Diurnal brooding behavior of long-tailed tits (*Aegithalos caudatus glaucogularis*)

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ABSTRACT
Brooding is a major breeding investment of parental birds during the early nestling stage, and has important effects on the development and survival of nestlings. Investigating brooding behavior can help to understand avian breeding investment strategies. From January to June in 2013 and 2014, we studied the brooding behaviors of long-tailed tits (*Aegithalos caudatus glaucogularis*) in Dongzhai National Nature Reserve, Henan Province, China. We analyzed the relationships between parental diurnal brooding duration and nestling age, brood size, temperature, relative breeding season, time of day and nestling frequencies during brooding duration. Results showed that female and male long-tailed tit parents had different breeding investment strategies during the early nestling stage. Female parents bore most of the brooding investment, while male parents performed most of the nestling feedings. In addition, helpers were not found to brood nestlings at the two cooperative breeding nests. Parental brooding duration was significantly associated with the food delivered to nestlings ($F=86.10$, $df=1$, $193.94$, $P<0.001$), and was longer when the nestlings received more food. We found that parental brooding duration declined significantly as nestlings aged ($F=5.99$, $df=1$, 50.13, $P=0.018$). When nestlings were six days old, daytime parental brooding almost ceased, implying that long-tailed tit nestlings might be able to maintain their own body temperature by this age. In addition, brooding duration was affected by both brood size ($F=12.74$, $df=1$, 32.08, $P=0.001$) and temperature ($F=5.83$, $df=1$, 39.59, $P=0.021$), with it being shorter in larger broods and when ambient temperature was higher.

Keywords: Long-tailed tit; *Aegithalos caudatus glaucogularis*; Brooding; Daytime; Early nestling stage

INTRODUCTION
A stable thermal environment is essential for egg hatching and nestling development during avian reproduction (Zhao et al., 2002). Brooding, whereby adult birds settle down with nestlings to provide heat (Johnson & Best, 1982), is an important investment in addition to feeding during the nestling period. For young nestlings, whose abilities to maintain body temperature are generally weak (especially that of altricial birds), brooding is extremely important to maintain a constant body temperature (Johnson & Best, 1982; Visser, 1998; Zheng, 2012).

The energy investments of caring for nestlings have profound effects on adult fitness (Monaghan, 2004). During the first days of the nestling period, brooding behavior can directly affect adults through loss of body mass, a phenomenon that has often been found in females of species in which the females undertake most of the brooding investment (Chastel & Kersten, 2002; Moreno, 1987, 1989a). Although brooding is important during the nestling stage, current studies on parental investment during this period have primarily focused on nestling feeding behavior. In some altricial birds, such as the long-tailed tit (*Aegithalos caudatus*) (MacColl & Hatchwell, 2003), twite (*Acanthis flavirostris*) (Zhao et al., 2003), and azure-winged magpie (*Cyanopica cyanus*) (Valencia et al., 2006), the feeding frequencies of female parents are significantly less than those of male parents at the early nestling stage, though this difference gradually disappears by the late nestling stage. It is worth noting that brooding investment is often borne by the...
females in these species (Ru et al., 1997; Song, 1981; Zhao et al., 2003), and focusing on adult nestling feeding alone may not completely reflect parental investments and strategies.

The long-tailed tit is a small passerine bird in the family Aegithalidae (Zheng, 2006). Its breeding biology (Fang & Ding, 1997; Gaston, 1973; Li et al., 2012; Song, 1981) and nestling feeding behaviors (Hatchwell & Russell, 1996; Hatchwell, 1999; MacColl & Hatchwell, 2003) have been extensively investigated. Brooding behavior, however, has only been described briefly (Song, 1981). In this study, we investigated the differences in diurnal brooding investment between female and male long-tailed tits and analyzed the associations between diurnal brooding duration and nestling age, brood size, ambient temperature, breeding season, time of day and nestling feeding frequency during brooding.

MATERIALS AND METHODS

Study area and study population

This study was conducted in the Dongzhai National Nature Reserve (N31°28′-32°09′, E114°18′-114°30′) between January and June in 2013 and 2014. The reserve, characterized by its rich avian diversity, is located in the south of Henan Province and west of the Dabieshan Mountains, and is at the transitional region between subtropical and temperate zones. Annual mean temperature and precipitation are 15.1°C and 1 209 mm, respectively (Song & Qu, 1996).

There are three subspecies of long-tailed tits in China, that is, A. c. caudatus, A. c. vinaceus and A. c. glaucogularis (Zheng, 2006). The long-tailed tit population at our study site belongs to the subspecies A. c. glaucogularis. According to morphological characteristics and breeding isolation, the subspecies A. c. glaucogularis and A. c. vinaceus have recently been treated as the independent silver-throated tit, A. glaucogularis (Harrap, 2008). In this study, however, we followed the traditional classification (Zheng, 2006) and treated the Dongzhai National Nature Reserve population as a subspecies of the long-tailed tit. The long-tailed tits are resident in the study area and occur in conifer, broadleaf forests and shrubs. The breeding season is usually from late January to early June, and annual nesting success of this population is about 30% (Li et al., 2012). Helpers exist at about 30% of the nests during the nestling stage, with most helpers being male failed breeders (Li et al., 2012).

Data collection and analyses

Each adult long-tailed tit involved in this study was banded with a metal ring and a unique combination of colored rings so that each individual could be identified. Banding was usually conducted at two periods: (1) before the breeding season (late December to January) when tits were still flocking and easily caught, and (2) during the breeding season when we continued to band the unbanded adults and nestlings (around 10 days old) born in that season (see Li et al., 2012 for banding method details). As we have studied the long-tailed tit population for several years, the birds involved in this study included those banded between 2011 and 2014 (2011: n=9; 2012: n=27; 2013: n=35; 2014: n=16). When banding a bird, we collected a 20-50 µL blood sample via venipuncture of the brachial vein for molecular sexing (see Li et al., 2010 for details). During the breeding seasons, we looked for long-tailed tit nests by following adults carrying nestling material and food, and by searching potential nesting sites. When a nest was found, we gave it a unique nest number as its nest identity, and checked it every one to three days to ascertain its breeding status. After nestlings hatched, we used video cameras (Samsung SMX-F40, Sony HDR-XR160E, Sony HDR-SR10 and Sony HDR-HC5) placed 0.5-3.0 m from the nests to film the brooding and feeding behaviors of adults every one to three days. Filming was usually carried out between 0800-1800 h and usually lasted for at least 1 h. The tit banding and sampling methods used in this study are common in ornithological research and our operation strictly followed current regulations. Among the nests involved in the analyses of brooding behavior, no adults or nestlings were banded during the early nestling period (i.e., the period when brooding normally occurred) and therefore our analyses were not affected by banding or sampling. Camera concealment was also carefully considered and the tits resumed their activities soon after the cameras were set up (usually within 20 min), with no apparent adverse effects detected. No adults or nestlings were banded during the brooding stage.

To explore the factors affecting diurnal brooding duration, we analyzed whether brooding duration was related to nestling age, brood size, ambient temperature, relative breeding season, time of day or nestling feeding frequency during brooding. Nestling age was measured in days after hatching, with day 0 being the day of hatching (long-tailed tit broods hatch synchronously). Based on field observations, brooding behavior of the long-tailed tit population mostly occurred between nestling age of one and five days. We therefore included videos filmed for nestlings up to six days old. Brood size was the number of nestlings observed when filming the nest. We used the mean daily temperature recorded by the Xinyang Weather Station as the ambient temperature of the filming day. Xinyang Weather Station is the closest weather station to our study site (about 25 km) and the weather data were downloaded from the China Meteorological Data Sharing Service System (http://cdc.nmic.cn/home.do). Relative breeding season was the number of days after the hatching date of the first nest in each breeding season, with this variable used to reflect the relative position of the nest in the breeding season. When analyzing brooding duration in relation to time of day, we divided daytime (0800-1800h) into ten 1-h periods (0800-0900h, 0900-1000h, etc.) and assigned the brooding behavior into the corresponding time period based on the time of its occurrence. If the brooding behavior occurred across time periods, the time period was considered the period of longer brooding duration. For example, if brooding behavior occurred from 0940h to 1005h, its duration in period 0900-1000h (20 min) was longer than that in period 1000-1100h (5 min), and therefore the brooding behavior was assigned to period 0900-1000h.

The nests of long-tailed tits are dome-shaped, with an entrance hole on the side near the top (Li et al., 2012). We treated a behavior as brooding when an adult entered the nest...
and settled down on the nestlings; it was not treated as a brooding behavior if an adult entered the nest for a short period of time without settling down. Brooding duration was the time between an adult's entry and leaving of the nest (min). Nestling feeding frequency included both feeding by the brooding adult who carried food to the nest and then brooded and feeding by non-brooding adults whose food was usually transferred by the brooding adult to the nestlings.

Data were analyzed using linear mixed models fitted by restricted estimation maximum likelihood. Linear mixed models can account for both fixed and random effects (Bolker et al., 2009). In the analysis, the duration of each brooding behavior was treated as the dependent variable. Year, nest identity, video identity and adult identity were included as random factors to control for non-independence between observations of the same year, same nest, same observation and same adult, respectively. Explanatory variables were the sex of parents, nestling age, square of nestling age (brooding duration may vary with age in an asymptote way), brood size, temperature, relative breeding season, time of day and nestling feeding frequency by adults during brooding. Two-way interactions of the above factors were also considered, except for that between nestling age and square of nestling age (biologically meaningless). Significance of terms was assessed from type III F tests. The best fitting model was obtained by sequentially dropping nonsignificant terms from the initial model until all terms were significant ( Crawley, 2007). All statistical analyses were performed in R v.2.14 (R Development Core Team, 2012) using package lme4 (Bates et al., 2014). Mean values were reported with standard deviation (mean±SD).

RESULTS

A total of 48 nests with 1-6 day-old nestlings were analyzed. Each nest was filmed 1.54±0.71 times, and each filming lasted for 1.23±0.57 h. Among all nests, two exhibited cooperative breeding, with each nest having a male helper. Among the 206 brooding behaviors recorded, female parents engaged in brooding behavior at a much higher rate (87.86%, n=181 times). The brooding duration of female parents was also significantly longer than that of male parents (female parents: 9.50±8.13 min, male parents: 4.70±5.38 min) (Table 1 and Figure 1A). For the two cooperative breeding nests, helpers were not observed to brood nestlings.

Table 1 Linear mixed model analysis results of parental brooding duration of long-tailed tits ( Aegithalos caudatus glaucogularis )

<table>
<thead>
<tr>
<th>Factors</th>
<th>Estimate</th>
<th>SE</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex of parents</td>
<td>−3.86</td>
<td>−2.27</td>
<td>1.20</td>
<td>9.30</td>
<td>0.009</td>
</tr>
<tr>
<td>Nestling age</td>
<td>−1.41</td>
<td>0.58</td>
<td>1.50</td>
<td>5.99</td>
<td>0.018</td>
</tr>
<tr>
<td>Brood size</td>
<td>0.64</td>
<td>32.08</td>
<td>1.32</td>
<td>12.74</td>
<td>0.001</td>
</tr>
<tr>
<td>Ambient temperature</td>
<td>−1.60</td>
<td>0.66</td>
<td>1.39</td>
<td>5.83</td>
<td>0.021</td>
</tr>
<tr>
<td>Total feeding frequencies</td>
<td>3.91</td>
<td>1.34</td>
<td>1.193</td>
<td>86.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nestling age × total feeding frequencies</td>
<td>1.42</td>
<td>0.42</td>
<td>1.198</td>
<td>11.35</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The best fitting model for fixed factors is shown above. Year, nest identity, video identity and adult identity were included in the model as random factors. Estimates of their variances±SD were 0.00±0.00, 1.25±1.12, 2.27±1.51, and 9.99±3.16, respectively. The estimate±SE of adults’ sex was estimated for males, with females being the reference.

Female and male parents also differed in their investment in nestling feeding during brooding. In the non-cooperative breeding nests (n=146), nestling feedings (n=22) during brooding of male parents (n=24 times) were all conducted by the male parents themselves who brought food when returning to the nest. However, during brooding of female parents (n=177 times), only 39.94% (n=143 times) of nestling feedings were performed by females carrying food back to the nest, with the remaining nestling feeding conducted by the male parents (60.06%, n=215 times). In the two cooperative breeding nests, male parent brooding was only recorded once, during which the male parent and a male helper each fed the nestlings once; during female parent brooding (n=4 times), nestlings were fed 21 times, with the brooding female parent, male parent and helper bird accounting for four, nine, and eight times, respectively.

At the early nestling stage, brooding duration was negatively affected by nestling age, brood size and temperature (Table 1). As nestlings aged, brooding duration significantly declined (Figure 1A). Brooding duration was 10.43±7.84 min (n=58) when nestlings were one day old, but only 1.08±0.18 min (n=2, based on 9 h of filming of seven nests) when nestlings were six days old. Brooding duration was significantly shorter in larger broods (Figure 1B) and when the ambient temperature was higher (Figure 1C).

In addition, brooding duration increased significantly with the increase in nestling feeding rates (Table 1 and Figure 1D). Brooding duration was also affected by the interaction between nestling age and adult feeding frequencies during brooding (Table 1). As nestlings aged from one to four days old, the effects of feeding frequency on brooding duration increased, as indicated by the slope of fitted lines (Figure 1D). When nestlings were five days old, the effect of feeding frequencies on brooding duration became smaller than that during the first four days, and had almost had no effect on brooding duration when nestlings were six days old (Figure 1D).

DISCUSSION

During the early nestling stage, both brooding duration and brooding frequencies of the long-tailed tit female parents were
higher than those of the male parents, indicating that female parents bore most of the brooding investment. This is similar to that observed in most passerine birds, in which female parents assume all or most brooding investment (Chastel & Kersten, 2002; Moreno, 1989b).

Unlike female parents, long-tailed tit male parents invested more time in feeding nestlings during the early nestling stage. MacColl & Hatchwell (2003) also found that female long-tailed tits in a British population fed nestlings significantly less frequently than the male parents did during the early nestling stage, though these differences disappeared by the late nestling stage. They suggested that the differences might be due to the female taking on egg laying and incubation duties, and therefore investing less time in nestling feeding than that undertaken by male parents (MacColl & Hatchwell, 2003). Our study suggests that the division of labor (i.e., brooding or feeding) could be an alternative explanation to female and male differences in nestling feeding frequencies at the early nestling stage. The division of labor might be a breeding strategy formed during the long-term evolution of birds, and may be beneficial to female parents who can focus on brooding and protecting nestlings (Katzenberger et al., 2015).

Although long-tailed tits are a cooperative breeding species, this study, like previous researches (Gaston, 1973; Hatchwell et al., 2004; Li et al., 2012), did not find helpers engaging in the brooding of nestlings. Again, this might be related to the division of labor between females and males. In long-tailed tits, most helpers are failed male breeders (Hatchwell et al., 2004; Li et al., 2012; MacColl & Hatchwell, 2002), with males more likely to engage in nestling feeding than brooding. Considering that only two cooperative breeding nests were involved in our study, however, the phenomenon that long-tailed tit helpers in the Dongzhai population do not participate in brooding needs to be confirmed with more observations in future studies.

During the early nestling stage in long-tailed tits, parental brooding duration declined as nestlings aged. This is probably related to the enhancement of the nestlings’ ability to maintain their own body temperatures (Jia et al., 2001; Katzenberger et al., 2015; Zheng, 2012). According to our observations, the nestlings of the long-tailed tit population in Dongzhai National Nature Reserve likely developed the ability to maintain body temperature at six days old, a little earlier than that recorded from Changbaishan Mountain, which is ~7.5 days old (Song, 1981). The phenomenon that brooding duration declines with nestling age also commonly exists in other bird species, such as willow grouse (Lagopus lagopus) (Pedersen & Steen, 1979).

Figure 1 Effects of nestling age (A), brood size (B), ambient temperature (C) and total feeding frequencies during brooding (D) on parental brooding duration in long-tailed tits (Aegithalos caudatus glaucogularis)
and black sparrowhawk (Accipiter melanoleucus) (Katzenberger et al., 2015).

Our results showed that the increase in nestling feeding frequency during brooding significantly lengthened brooding duration in long-tailed tits, implying that brooding adults may adjust brooding duration according to their partner’s feeding investment and nestling demands. Moreover, the interaction between nestling age and adult feeding frequencies during brooding also significantly affected brooding duration. During the first four days after nestlings hatched, the effects of feeding frequencies on brooding duration increased with nestling age. This may be due to the increase in food demands of nestlings with age, with brooding adults being able to adjust brooding investment according to nestling demands, leading to stronger effects of feeding frequency on brooding duration. When nestlings were five days old, the effects of feeding frequency were smaller than those during the first four days, and almost no effects were observed when nestlings were six days old. This might be due to nestlings gaining the ability to maintain body temperature at five to six days old, with the importance of brooding and the relationship between brooding duration and other factors becoming weaker compared with that during the first four days. Alternatively, it might also be due to that fewer brooding behaviors were recorded when nestlings were five and six days old (13 and two times, respectively) as parents reduced their investment in brooding older nestlings.

In addition, brooding duration of long-tailed tit was shorter in larger broods, a pattern that has also been found in other birds (Chastel & Kersten, 2002; Koenig & Walters, 2011). The effects of brood size on brooding duration is probably related to the thermal preservation and production of nestlings. In larger broods, total heat produced increases with nestling number (Clark, 1984), enabling larger broods to better regulate body temperature.

The brooding behavior of birds can be affected by ambient temperature. For example, brooding duration is negatively correlated with ambient temperature in species like blood pheasants (Ithaginis cruentus) (Jia et al., 2001) and acorn woodpeckers (Melanerpes formicivorus) (Koenig & Walters, 2011). In this study, daily mean temperature had similar effects on brooding duration of the long-tailed tit. This phenomenon could be related to nestling heat demands (Koenig & Walters, 2011): when ambient temperature is low, the temperature difference inside and outside the nest is greater and heat is lost quickly, resulting in adults spending a longer time in brooding behavior; by contrast, when the ambient temperature is high, the heat of the nest is lost slowly and adults can spend a relatively shorter time in brooding behavior.

In summary, long-tailed tit females and males exhibited different brooding behavior and division of labor in the early nestling stage: female parents assumed most of the brooding investment, while male parents engaged in more nestling feeding investment. The brooding behavior of long-tailed tit was related to many factors, with brooding duration affected by nestling age, brood size and ambient temperature.

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