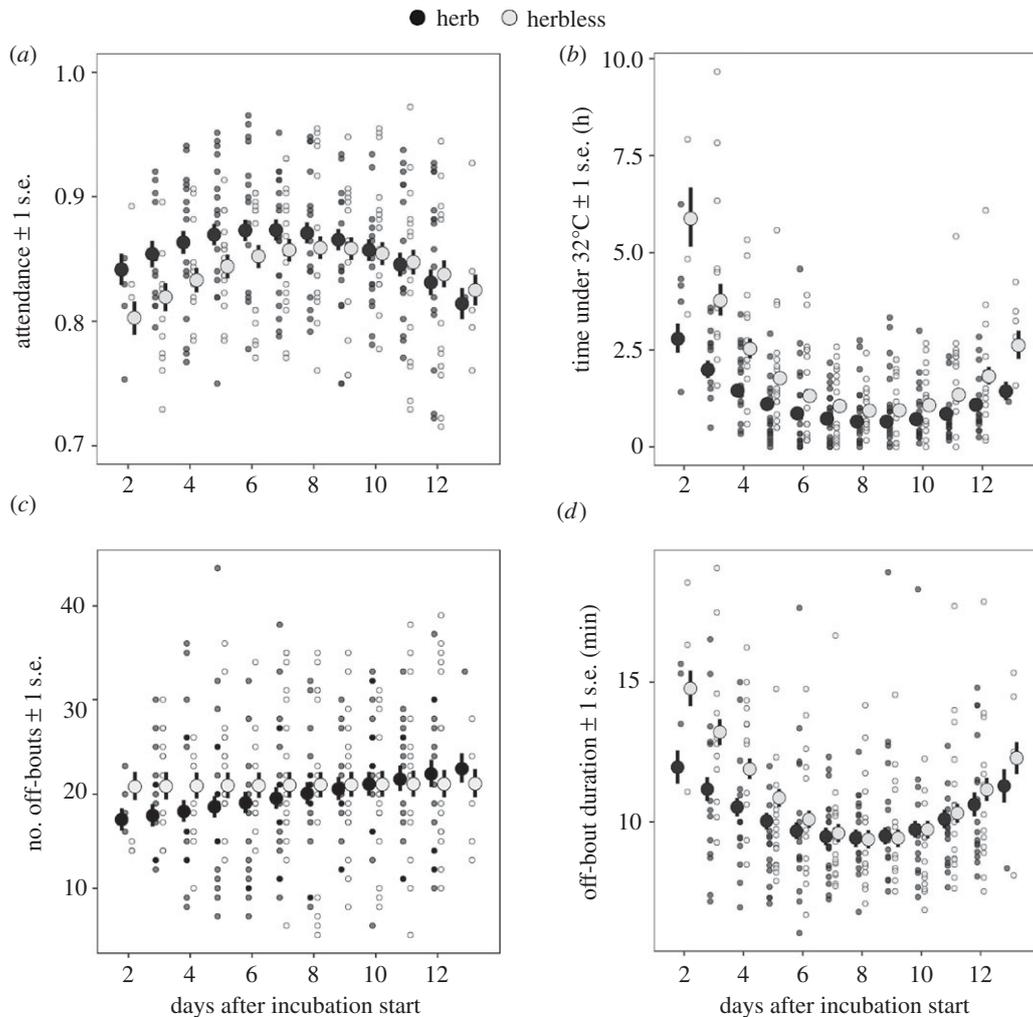
Figure 2. Inferred behaviour of starlings incubating herb and herbless nests over the course of incubation. (a) Nest attendance, (b) duration of daytime egg temperature drops less than 32°C (h), (c) number of daytime off-bouts per day and (d) duration of daytime off-bouts (min). Details as in figure 1.

Off-bouts of parents were initially much shorter for herb than for herbless nests. Consequently, during early incubation eggs in herb nests experienced far shorter cooling to low temperatures than those in herbless nests. Unexpectedly, however, starlings departed from herb nests earlier in the morning than from herbless nests. Because evening return times did not differ, starlings on herb nests had a longer active day of shuttling than those on herbless nests. Despite shortening their uninterrupted nocturnal incubation phase, parents on herb nests had overall higher nest attendance than those on herbless nests.

The starlings' behaviour changed substantially during the incubation period. Highly dynamic incubation behaviour, and corresponding substantial changes in temperatures experienced by embryos as they develop, are widespread among birds [3,13,21]. Many species display partial incubation during laying [21] and develop full incubation only gradually after clutch completion. Consequently, incubation temperatures and thermal precision (i.e. low variance) commonly improve as embryonic development progresses [3,21]. In the starlings, initial egg temperatures can be characterized as showing a 'slow rise' time profile [21]. In parallel, the effects of herbs on incubation also changed over time, being most pronounced during early embryogenesis. Thus, daytime and night-time egg temperatures initially increased more steeply in herb compared to herbless nests. These and associated behavioural differences gradually receded, so

that herb and herbless nests were similar during the second half of the incubation period. In both nest types temperatures and attendance dropped and absences lengthened during late incubation stages, leading to overall curved trajectories. Dropping temperatures during late incubation stages have been previously reported in birds [3,10], but curved trajectories may be under-reported because analyses often consider only linear effects [22].

Incubating birds usually reduce exposure of their eggs to cooling if they energetically can [11,16–18]. Brief egg temperature drops have been associated with delayed embryonic development, and extended cooling during vulnerable periods is considered detrimental [3,10]. For example, some studies refer to egg temperatures below 27°C as physiological zero when development is halted [3,4]. Nonetheless, similarly to an earlier study [19], we found that the starlings incubated at potentially suboptimal temperatures. Cooling of eggs to low temperature occurred in particular in herbless nests. For example, during early incubation (day 3), egg temperatures dropped below 32°C for about 3 h in herbless nests, but only for 1.75 h in herb nests. Effects of herbs were statistically similar for drops below 27°C, where temperatures fell below 27°C for about 1.5 h per day in herbless nests, but only for 1 h in herb nests. In our study, herb-incubating starlings reduced egg cooling by shorter off-bouts, potentially saving energy through their more steady incubation patterns [13].
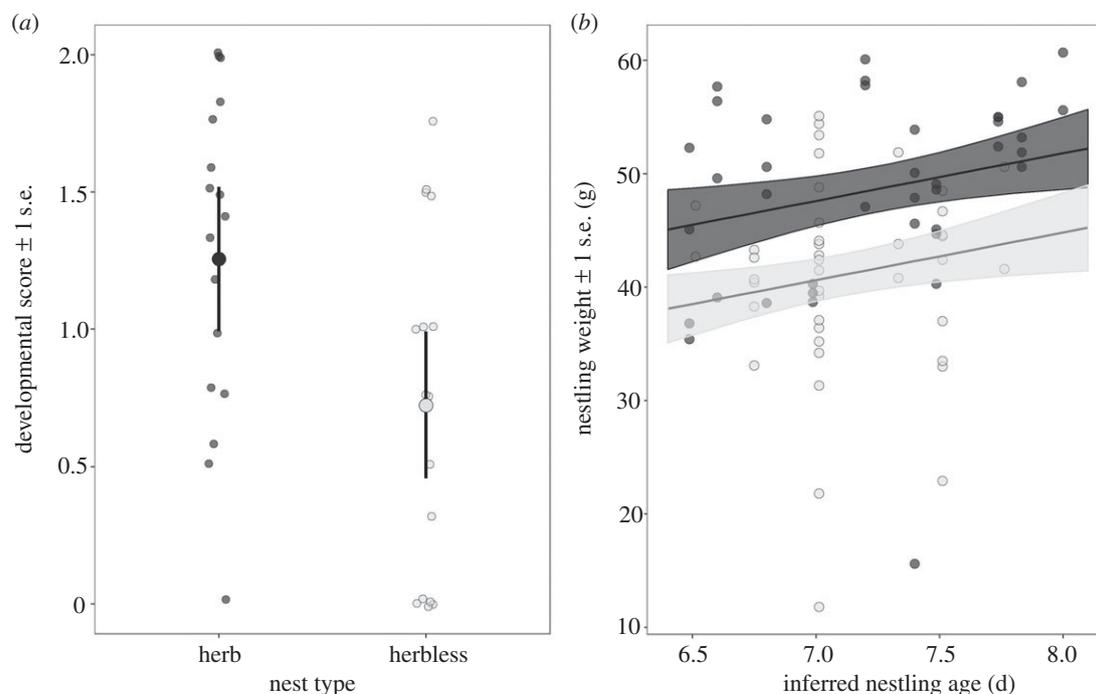
Figure 3. Offspring development in herb and herbless nests. (*a*) Mean developmental score of offspring in herb and herbless nests on day 13 after the start of incubation, calculated by scoring each nestling as either egg, or freshly hatched, or hatched the previous day. (*b*) Nestling body mass in nests with herbs and without herbs, at mean clutch ages from 6.5 to 8 d. Details as in figure 1.

We had proposed several hypotheses for how aromatic, green herbs could facilitate increased egg temperatures. We speculated that fresh herbs could provide direct thermal benefits through improved insulating properties and through heat produced by decomposition ('ambient incubation' [2]). Such herb-induced thermal benefits should be detected during the birds' extended midday outings by a slower cooling rate and faster rewarming after their return. Furthermore, if fresh herbs brought thermal gains, these should change in herb nests over incubation. None of these predictions were supported by the data. Measurements of artificial nests in an incubator confirmed our findings (see the electronic supplementary material, methods), as cooling of logger eggs also did not differ with nest type. Thus, we conclude that herbs most probably affect egg temperature by influencing the incubating parents.

Effects of herbs on parents could be mediated by reduced parasite load associated with nest herbs [30–32], as exemplified by increased sleep in great tits exposed to lower hen flea infestation [20]. However, in our starlings night rest was conversely shorter in herb females. Lack of the predicted effect could relate to parasite species. In our starling population, the main ectoparasite in nests is the red fowl mite (*Dermanyssus gallinae*), a blood-sucking species, which according to studies on several songbird species including starlings is unaffected by herbs [24,25,42] (but see [43]). Nonetheless, herbal effects on other parasites and other aspects of parental health are possible. For example, bacterial growth is inhibited by herbs [32,44], and the greater heat applied to eggs in herb nests could further reduce bacterial load, thereby improving parental condition [45].

Herbs could also affect incubation via sexual and social signalling [29,33]. In starlings the males' provisioning with herbs during courtship may signal their quality [35,46]. According to the 'differential allocation hypothesis' [47], for a female paired with an attractive mate, increased reproductive investment could help maintain the pair bond. Hence, females could respond to herbal quality signals of the nest-building male by increased incubation efforts. Starlings in our colony rarely form long-term pair bonds, but differential allocation could pay off to females via high-quality paternal genes [46]. However, our experimental nest exchange after laying uncoupled herb presence from courtship displays. Thus, signalling effects on females would be largely reduced to olfactory perception of volatile compounds of herbs, which are perceptible to the starlings' fine sense of smell in particular during the breeding season [28]. Alternatively, volatiles could also affect incubating birds directly. Effects of herbs on female physiology are supported for European starlings in our colony, where provisioned herbs correlated with the deposition of androgens in the egg yolk [29], and for spotless starlings (*Sturnus unicolor*), where experimental inclusion of herbs increased female plasma androgen levels [48]. These findings could be interpreted equally as arising from signalling or from pharmacological action of herbs [29,48].

In support for a role of volatile herb compounds, effects of herbs on incubation were greatest at early incubation and then steadily declined. This paralleled the decreasing volatile release which we had found to decline by a factor of 10 over 9 days in unoccupied starling nests [28]. One specific mechanism by which volatile compounds may have enhanced egg temperatures involves pharmacologically sedating the incubating bird. The starlings' initial steady incubation patterns in herb nests support such an interpretation. Parents on herb nests showed longer incubation bouts, less variance and a tendency for more static phases than those from herbless nests. Some compounds which we have previously identified from nest air [28], like limonene, sabinene or caryophyllene, are released by herbs used in traditional herbal medicine [49]. Milfoil, the starlings' most preferred herb, is considered a mild sedative [50].

Whatever the mechanism, the observed steady incubation in herb nests has potential benefits for the incubating birds [11,16–18] as well as for the developing embryos. Benefits for herb-incubating parents arise from reduced variation in egg temperature, which has been experimentally shown to be more energy-efficient due to lower thermal flux [13]. Energy costs to warm the eggs correlate with the drop in temperature during a preceding absence [51], so that the shorter off-bouts of herb incubators should lead to a more positive energy balance. A more positive energy balance could also explain the unexpected earlier morning departure of parents from herb compared to herbless nests. Parents in herb nests started their day consistently early, whereas herbless incubators delayed morning activities [14], in particular around mid-incubation when incubation temperatures and hence energy-demand were highest. An early start to the day in birds has often been associated with advantages [14,15,52]. Thus, herb-induced energy savings might have enabled starlings to exploit feeding conditions early in the morning when low ambient temperatures impose high energetic costs. Energy balance is known to influence the timing of animal activities (e.g. [53]), but pharmacological effects on biological timing are also possible.

Steady incubation also has potential benefits for the developing avian embryo. For example, slight differences in incubation temperature affected early growth and stress physiology of wood ducks [5], and growth and energy metabolism in blue tits [7]. Zebra finches (*Taeniopygia guttata*) that experienced drops of incubation temperature were smaller on day 12 than those from nests with constant temperature [54]. Beneficial effects of incubation could also extend across the lifespan, as shown for zebra finches, where offspring from eggs incubated under higher temperatures had higher long-term survival [55]. In our study, steady incubation in herb compared to herbless nests significantly accelerated embryogenesis. Furthermore, nestlings from herb nests were heavier seven days after hatching, indicating either carry-over effects from incubation or continued beneficial effects of herbs.

Our study thus corroborates previous findings that young starlings raised in herb nests had advantages over those raised in herbless nests [24]. We extend these earlier findings by showing that positive effects already started during incubation. Several previously reported benefits, such as reduced bacterial load, higher fledging weight and enhanced immune responses [24,30,44], were not assessed in our study but may add to the benefits we report. Conversely, in earlier studies, advantages of nestlings in herb nests were interpreted as direct herbal effects on the young [24]. However, incubation temperature can also affect the nestlings' immune system [17,56], weight [5,7] or bacterial infestation [45]. Therefore, for some of the earlier-reported benefits of herb nests, egg temperature may have also played a role.

Taken together, our study highlights 'green incubation' on nests that contain selected volatile herbs. Inclusion of herbs conveys multiple benefits to starlings, from male courtship success and increased reproductive investment of females to enhanced offspring health. To this we add our findings that green herbs also benefit offspring during the egg stage through improved parental incubation.

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