Differently sized cuckoos pose different threats to hosts

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Abstract

Hole-nesting tits \textit{Parus} spp. have been classified as “unsuitable” hosts for cuckoo parasitism because cuckoos cannot enter a cavity if the entrance is too small. However, Chinese tits could reject alien eggs and egg ejection rate increased with the local diversity of parasitic cuckoo species. Antiparasitic behavior among Chinese tits may have evolved due to greater size variation among sympatric cuckoo species. This raises the question of whether differently sized parasitic cuckoos pose different threats to Chinese tits. A green-backed tit \textit{Parus monticolus} population that is sympatric with Asian emerald cuckoo \textit{Chrysococcyx maculatus} (eme-cuckoo, small-sized parasite) and common cuckoo \textit{Cuculus canorus} (com-cuckoo, large-sized parasite), and a cinereous tit \textit{P. cinereus} population that is only sympatric with com-cuckoo were chosen as study organisms. We observed behavioral response and recorded alarm calls of the 2 tit species to eme-cuckoo, com-cuckoo, chipmunk \textit{Tamias sibiricus} (a nest predator) and dove \textit{Streptopelia orientalis} (a harmless control), and subsequently played back alarm calls to conspecific incubating females. In dummy experiments, both tit species performed intense response behavior to chipmunk, but rarely responded strongly to the 3 avian species. In playback experiments, both tit species responded strongly to conspecific chipmunk alarm calls, but rarely responded to dove alarm calls. The intensity of response of incubating female green-backed tits to eme-cuckoo and com-cuckoo alarm calls were similar to that of chipmunk alarm calls, while the intensity to eme-cuckoo alarm calls was higher than the intensity to dove alarm calls which was similar to that of com-cuckoo alarm calls. In contrast, few female cinereous tits responded to eme-cuckoo and com-cuckoo alarm calls. These findings indicated that the threat level of eme-cuckoo was slightly greater than that of com-cuckoo for sympatric green-backed tits, but not for allopatric cinereous tits.

Key words: body size, cinereous tit, cuckoo parasitism, green-backed tit, play-back, sympatry with multiple cuckoo species

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The process of natural selection produces the vast diversity of behavior we see within and among animal species. For the complex behavioral phenomenon of variation in behavior, ethologists suggested that a comprehensive understanding requires a balanced and integrated approach to proximate and ultimate causation (Tinbergen 1963). Proximate causes refer to mechanisms and development (how a behavior works), while ultimate causes refer to function, origins, and selection mechanisms (why a behavior exists) (Scott-Phillips et al. 2011; Dezeche et al. 2013). Overall, behavioral consequences should benefit for individual survival and reproduction (its adaptive significance).

In birds, obligate brood parasites lay their eggs in the nests of other species (hosts) and transfer the reproductive costs to hosts (Davies 2011; Soler 2014). Under selection from brood parasitism, hosts have evolved several antiparasite strategies to avoid parasitism, such as recognition and attack of parasites, recognizing and rejecting foreign eggs or chicks (e.g., Rothstein and Robinson 1998; Davies and Welbergen 2008; Davies 2011). However, variation among hosts in their anti-parasite behaviors is often found (e.g., Moksnes et al. 1991; Liang et al. 2016). For example, some hosts eject alien parasitic eggs or desert parasitized nests and re-nest (re-jester species), and many hosts accept them as they cannot distinguish their own eggs from alien eggs, or they cannot remove alien eggs from their nests (accepter species, see details in Neudorf and Sealy 1992).

Hole-nesting tits Parus spp. have been classified as “unsuitable” hosts for cuckoo parasitism because cuckoos cannot enter a cavity if the entrance is too small (van Balen et al. 1982; Moksnes et al. 1991; Davies 2000; but see Grim et al. 2014; Liang et al. 2016). Previous studies suggested that only hosts under pressure from brood parasitism evolved antiparasitic strategies to avoid parasitism (e.g., Rothstein and Robinson 1998; Davies and Welbergen 2008; Davies 2011). Thus, tits were assumed not to evolve such antiparasite behaviors. This hypothesis was supported by studies of European tits as they accepted 100% alien eggs (Moksnes et al. 1991). However, recent studies showed that green-backed tits P. monticulus (Yang et al. 2019) and cinereous tits P. cinereus (Liang et al. 2016) in China are rejecters, and egg ejection rate in cinereous tits increased strongly with the diversity of parasitic cuckoo species (Liang et al. 2016). Egg recognition of Chinese tits implied that they most likely are currently parasitized, or historically interacted with parasites (Lahni 2006; Peer et al. 2013; Liang et al. 2016; Yang et al. 2014a, 2019). Here, we assumed that antiparasite behavior among Chinese tits may have evolved due to greater size variation among Chinese cuckoo species, which is not the case in Europe.

Cuckoos can enter cavities to utilize hole-nesting species as hosts (Rutila et al. 2002; Thomson et al. 2016), and natural tit cavities have been reported to have many cases of cuckoo parasitism (Møller et al. 2011; Grim et al. 2014; Liang et al. 2016). In addition, tits are able to rear cuckoo chicks to fledging if they are successfully parasitized by cuckoos (Grim et al. 2014). The frequency of cuckoo parasitism in tits may be underestimated (Grim et al. 2014; Liang et al. 2016). Therefore, it is possible that coevolution between tits and cuckoos take place. Tits living in Europe either lost or even did not evolve specific anti-cuckoo adaptations in the ecological context where only a single large cuckoo (common cuckoo Cuculus canorus) does not represent a threat to tits usually breeding in small-sized holes (Liang et al. 2016). In contrast, there are up to 17 species of cuckoos in China (Yang et al. 2012; Zheng 2017), and their body sizes range from 16 to 45 cm (MacKinnon and Phillips 1999; Chen et al. 2017; Zheng 2017). Then, in theory, tits living in China cannot effectively prevent all parasitic cuckoos from entering their nests. This raises the ultimate question of whether differently sized parasitic cuckoos pose different threats to Chinese tits.

Here, we considered that large cuckoos pose low risk of parasitism to tits as cavities with small entrances could prevent them (Grim et al. 2014; Liang et al. 2016), while small cuckoo pose high parasitic threat to tits as they can enter cavities. Previous studies have shown that tits responded slightly aggressively to large cuckoos and other avian species (Davies and Welbergen 2008; Yu et al. 2017a), but tits can convey different threat information to conspecifics about cuckoos and other intruders to conspecifics by their alarm calls (Yu et al. 2017a). Combined with previous studies, we hypothesized that (1) tits do not perform aggressive behavior to large parasitic cuckoos, but they might be aggressive toward small cuckoos; (2) tits’ alarm calls to small and large cuckoos may convey different information to conspecifics. Thus an individual’s lifetime experience, or the history of evolutionary exposure to parasites influences the response behavior of birds (Peer et al. 2011; Kuehn et al. 2016). Finally, we hypothesized that (3) only tits that are sympatric with small cuckoos could perform aggressive behavior and utter referential alarm calls. In this study, we used a combination of model presentation and playback experiments in 2 tit populations sympatric and allopatric with small cuckoos, respectively, to examine the 3 hypotheses listed above.

Materials and Methods

Study species and study area

We chose a population of green-backed tits with 100% alien egg ejection rate in Guizhou (Yang et al. 2019), Southwestern China as a study system. Green-backed tits are sympatric with the small-sized Asian emerald cuckoo Chrysococcyx maculatus (about 17 cm, hereafter em-cuckoo) and the large-sized common cuckoo (about 32 cm, hereafter com-cuckoo) (MacKinnon and Phillips 1999; Yang et al. 2012). The green-backed tit is, therefore, an ideal species for the study of behavioral responses to small and large cuckoos. Second, we chose a population of cinereous tits (morphology and habits very similar with green-backed tit, Zheng 2017; Yang and Liang 2018) with 70% alien egg ejection rate in Jilin (Liang et al. 2016), Northeastern China as a second model system. Cinereous tits are sympatric with com-cuckoo, but allopatric with the em-cuckoo (Yu et al. 2017a). We observed the response behavior and recorded the alarm calls of the 2 species of tits to small em-cuckoo and large com-cuckoo. Subsequently, we played back conspecific alarm calls to incubating females by adopting the method of playback experiments of Suzuki (2015).

Our experiments were carried out in 2 nature reserves: Kuankuoshui National Nature Reserve (hereafter KKS, 28°06′–28°19′ N, 107°02′–107°14′ E) in Guizhou (for details, see Yang et al. 2010 and Zuojia Nature Reserve (hereafter ZJ, 44°1′–45°0′ N, 126°0′–126°8′ E) in Jilin (for details, see Yu et al. 2017a). A total of 11 parasitic cuckoo species are distributed in Guizhou, and their body sizes range from 16 to 45 cm (MacKinnon and Phillips 1999; Yang et al. 2012). In contrast, only 5 large-sized cuckoo species are distributed in Zuojia (body sizes range from 26 to 32 cm, MacKinnon and Phillips 1999; Yang et al. 2012; Yu et al. 2017a). We attached the nest-boxes to trees about 3 m above the ground in the 2 nature reserves. The number of nest boxes distributed in KKS and ZJ was kept at about 180 and 450 per year, respectively. We monitored a population of green-backed tits in KKS and a population of cinereous tits in ZJ. Both tits were nesting in nest-boxes,
and we visited the nest-boxes at least once a week to ascertain the first egg date and clutch size (Yu et al. 2017b; Yang et al. 2019). Experiments were conducted during the breeding season of tits in 2012, 2013, 2016, and 2018 (see details below). We identified different individuals by banding (Yu et al. 2017a).

Previous studies found that tits performed more aggressive behaviors to nest predators, such as hovering over a snake while spreading out their wings and tail (Suzuki 2011; Yu J et al., unpublished data). Even the parasites are not nest predators, but they might perform nest entering behavior. Thus, we also chose nest predator common chipmunks Tamias sibiricus (hereafter chipmunk) as one stimulus to test whether tits perform different behaviors and encode different alarm information between nest-predators and parasites. Common chipmunks are major nest predators of cinereous tits in ZJ as they entered nests to destroy the nest cup and bite the eggs and chicks. In KKS, Swinhoe’s striped squirrel Tamioptes swinhoei is a nest predator (Cai et al. 2018), and the appearance of Swinhoe’s striped squirrel is similar to common chipmunks.

**Dummy experiment**

During the incubation period of green-backed tits and cinereous tits, we presented taxidermic dummies of a com-cuckoo (large parasite, 2 models), an eme-cuckoo (small parasite, 1 model), a chipmunk (nest predator, 2 models), and an oriental turtle dove Streptopelia orientalis (neutral control, 2 models, hereafter dove) above the nest boxes, posed as naturally standing with wings naturally closed. We followed the method in Yu et al. (2017a) to score the dummy response (dummy response scores hereafter) of tits on a 3-point scale: (i) entered the nest; (ii) produced alarm calls while stationary observing; (iii) produced alarm calls with agitated skipping and flicking of wings; (iv) performing attack behavior with no physical encounter; and (v) performing attack behavior with physical impact (see also Liang and Møller 2015). In addition, we recorded the alarm calls of tits to these 4 intruders for playbacks. A TASCAM HD-P2 portable digital recorder (TEAC Corporation, Tokyo, Japan) and a Sennheiser MKH P48 external directional microphone (Sennheiser electronic GmbH & Co. KG, Wedemark, Germany) were used to carry out sound recordings. The recording parameters were set as follows: 44.1 kHz frequency and 24 bits accuracy. The trials in green-backed tits (n = 17 nests) were conducted during sunny days between 8:30 AM and 5:00 PM, from 16 May to 9 June 2016, and each nest was presented with all 4 model treatments. While the trials in cinereous tits were conducted during sunny days between 8:30 AM and 5:00 PM, from May to June 2012 (n = 17 nests in chipmunks dummy experiments, 6 May–2 June), 2013 (n = 14 nests in com-cuckoo and dove experiment, each nest received all 2 model treatments, May 13–June 6, see details in Yu et al. 2017a) and 2018 (n = 19 nests in eme-cuckoo experiments, 4–9 May), no recaptured individuals was found in different years.

**Playback experiment**

The alarm calls for playback were those of tits to com-cuckoo specimens (referred to as “com-cuckoo alarm calls”), eme-cuckoo specimens (“eme-cuckoo alarm calls”), common chipmunk specimens (“chipmunk alarm calls”), and dove specimens (“dove alarm calls”). A total of 16 green-backed tit alarm call records from 7 nests (3 com-cuckoo alarm calls, 4 eme-cuckoo alarm calls, 5 chipmunk alarm calls, 4 dove alarm calls) and 23 cinereous tit alarm call records from 20 nests (6 com-cuckoo alarm calls, 5 eme-cuckoo alarm calls, 6 chipmunk alarm calls, 6 dove alarm calls) were used to reduce pseudo-replication (Kroodsma 1989). Avisoft SASLab Pro 5.2 software (Avisoft Bioacoustics, Glienicke, Germany) was used to remove background noises <1 kHz from selected recordings. When recordings had overlapping calls, we deleted them. We tried our best not to change the call types and calling rates of the stimuli. The alarm calls of green-backed tits were played back to green-backed tits (referred to as green-backed tit playback hereafter), and the alarm calls of cinereous tits were played back to cinereous tits (referred to as cinereous tit playback hereafter). Stimuli that were played back to the focal female were recorded from strangers other than their mates or neighbors (Suzuki 2015). The method of constructing the playback stimuli is described in details in Yu et al. (2016). The sound equipment RoyQueen M300 (ShenZhen RoyQueen Audio Technology Co., Ltd, Shenzhen, China) was attached atop a tripod (1.20 m height) and placed 2.0 m in front of the nest box. Researchers stayed quiet at a distance of 10–15 m from the nest box. After observed the female returned to her nestbox at least 2 min, the researcher J.Y. conducted the 1 min playback. The green-backed tit playback experiments in KKS (n = 17 focal female individuals, each nest was presented with all 4 treatments) were conducted on clear and windless days from 8:30 AM to 5:00 PM, 7 June–7 July 2016. For cinereous tit playback experiments in ZJ, com-cuckoo alarm calls, chipmunk alarm calls, and dove alarm calls (n = 23 focal female individuals, each nest was presented with 3 treatments) were played back from 5 to 27 May 2016, and eme-cuckoo alarm calls (n = 26 focal female individuals, no individuals in 2016 have been found) were played back from 6 to 15 May 2018. All playback experiments were carried out from 7:30 AM to 5:00 PM under clear and windless weather conditions. Each stimulus was played at the same volume and the sound pressure level at 1 m ≈ 75 dB for all trials. The playback order of alarm calls was selected at random to avoid behavioral differences caused by fixed orders, and the intervals of >1.5 h between trials for each focal individual. Before experiments started, it was confirmed that the female tit was incubating and inside the nest. We recorded female responses to alarm calls by using a Finze PD6 mini digital video camera (Yun Fei Yang Co., Ltd, Shenzhen, China). The behavioral responses of female tits within a 1-min playback period were analyzed indoors. We scored the response of a female tit (playback response scores hereafter) on a 4-point scale with (1) no response; (2) standing up; (3) looking out of the nest entrance; and (4) leaving the nest (see details in Suzuki 2015). It was not possible for us to record data blind because this study involved focal animals in the field.

**Statistical analysis**

All data were analyzed using R 3.4.3 software (http://www.r-project.org). Because response scores were ordinal dependent variables, cumulative link mixed models (CLMMs function in R package ordinal) were used. We ran separate models for dummy response scores and playback response scores of 2 tit species, respectively. In the model, dummy response scores or playback response scores were the dependent variable, whereas treatment, order of treatment exposure, and time of day (control for the effect of time on response behaviors of tits) were treated as fixed terms and individual identity of focal birds as random terms. For cinereous tits, we included sampling year instead of order of treatment exposure in statistical models as we collected data at ZJ in different years. We used 2-tailed likelihood ratio tests to obtain P-values. When the result of multiple comparisons was significant, we conducted the post hoc pairwise
comparison between treatments. The level of significance was first set to \( \alpha = 0.05 \), while 2 group comparisons after multiple comparisons will increase the probability of type I errors, we used false discovery rate control to adjust \( P \)-values (Benjamini and Hochberg 1995) using the p.adjust function.

**Results**

In dummy experiments, there was a significant effect of treatment on dummy response scores of green-backed tits (CLMMs, \( \chi^2 = 9.97, P = 0.019 \)) and cinereous tits (\( \chi^2 = 26.31, P < 0.001 \)). In contrast, there were no significant effects of trial order (\( \chi^2 = 0.05, P = 0.82 \)) or time of day (\( \chi^2 = 0.21, P = 0.65 \)) on dummy response scores of green-backed tits, and no significant effects of sampling year (\( \chi^2 = 0.00, P = 1.00 \)) or time of day (\( \chi^2 = 0.47, P = 0.49 \)) on dummy response scores of cinereous tits. Both tit species performed stronger response behavior (higher dummy response scores) to chipmunk than to the 3 avian species (Table 1 and Figure 1). In contrast, there was no difference among the dummy response scores to com-cuckoo, eme-cuckoo, or dove (Table 1 and Figure 1).

In playback experiments, there was a significant effect of playback stimuli on playback response scores of incubating female green-backed tits (\( \chi^2 = 12.30, P = 0.006 \)) and incubating female cinereous tits (\( \chi^2 = 22.39, P < 0.001 \)). In contrast, there was no significant effects of trial order (\( \chi^2 = 0.06, P = 0.80 \)) or time of day (\( \chi^2 = 0.11, P = 0.74 \)) on playback response scores of female green-backed tits, and no significant effects of sampling year (\( \chi^2 = 0.00, P = 1.00 \)) or time of day (\( \chi^2 = 0.53, P = 0.47 \)) on playback response scores of female cinereous tits. For female green-backed tits, there was no difference among the playback response scores to com-cuckoo, eme-cuckoo, and chipmunk alarm calls, or between the playback response scores to com-cuckoo and dove alarm calls, but playback response scores to eme-cuckoo and chipmunk alarm calls were significantly higher than those to dove alarm calls (Table 2 and Figure 2). For female cinereous tits, playback response scores to com-cuckoo, eme-cuckoo, and dove alarm calls were similar, but significantly lower than those to chipmunk alarm calls (Table 2 and Figure 2).

**Discussion**

In dummy experiments, green-backed tits and cinereous tits only performed significantly more attacks on chipmunks than on the 3 avian species. Generally, direct attacks and defensive displays are nest defense behavior that may enhance a parent’s reproductive success (Montgomerie and Weatherhead 1988). Chipmunks as nest predators particularly threaten eggs and offspring, and both tit species performed strong attacks on them (Figure 1). Doves are not predators and such an open-cup nesting species presents no threat to tits, causing a slight behavioral response of tits. The results indicated that tits could depend on the threat of invaders to make appropriate response behaviors. Contrary to our hypothesis (1) tits might be aggressive toward small cuckoos, while there was no difference in response behaviors of green-backed tits and cinereous tits when they were facing com-cuckoo and eme-cuckoo, which look alike, the threat of these 3 avian species to tits were similar. However, similar response behavior might be caused by different ultimate causation. The defense strategy of some hosts in China adopted against brood parasitism might be to remain tolerant in the first line of defense to reduce the cost of breeding (such as misidentification of a hawk as a cuckoo, see details in Yang et al. 2014b; Yu et al. 2016), and putting more effort into identification and rejection of alien eggs (Rothstein 1990; Davies 2000, 2011; Kilner and Langmore 2011; Liang et al. 2013; Yang et al. 2015a, 2015b). Considering that both tit populations in our study have high alien egg ejection rate, we thus could not exclude the possibility that the threat from the com-cuckoo and eme-cuckoo to tits is different.

**Table 1.** Results of post hoc comparisons for dummy response scores of green-backed tits and cinereous tits to eme-cuckoo, com-cuckoo, chipmunk and dove specimen in dummy experiments

| Dummy response scores of | Eme-cuckoo | Com-cuckoo | Dove |
|-------------------------|------------|------------|------|
| Green-backed tit        |            |            |      |
| com-cuckoo              | 0.40       |            |      |
| Dove                    | 0.31       | 0.79       |      |
| Chipmunk                | 0.04       | 0.04       | 0.04 |
| Cinereous tit           |            |            |      |
| com-cuckoo              | 0.36       |            |      |
| Dove                    | 0.36       | 0.10       |      |
| Chipmunk                | <0.001     | 0.004      | <0.001|

\( P \)-values were adjusted by false discovery rate control.

**Figure 1.** Responses of green-backed tits (A) and cinereous tits (B) to dove, common cuckoo, emerald cuckoo, and chipmunk.
In playback experiments with green-backed tits, the response behavior of incubating female tits to the conspecific com-cuckoo, eme-cuckoo, and chipmunk alarm calls were similar, and some females stepped onto the nest entrance and left the nest-box (Figure 2). The chipmunk as a nest predator might even attack adult birds if they were inside boxes. Stepping onto the nest entrance and leaving the nest-box could help tits gather information on the locations of invaders and avoid encounters with them inside the nest (Martin et al. 2000; Schneider and Griesser 2012; see details in Suzuki 2015). Therefore, incubating females might receive information about threat from an invader that could enter the nest from conspecific eme-cuckoo and com-cuckoo alarm calls (Figure 2). Although there was no statistical difference among the playback response scores of female green-backed tits to com-cuckoo and eme-cuckoo alarm calls, we still suggested that the response intensity to com-cuckoo alarm calls was slightly lower than that to eme-cuckoo alarm calls. Playback response scores of female green-backed tits to eme-cuckoo were significantly higher than those to dove alarm calls, while playback response scores to com-cuckoo were similar with those to dove alarm calls (Table 2 and Figure 2). These results indicate that cinereous tits did not receive threat information from eme-cuckoo and com-cuckoo alarm calls. Although the cinereous tits is a potential host of the com-cuckoo, parasitism rate is very low (Liang et al. 2016). The eme-cuckoo is allopatric with the cinereous tits in our study area (Yu et al. 2017a), which implies that cinereous tits likely have no history of evolutionary exposure to small sized parasitic eme-cuckoos and do not know supported our hypothesis (2) that tit alarm calls to small and large cuckoos may convey different information to conspecifics.

Previous studies found that female yellow warblers *Setophaga petechia*, an open-cup nesting bird species, sat tightly on their nests to prevent parasitism (Hobson and Sealy 1989; Gill and Sealy 1996, 2004). Why did incubating female green-backed tits not remain in the nest like yellow warblers? Here, we suggested that hole-nesting birds could avoid encounters with any potential enemy in the nest chamber. Although parasites do not pose a lethal threat to hosts, there is still the risk of injury during combat if the female encountered a nest invader inside a cavity, such as a female Mandarin duck *Aix galericulata* having a physical conflict with one conspecific brood parasite in her nest box (our field observations, unpublished data). In addition, green-backed tits can reject foreign eggs to avoid parasitism if cuckoos successfully lay eggs in their nests (Liang et al. 2016; Yang et al. 2019).

Consistent with our hypothesis (3), only tits that are sympatric with small cuckoos could utter referential alarm calls. In playback experiments with cinereous tits, the response behavior of incubating female tits to the conspecific com-cuckoo and the eme-cuckoo alarm calls were similar to that of conspecific dove alarm calls, and only a few females stepped onto the nest entrance and left the nest-box (Figure 2). These results indicate that cinereous tits did not receive threat information from eme-cuckoo and com-cuckoo alarm calls. Although the cinereous tits is a potential host of the com-cuckoo, parasitism rate is very low (Liang et al. 2016). The eme-cuckoo is allopatric with the cinereous tits in our study area (Yu et al. 2017a), which implies that cinereous tits likely have no history of evolutionary exposure to small sized parasitic eme-cuckoos and do not know

### Table 2. Results of post hoc comparisons for playback response scores of green-backed tits and cinereous tits to conspecific eme-cuckoo, com-cuckoo, chipmunk, and dove alarm calls in playback experiments

| Playback response scores | Eme-cuckoo | Com-cuckoo | Dove |
|--------------------------|------------|------------|------|
| Female green-backed tit   | Com-cuckoo | 0.14       |      |
|                          | Dove       | 0.02       | 0.053|
|                          | Chipmunk   | 0.41       | 0.34 |
| Female cinereous tit     | Com-cuckoo | 0.84       |      |
|                          | Dove       | 0.96       | 0.84 |
|                          | Chipmunk   | 0.02       | <0.001|

P-values were adjusted by false discovery rate control.
that the eme-cuckoos could enter holes. Thus, sympatric con-cuckoo and allopatric eme-cuckoos might not pose a threat (or a very low threat) to cinereous tits. Our results supported that an individual’s lifetime experience or history of evolutionary exposure could influence the response behavior of tits to brood parasites (Peer et al. 2011; Kuehn et al. 2016).

Few incubating female green-backed tits and cinereous tits showed any response to conspecific dove alarm calls. In contrast, incubating female tits inside a cavity looked out of the nest entrance or left the nest-box in response to chipmunk alarm calls (Figure 2). The chipmunk as a nest predator might even attack adult birds if they were inside boxes. Thus, incubating female tits should gather information on the locations of invaders and avoid encounters with nest predators inside the nest (Martin et al. 2000; Schneider and Griesser 2012; see details in Suzuki 2015). Our experimental play-back results were consistent with previous findings that incubating female tits could assess the threat outside the nest cavity by using conspecific alarm calls followed by appropriate response behavior (Suzuki 2015). However, we did not examine and confirm the rules of information encoded in green-backed tit and cinereous tit alarm calls in this study, although that could be explored in future research.

Several studies have shown that hosts performed different anti-parasite behaviors in single- and multiple-cuckoo species systems (Yang et al. 2014b; Liang and Møller 2015) and among different diversities of brood parasite hosts (Liang et al. 2016, 2017). Such heterogeneity in host behavior may have evolved as a means of efficient defense against brood parasites. The response behaviors of incubating tits to conspecific com-cuckoo and eme-cuckoo alarm calls were similar, which was inconsistent with our predictions. However, the threat level of eme-cuckoo was slightly greater than that of com-cuckoo for sympatric green-backed tits, but not for allopatric cinereous tits (see above). Parasitism status by brood parasites was correlated with egg recognition in hosts (Soler and Møller 1999; Langmore et al. 2005), the different egg ejection rates implied that the intensity of parasite pressure in those 2 tit populations differed. Here, we suggest that body size of brood parasites might be a predictor of risk for parasitism to hole-nesting birds. Anti-parasite behavior should constitute a strong selective force driving the evolution of the behavior of both hosts and parasites (Darwin 1859), and anti-parasite behavior of Chinese tits may evolve due to the diversity of sympatry brood parasitic hosts, especially among those varying in body sizes.

**Ethical Standards**

The experiments comply with the current laws of China. Fieldwork was carried out under the permission from the Kuankuoshui National Nature Reserve and Zuojia Nature Reserve. Experimental procedures were permitted by the National Animal Research Authority in Northeast Normal University (approval number: NENU-20080416).

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**Conflict of Interest**

We declare that all authors have no competing interest.

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