

## Differently sized cuckoos pose different threats to hosts

Jiangping Yu, Mingju E, Wei Sun, Wei Liang, Haitao Wang, Anders Møller

► **To cite this version:**

Jiangping Yu, Mingju E, Wei Sun, Wei Liang, Haitao Wang, et al.. Differently sized cuckoos pose different threats to hosts. *Current zoology*, Institute of zoology, Chinese academy of sciences, 2019, 66, pp.247 - 253. 10.1093/cz/zoz049 . hal-03024722

**HAL Id: hal-03024722**

**<https://hal-cnrs.archives-ouvertes.fr/hal-03024722>**

Submitted on 25 Nov 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Article

# Differently sized cuckoos pose different threats to hosts

Jiangping YU<sup>a,b</sup>, Mingju E<sup>c</sup>, Wei SUN<sup>b</sup>, Wei LIANG <sup>d,\*</sup>, Haitao WANG<sup>a,\*</sup>, and Anders Pape MØLLER <sup>e,f</sup>

<sup>a</sup>Jilin Engineering Laboratory for Avian Ecology and Conservation Genetics, School of Life Sciences, Northeast Normal University, Changchun 130024, China, <sup>b</sup>Ministry of Education Key Laboratory of Vegetation Ecology, School of Life Sciences, Northeast Normal University, Changchun 130024, China, <sup>c</sup>Jilin Provincial Key Laboratory of Animal Resource Conservation and Utilization, School of Life Sciences, Northeast Normal University, Changchun 130024, China, <sup>d</sup>Ministry of Education Key Laboratory for Ecology of Tropical Islands, College of Life Sciences, Hainan Normal University, Haikou 571158, China, <sup>e</sup>Ecologie Systématique Evolution, Université Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, Orsay Cedex F-91405, France and <sup>f</sup>Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, College of Life Sciences, Beijing Normal University, Beijing 100875, China

\*Address correspondence to Wei Liang. E-mail: liangwei@hainnu.edu.cn and Haitao Wang.

E-mail: wanght402@nenu.edu.cn.

Handling editor: Zhi-Yun Jia

Received on 10 June 2019; accepted on 3 October 2019

## Abstract

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. 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 *Parus monticolus* population that is sympatric with Asian emerald cuckoo *Chrysococcyx maculatus* (eme-cuckoo, small-sized parasite) and common cuckoo *Cuculus canorus* (com-cuckoo, large-sized parasite), and a cinereous tit *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 *Tamias sibiricus* (a nest predator) and dove *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

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; Dezecache 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 (rejecter 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 antiparasitism 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. monticolus* (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 (Lahti 2006; Peer et al. 2011; Liang et al. 2016; Yang et al. 2014a, 2019). Here, we assumed that antiparasitic 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 (Rutala 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 Phillipps 1999; Liang 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 eme-cuckoo) and the large-sized common cuckoo (about 32 cm, hereafter com-cuckoo) (MacKinnon and Phillipps 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 eme-cuckoo (Yu et al. 2017a). We observed the response behavior and recorded the alarm calls of the 2 species of tits to small eme-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 Zuoqia 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 Phillipps 1999; Yang et al. 2012). In contrast, only 5 large-sized cuckoo species are distributed in Zuoqia (body sizes range from 26 to 32 cm, MacKinnon and Phillipps 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 *Tamias swinhoi* 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 5-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 (Kroodsmas 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 experiments.

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  $\approx$  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 Pinze 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

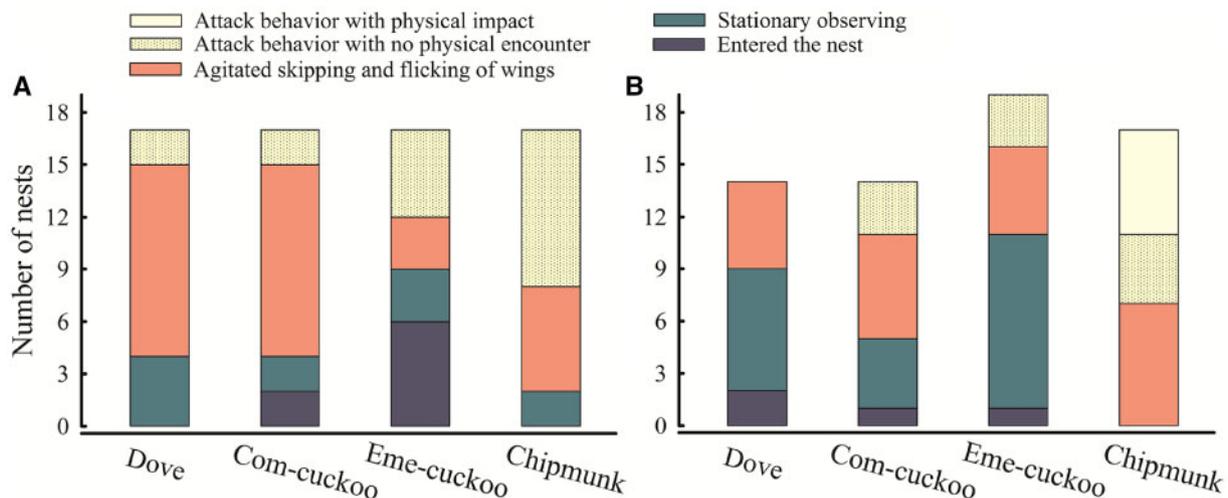
In dummy experiments, there was a significant effect of treatment on dummy response scores of green-backed tits (CLMMs,  $\chi^2_3 = 9.97$ ,  $P = 0.019$ ) and cinereous tits ( $\chi^2_3 = 26.31$ ,  $P < 0.001$ ). In contrast, there were no significant effects of trial order ( $\chi^2_1 = 0.05$ ,  $P = 0.82$ ) or time of day ( $\chi^2_1 = 0.21$ ,  $P = 0.65$ ) on dummy response scores of green-backed tits, and no significant effects of sampling year ( $\chi^2_1 = 0.00$ ,  $P = 1.00$ ) or time of day ( $\chi^2_1 = 0.47$ ,  $P = 0.49$ ) 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 ( $\chi^2_3 = 12.30$ ,  $P = 0.006$ ) and incubating female cinereous tits ( $\chi^2_3 = 22.39$ ,  $P < 0.001$ ). In contrast, there was no significant effects of trial order ( $\chi^2_1 = 0.06$ ,  $P = 0.80$ ) or time of day ( $\chi^2_1 = 0.11$ ,  $P = 0.74$ ) on playback response scores of female green-

**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.

backed tits, and no significant effects of sampling year ( $\chi^2_1 = 0.00$ ,  $P = 1.00$ ) or time of day ( $\chi^2_1 = 0.53$ ,  $P = 0.47$ ) 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).

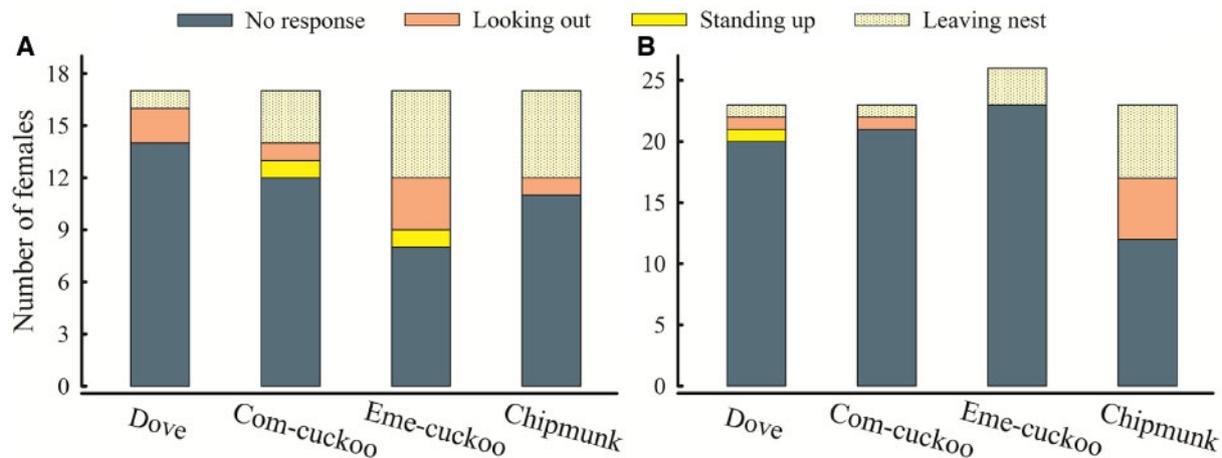
## Discussion

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 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 of		Eme-cuckoo	Com-cuckoo	Dove
Female green-backed tit	Com-cuckoo	0.14		
	Dove	0.02	0.053	
	Chipmunk	0.41	0.34	<0.001
Female cinereous tit	Com-cuckoo	0.84		
	Dove	0.96	0.84	
	Chipmunk	0.02	<0.001	0.003

*P*-values were adjusted by false discovery rate control.

**Figure 2.** Responses of incubating female green-backed tits (A) and cinereous tits (B) to playback of conspecific dove alarm calls (dove), common cuckoo (com-cuckoo) alarm calls, emerald cuckoo (eme-cuckoo) alarm calls, and chipmunk (chipmunk) alarm calls.

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). In a typical multiple-cuckoo system, where several brood parasitic cuckoos co-occur, hosts encounter a more diverse selective pressure from parasitic cuckoos because some brood parasites may overlap in host use (Yang et al. 2012; Liang et al. 2017). However, the size of the nest hole of an artificial nest-box could effectively prevent larger-sized parasitic cuckoos from entering the nest (Liang et al. 2016). For the population of green-backed tits in this study, the threat level of a small-sized parasite like the eme-cuckoo should be higher than that of the large-sized parasitic com-cuckoo. Thus, our results partly

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

that the eme-cuckoos could enter holes. Thus, sympatric com-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 playback 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 1990; 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 Zuoqia Nature Reserve. Experimental procedures were permitted by the National Animal Research Authority in Northeast Normal University (approval number: NENU-20080416).

## Acknowledgments

We would like to thank Dr. Zhi-Yun Jia and 3 anonymous reviewers for their constructive and helpful comments on our manuscript. We are grateful to Hailin Lu, Weiwei Lv, Juan Huo, Tongping Su, Laikun Ma and Ye Gong for their assistance with fieldwork. We also thank Kuankuoshui National Nature Reserve and Zuoqia Nature Reserve for their support and permission to carry out this study.

## Funding

This work was supported by the National Natural Science Foundation of China (Nos. 31770419 and 31470458 to H.W., 31772453 and 31970427 to W.L.), the Open Project Program of Jilin Provincial Key Laboratory of Animal Resource Conservation and Utilization (130028823), the Fundamental Research Funds for the Central Universities (2412018QD009), and the Project funded by China Postdoctoral Science Foundation (2018M631854).

## Conflict of Interest

We declare that all authors have no competing interest.

## References

- Benjamini Y, Hochberg Y, 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 57:289–300.
- Cai Y, Zhang H, Hu C, Chen G, Su H, 2018. A survey of animal resources of Callosiurinae in Guizhou Province. *Chin J Wildl* 39:529–535.
- Darwin C, 1859. *On the Origin of species by Means of Natural Selection*. London: John Murray.
- Davies NB, 2000. *Cuckoos, Cowbirds and Other Cheats*. London: T and AD Poyser.
- Davies NB, 2011. Cuckoo adaptations: trickery and tuning. *J Zool* 284:1–14.
- Davies NB, Welbergen JA, 2008. Cuckoo-hawk mimicry? An experimental test. *Proc R Soc Lond B* 275:1817–1822.
- Dezecache G, Mercier H, Scott-Phillips TC, 2013. An evolutionary approach to emotional communication. *J Pragmatics* 59:221–233.
- Gill SA, Sealy SG, 1996. Nest defence by yellow warblers: recognition of a brood parasite and an avian nest predator. *Behaviour* 133:263–282.
- Gill SA, Sealy SG, 2004. Functional reference in an alarm signal given during nest defence: set calls of yellow warblers denote brood-parasitic brown-headed cowbirds. *Behav Ecol Sociobiol* 56:71–80.
- Grim T, Sams P, Procházka P, Rutila J, 2014. Are tits really unsuitable hosts for the common cuckoo? *Ornis Fenn* 91:166–177.
- Hobson KA, Sealy SG, 1989. Responses of yellow warblers to the threat of cowbird parasitism. *Anim Behav* 38:510–519.
- Kilner RM, Langmore NE, 2011. Cuckoos versus hosts in insects and birds: adaptations, counter-adaptations and outcomes. *Biol Rev* 86:836–852.
- Kroodsma DE, 1989. Suggested experimental designs for song playbacks. *Anim Behav* 37:600–609.
- Kuehn MJ, Peer BD, McCleery RA, Rothstein SI, 2016. Yellow warbler defenses are retained in the absence of brood parasitism but enhanced by experience with cowbirds. *Behav Ecol* 27:279–286.
- Lahti DC, 2006. Persistence of egg recognition in the absence of cuckoo brood parasitism: pattern and mechanism. *Evolution* 60:157–168.
- Langmore NE, Kilner RM, Butchart SHM, Maurer G, Davies NB et al., 2005. The evolution of egg rejection by cuckoo hosts in Australia and Europe. *Behav Ecol* 16:686–692.
- Liang W, Møller AP, 2015. Hawk mimicry in cuckoos and anti-parasitic aggressive behavior of barn swallows in Denmark and China. *J Avian Biol* 46:216–223.
- Liang W, Møller AP, Stokke BG, Yang C, Kovařík P et al., 2016. Geographic variation in egg ejection rate by great tits across 2 continents. *Behav Ecol* 27:1405–1412.
- Liang W, Yang C, Møller AP, 2017. High diversity of brood parasites in China and coevolution between cuckoos and their hosts. In Soler M, editor. *Avian Brood Parasitism: Behaviour, Ecology, Evolution and Coevolution*. Cham, Switzerland: Springer International Publishing AG.
- Liang W, Yang C, Wang L, Møller AP, 2013. Avoiding parasitism by breeding indoors: cuckoo parasitism of hirundines and rejection of eggs. *Behav Ecol Sociobiol* 67:913–918.
- MacKinnon J, Phillips K, 1999. *A Field Guide to the Birds of China*. New York: Oxford University Press.

- Martin TE, Scott J, Menge C, 2000. Nest predation increases with parental activity: separating nest site and parental activity effects. *Proc R Soc Lond B* 267:2287–2293.
- Moksnes A, Røskaft E, Braa AT, Korsnes L, Lampe HM, Pedersen HC, 1991. Behavioural responses of potential hosts towards artificial cuckoo eggs and dummies. *Behaviour* 116:64–89.
- Møller AP, Saino N, Adamik P, Ambrosini R, Antonov A et al., 2011. Rapid change in host use of the common cuckoo *Cuculus canorus* linked to climate change. *Proc Biol Sci* 278:733–738.
- Montgomerie RD, Weatherhead PJ, 1988. Risks and rewards of nest defence by parent birds. *Q Rev Biol* 63:167–187.
- Neudorf DL, Sealy SG, 1992. Reactions of four passerine species to threats of predation and cowbird parasitism: enemy recognition or generalized responses? *Behaviour* 123:84–105.
- Peer BD, Kuehn MJ, Rothstein SI, Fleischer RC, 2011. Persistence of host defence behaviour in the absence of avian brood parasitism. *Biol Lett* 7: 670–673.
- Rothstein SI, 1990. A model system for coevolution: avian brood parasitism. *Annu Rev Ecol Syst* 21:481–508.
- Rothstein SI, Robinson SK, 1998. *Parasitic Birds and Their Hosts: Studies in Coevolution*. Oxford: Oxford University Press.
- Rutila J, Latja R, Koskela K, 2002. The common cuckoo *Cuculus canorus* and its cavity nesting host, the redstart *Phoenicurus phoenicurus*: a peculiar cuckoo-host system? *J Avian Biol* 33:414–419.
- Schneider NA, Griesser M, 2012. Incubating females use dynamic risk assessment to evaluate the risk posed by different predators. *Behav Ecol* 24: 47–52.
- Scott-Phillips TC, Dickins TE, West SA, 2011. Evolutionary theory and the ultimate–proximate distinction in the human behavioural sciences. *Perspect Psychol Sci* 6:38.
- Soler M, 2014. Long-term coevolution between avian brood parasites and their hosts. *Biol Rev* 89:688–704.
- Soler M, Møller AP, 1990. Duration of sympatry and coevolution between the great spotted cuckoo and its magpie host. *Nature* 343:748–750.
- Suzuki TN, 2011. Parental alarm calls warn nestlings about different predatory threats. *Curr Biol* 21:R15–R16.
- Suzuki TN, 2015. Assessment of predation risk through referential communication in incubating birds. *Sci Rep* 5:10239.
- Thomson RL, Tolvanen J, Forsman JT, 2016. Cuckoo parasitism in a cavity nesting host: near absent egg-rejection in a northern redstart population under heavy apparent (but low effective) brood parasitism. *J Avian Biol* 47: 363–370.
- Tinbergen N, 1963. On aims and methods of ethology. *Z Tierpsychol* 20: 410–433.
- van Balen JH, Booy CJH, Van Franeker JA, Osieck ER, 1982. Studies on hole-nesting birds in natural nest sites. I. Availability and occupation of natural nest sites. *Ardea* 70:1–24.
- Yang C, Chen M, Wang L, Liang W, Møller AP, 2015a. Nest sanitation elicits egg discrimination in cuckoo hosts. *Anim Cogn* 18:1373–1377.
- Yang C, Liang W, 2018. *Bird Nests in Kuankuoshui Nature Reserve*. Beijing: Science Press.
- Yang C, Liang W, Antonov A, Cai Y, Stokke BG et al., 2012. Diversity of parasitic cuckoos and their hosts in China. *Chin Birds* 3:9–32.
- Yang C, Liang W, Cai Y, Shi S, Takasu F et al., 2010. Coevolution in action: disruptive selection on egg colour in an avian brood parasite and its host. *PLoS One* 5:e10816.
- Yang C, Liang W, Møller AP, 2019. Egg retrieval versus egg rejection in cuckoo hosts. *Philos Trans R Soc Lond B Biol Sci* 374:20180200.
- Yang C, Liu Y, Zeng L, Liang W, 2014a. Egg color variation, but not egg rejection behavior, changes in a cuckoo host breeding in the absence of brood parasitism. *Ecol Evol* 4:2239–2246.
- Yang C, Wang L, Cheng S-J, Hsu Y-C, Liang W, Møller AP, 2014b. Nest defenses and egg recognition of yellow-bellied prinia against cuckoo parasitism. *Naturwissenschaften* 101:727–734.
- Yang C, Wang L, Liang W, Møller AP, 2015b. Nest sanitation behavior as a pre-adaptation of egg rejection to counter parasitism in hirundines. *Anim Cogn* 18:355–360.
- Yu J, Lv W, Xu H, Bibi N, Yu Y et al., 2017b. Function of note strings in Japanese tit alarm calls to the common cuckoo: a playback experiment. *Avian Res* 8:22.
- Yu J, Wang L, Xing X, Yang C, Ma J et al., 2016. Barn swallows (*Hirundo rustica*) differentiate between common cuckoo and sparrowhawk in China: alarm calls convey information on threat. *Behav Ecol Sociobiol* 70: 171–178.
- Yu J, Xing X, Jiang Y, Liang W, Wang H, Møller AP, 2017a. Alarm call-based discrimination between common cuckoo and Eurasian sparrowhawk in a Chinese population of great tits. *Ethology* 123:542–550.
- Zheng G, 2017. *A Checklist on the Classification and Distribution of the Birds of China*. 3rd edn. Beijing: Science Press.

