Comparative analysis of hissing calls in five tit species

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Title	Comparative analysis of hissing calls in five tit species
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Abstract

Nest predation often leads to breeding failure and is an important component of natural selection that affects the evolution of nest defense behavior in birds. Many tit species give a hissing call as nest defense, but there are few studies of interspecific variation in hissing calls, and whether these are related to nest predation and nesting success. In this study, we compared the hissing calls of five tit species including cinereous tits (Parus cinereus), marsh tits (Poecile palustris), varied tits (Sittiparus varius), willow tits (Poecile montanus), and coal tits (Periparus ater) in Saihanba National Forest Park in Hebei and Xianrendong National Nature Reserve in Liaoning. In Saihanba of Hebei, the proportion of cinereous, willow, and coal tit individuals giving a hissing call differed significantly but the rate of nest predation was similar. It was also true for the three tit species (cinereous, varied, and marsh tits) in Xianrendong of Liaoning. Cinereous and varied tits showed no differences in clutch size, date of the first egg, nest predation and nesting success between individuals that gave and those that did not give a hissing call. These results indicate that for tit species that breed in nest boxes distributed within the same area, there is interspecific variation in hissing calls but this variation is not significantly correlated with nest predation risk.

Keywords	hissing call; nest defense; nest predation; repeatability; Paridae.

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

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Dear Prof. J. J. Bolhuis,

Please find enclosed a manuscript entitled "**Comparative analysis of hissing calls in five tit species**" for possible publication in *Behavioral Processes*.

Many tit species give a hissing call for nest defense, but there are few studies on inter-specific variations in hissing calls and whether these are related to nest predation and nesting success. In this study, we compared the hissing calls of five tit species including cinereous tit (*Parus cinereus*), marsh tit (*Poecile palustris*), varied tit (*Sittiparus varius*), willow tit (*Poecile montanus*), and coal tit (*Periparus ater*) in China.

The main finding of this study is that **for tit species that breed in nest boxes distributed within the same area, there are inter-specific variations in hissing call but these are not significantly correlated with nest predation risk and nesting success**.

We believe that these findings will be of interest to a wide range of researchers, who constitute the readership of your prestigious journal.

This manuscript has not been published or presented elsewhere in part or in entirety, and it is not under consideration for publication by another journal. We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violates any of these. There are no competing interest to declare.

Thank you for your consideration. I am looking forward to hearing from you.

On behalf of the authors, sincerely yours,

Dongmei Wan and Wei Liang

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Response to comments (Ref: BEPROC_2019_100R3)

Dear Prof. Bolhuis,
Editor-in-Chief,
Behavioural Processes

Thank you very much for kindly permitting us to revise our manuscript.
We have now read the comments carefully and have revised the paper as suggested by you and the reviewers.

Please find explained in blue bold font below how we have addressed the points.

Thank you very much in advance for your editorial assistance.

Yours sincerely, on behalf of the co-authors,

Wei Liang

Ref: BEPROC_2019_100_R3
Title: Comparative analysis of hissing calls in five tit species
Journal: Behavioural Processes

Dear Dr. Liang,

Thank you for submitting your manuscript to Behavioural Processes. Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, please submit your revision within 60 days of accepting to revise. A summary is appended below. While revising the paper please consider the reviewers' comments carefully. We look forward to receiving your detailed response and your revised manuscript.

Reviewer 2 has some final issues that you should address. As soon as I receive your revision I will accept it.

I look forward to receiving your revised manuscript as soon as possible.

Kind regards,

Professor. Bolhuis
Editor-in-Chief
Behavioural Processes

Comments from the editors and reviewers:

-Reviewer 1

- I focused mainly on previous problems/ suggestions. Well solved.

Thank you very much for your helpful comments. We appreciate your time and your kind help.

-Reviewer 2

The revisions the authors made are strong – the paper reads really well! I have only a few minor wording suggestions:

Thank you very much for taking your time and your helpful comments.

Highlights: Given the changes made, I suggest replacing "these are related" in both highlights with "this variation is related"

Revised as you kindly suggested.

Discussion:

Line 235-236: I suggest "there were few snakes and few Swinhoe's . . ."

Revised. Thanks.

Line 252: I suggest replacing "female birds (Koosa and Tilgar 2016). For example, females" with "female great tits (Koosa and Tilgar 2016) in that females"

Revised.

A nice study!

Thanks. We really appreciate all helpful suggestions from the Editor and Reviewers.

Highlights

Many tit species in the Paridae family give a hissing call for nest defense, but there are few studies on inter-specific variation in hissing calls and whether this variation is related to nest predation and nesting success.

This study showed that there is inter-specific variation in hissing calls of five tit species in China that breed in nest boxes distributed within the same area, but this variation is not significantly correlated with nest predation risk and nesting success.

Comparative analysis of hissing calls in five tit species

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Running headline: Hissing calls in tit species

Abstract

Nest predation often leads to breeding failure and is an important component of natural selection that affects the evolution of nest defense behavior in birds. Many tit species give a hissing call as nest defense, but there are few studies of interspecific variation in hissing calls, and whether these are related to nest predation and nesting success. In this study, we compared the hissing calls of five tit species including cinereous tits (*Parus cinereus*), marsh tits (*Poecile palustris*), varied tits (*Sittiparus varius*), willow tits (*Poecile montanus*), and coal tits (*Periparus ater*) in Saihanba National Forest Park in Hebei and Xianrendong National Nature Reserve in Liaoning. In Saihanba of Hebei, the proportion of cinereous, willow, and coal tit individuals giving a hissing call differed significantly but the rate of nest predation was similar. It was also true for the three tit species (cinereous, varied, and marsh tits) in Xianrendong of Liaoning. Cinereous and varied tits showed no differences in clutch size, date of the first egg, nest predation and nesting success between individuals that gave and those that did not give a hissing call. These results indicate that for tit species that breed in nest boxes distributed within the same area, there is interspecific variation in hissing calls but this variation is not significantly correlated with nest predation risk.

Keywords: hissing call; nest defense; nest predation; repeatability; Paridae.

Introduction

Nest predation often leads to reproductive failure and is a major selective force that affects nest defense behavior in birds (Ricklefs 1969; Martin 1995; Forstmeier and Weiss 2004; Fontaine and Martin 2006; Lima 2009; Tilgar and Moks 2015; Fu et al. 2016; Guppy et al. 2017). Birds have evolved complex anti-predation strategies to protect their nests and demonstrate specific behaviors when facing predators of different types and risk levels (Lima et al. 2005; Yorzinski and Vehrencamp 2009; Krams et al. 2010; Yorzinski and Platt 2012; Suzuki 2011, 2015; Daniela et al. 2018; Maziarz et al. 2018). Nest stage, sex, nest type and predator location may also have an effect on anti-predator behaviour of birds (Burger 1992; Ritchison 1993; Crisologo and Bonter 2017). For example, when a blue peacock (*Pavo cristatus*) encounters a raccoon during the day, it calls loudly, stretches its neck, opens its wings, and strikes a flying pose as it approaches the predator. However, at night it will instead remain silent and give a soft hissing call (Yorzinski and Platt 2012).

In animals, sound is often used to transmit predator information (Zuberbühler 2009; Fasanella and Fernández 2009; Suzuki 2011, 2014, 2015; Fuong et al. 2014; Townsend et al. 2014). Individuals of the same or different species use this acoustic information to evade predation (Sherman 1977; Pipia et al. 2009; Kitchen et al. 2010; Suzuki 2011, 2015; Gill and Bierema 2013; Townsend and Manser 2013). For example, Japanese tits (*Parus minor*) give different warning sounds to indicate the predator type: the “jar” sound is used to

indicate a Japanese rat snake (*Elaphe climacophora*) whereas the "chicka" sound indicates a jungle crow (*Corvus macrorhynchos*) (Suzuki 2014). When chicks close to fledging hear the "jar" sound coming from the female they jump out of the nest to evade predation by the snake, but upon hearing the "chicka" sound they instead huddle in the nest to avoid being preyed upon (Suzuki 2011).

Some birds have also evolved acoustic Batesian mimicry that simulates the sound of a toxic, inedible, or more dangerous species so as to gain security benefits (Gaul 1952; Sibley 1955; Klump and Shalter 1984; Apel and Weise 1986; Rowe et al. 1986; Owings et al. 2002; Kelly et al. 2008; Zub et al 2017). When a female bird like a great tit (*Parus major*) senses the presence of a predator, the bird stretches its wings forward and down rapidly in a curve, raises and extends its tail, and gives a spontaneous hissing call (Perrins 1979; Cramp and Perrins 1994) that leads to its misidentification as a snake by the predator, which is then discouraged from approaching (Cox 1930; Sibley 1955; Rowe et al. 1986; Perrins 1979; Krams et al. 2014). As an additional example, the hissing call of burrowing owls (*Athene cunicularia*) simulates the crackling sound of alerted Prairie rattlesnakes (*Crotalus viridis*) and is used to scare off its predator, the California ground squirrel (*Spermophilus beecheyi*) (Rowe et al. 1986).

Hissing calls are common in cavity breeding birds including tits (Odum 1942; Hinde 1952; Sibley 1955; Apel and Weise 1986; Broughton 2005, 2012). In many tit species, females incubate the eggs while males scarcely engage in

89 alerting behavior or assist with nest defense (Perrins 1979). A recent study
90 showed that hissing females of great tits survive better than silent females
91 (Krams et al. 2014). Playing recordings of the hissing calls of the great tit,
92 Eurasian blue tit (*Cyanistes caeruleus*), and marsh tit (*Poecile palustris*) could
93 lead to lower levels of nest exploration by their predators, the yellow-necked
94 mouse (*Apodemus flavicollis*) (Zub et al. 2017). Among different tit species
95 breeding in the same area, rates of nest predation are lower for those that exhibit
96 hissing behavior than for those that do not (Walankiewicz 2002; Wesołowski
97 2002; Czeszczewik 2004; Wesołowski and Rowiński 2012; Maziarz et al. 2016).
98 However, hissing calls of many tit species have not yet been investigated.
99 Moreover, there is little information on whether there is interspecific variation
100 in hissing calls of tit species located in the same area, and whether the hissing
101 call of different species is related to their life history traits.
102 To address these questions, in this study we compared the hissing calls of
103 five tit species located in the same area. For sympatrically breeding cinereous
104 and varied tits, we also investigated whether there were differences in the
105 breeding parameters of individuals with or without a hissing call, such as date of
106 the first egg, clutch size, nest predation rate and nesting success in order to
107 determine the relationship between hissing call and breeding performance.
108

109 **Materials and methods**

110 **Study area and study species**

The Saihanba National Forest Park (SHB) is located in Weichang, 240 km from Chengde City, Hebei Province (42°02'–42°36' N, 116°51'–117°39' E) at an altitude of 1,350–1,650 m. The park has a semi-arid/semi-humid cold-temperature continental monsoon climate and is the main natural secondary forest and plantation forest area in Hebei. Within the park there are plateaus, mountains, forests, and grasslands (Fig. 1; Liu et al. 2017). In 2017, 195 wooden nestboxes were put up at a height of 3–4 m on Mongolia scotch pines (*Pinus sylvestris var. mongolica*) and telegraph poles. These nestboxes have an entrance-hole diameter of 4.5 cm and a large chamber (14 cm × 17 cm × 34 cm).

The Xianrendong National Nature Reserve (XRD) is located in Zhuanghe, Liaoning Province (39°54'–40°03' N, 122°53'–123°03' E) at an altitude of 200–600 m. It is adjacent to the Yellow Sea and is located in a warm, temperate, humid monsoon climate zone (Fig. 1; Du et al. 2010). A total of 332 and 426 wooden nestboxes were put up in 2016 and 2018, with a height of 1.3–4.4 m in pine trees (*Pinus densiflora*), poplar trees (*Alnus japonica*) and Chinese ashes (*Pterocarya stenoptera*). These nestboxes have an entrance-hole diameter of 3.5-4.5 cm and a large chamber (outside 14 cm × 16 cm × 34 cm, inside 10 cm × 12 cm × 32 cm).

Tits belong to the Paridae family, which comprises small passerine birds that are mainly distributed in the Northern hemisphere and Africa. These small, stocky, woodland species have short bills and a length of 10–22 cm (Gosler and Peter, 2007). Great tits, which were originally distributed in Eurasia, are now

133 classified as three separate species: great tits from Europe to Northwestern Asia,
134 the cinereous tit (*Parus cinereus*) of South Asia, and the Japanese tit of East
135 Asia (Päckert et al. 2005).

136 In 2018, the birds attracted to artificial nest boxes hung in SHB were
137 mainly cinereous tits, willow tits (*Poecile montanus*), and coal tits (*Periparus*
138 *ater*) (Fig. 1, A-C). Between 2016 and 2018, the birds that were attracted to nest
139 boxes hung in XRD were mainly cinereous, marsh, and varied tits (*Sittiparus*
140 *varius*) (Fig. 1, D-F).

141

142 **Field data collection**

143 The nest boxes, particularly those used by tits, were routinely examined during
144 the breeding season. In SHB, we performed our first nest inspection on May 10,
145 2018, and the last nest inspection on August 2, 2018. In XRD, in 2016 our first
146 nest inspection was conducted on April 10, and the last nest inspection was
147 conducted on July 3; in 2018 our first nest inspection was performed on April 8,
148 and our last nest inspection was performed on July 1. During a day, we checked
149 nest boxes between 09:30 a.m. and 16:00 p.m. Hatching status was determined
150 according to clutch size and date of the first egg. The date at which the female
151 laid the last egg was defined as day 0 of the incubation period. In this study, the
152 incubation period of each of the five tit species was approximately 12 days. We
153 divided the incubation period into three stages: early, mid and late incubation.
154 The nest boxes were inspected once during each stage. When we opened the lid

of the nest box during inspection, some tits gave a hissing call instead of escaping from the nest. Depending on the response of the female upon opening the nest box, we divided birds into those with or without hissing calls. During field work, we found that the hissing call of the five tit species was highly repeatable—i.e., individuals that did not give a hissing call at the start of the study also did not give any hissing calls later during the breeding period.

Nest predation rate was defined as the proportion of depredated nests to the total nests monitored (Krams et al. 2014). Nesting success was defined as the proportion of successful nests (success to fledge at least one young), and it was a dichotomous variable for measuring predation intensity (Pribil 1998).

Statistical analysis

Statistical analysis was performed using SPSS v.16.0 for Windows (IBM, Armonk, NY, USA). The one-sample Kolmogorov-Smirnov test was used to analyze the normality of the data. When the data normality condition was met, the t-test or one-way analysis of variance was used to compare mean values. Otherwise, non-parametric tests—i.e., the Mann-Whitney U test and Kruskal-Wallis test—were used. All tests were two-tailed, with a significance level of $P < 0.05$. Data are expressed as mean ± standard deviation (mean ± SD).

Results

During 2018 and 2019 in SHB, 19 out of 32 (59.4%) cinereous tit, 14 out of 17 (82.3%) coal tit, and 4 out of 17 (23.5%) willow tit nests contained females that gave hissing calls. There were significant differences among hissing calls of the three tit species located in the same area (Fig. 2; $P = 0.002$, Fisher's exact test).

During 2018 in XRD, 16 out of 39 (41.0%) varied tits, 24 out of 40 (60.0%) cinereous tits, and 3 out of 17 (17.6%) marsh tits gave hissing calls. Significant differences were observed in the frequency of hissing calls of the three tit species located in the same area (Fig. 2; $P = 0.011$, Fisher's exact test).

In SHB, none of the 66 cinereous tits and 45 coal tits and only one of the 31 willow tit nests were depredated. There were no significant differences in predation rates among the three tit species ($P > 0.05$, Fisher's exact test).

In XRD, 18 out of 39 (46.2%) varied tits, 14 out of 40 (35.0%) cinereous tits, and 8 out of 19 (42.1%) marsh tit nests were targeted by predators. There were no significant differences in predation rates among the three tit species ($P = 0.597$, Fisher's exact test). For a total of 51 depredated nests, 30 nests were confirmed to be depredated by mice (10%; 3 out of 30 nests) or snakes (90%; including Korean rat-snakes *Elaphe anomala* and steppe rat-snakes *E. dione*).

For cinereous tits, 62 tits gave hissing calls whereas 23 tits did not. The date of the first egg and clutch size did not differ significantly between the two groups of tits [9.75 ± 1.31 (n = 60) vs. 9.77 ± 1.23 (n = 22)] ($P = 0.880$, Mann-Whitney U test). Similar rates of nest predation (19.4% vs. 30.4%) and nesting

197 success (64.5% vs. 52.2%) were also observed between the two groups (Fig. 3;
198 nest predation: P =0.379; nesting success: P =0.326, Fisher's exact test).
199 For varied tits, 52 tits gave hissing calls whereas 37 tits did not. The two
200 groups were similar in terms of date of the first egg and clutch size (7.31 ± 1.08
201 vs. 7.10 ± 1.12) (P = 0.264, Mann-Whitney U test) as well as nest predation
202 (21.6% vs. 30.8%) and nesting success (56.8% vs. 44.2%) (Fig. 3; nest
203 predation: P =0.468; nesting success: P =0.286, Fisher's exact test).
204
205 **Discussion**
206 Nest predation is a major cause of death in birds and has led to the evolution of
207 morphological, physiological, and behavioral strategies to avoid predation
208 (Lima 2009; Parejo et al. 2013; Fu et al. 2016). Previous work showed that
209 cavity breeding tits give hissing calls to frighten approaching predators (Rowe
210 et al. 1986; Zub et al. 2017) and thereby increase the survival rate of female
211 birds and fledglings (Krams et al. 2014). Our study indicated that in SHB, there
212 were significant differences in the proportions of individuals giving a hissing
213 call among cinereous, willow and marsh tits. Furthermore, in XRD, differences
214 were also observed in the proportions of cinereous, varied, and marsh tits giving
215 hissing calls. However, in both SHB of Hebei and XRD of Liaoning, nest
216 predation rates of tits breeding in nest boxes located in the same area did not
217 differ. Cinereous and varied tits showed no differences in clutch size, date of the

first egg, nest predation rate and nesting success between birds with and without hissing calls.

Tit species in the wild are known to give hissing calls (Pickens 1928; Sibley 1995). However, there have been few studies investigating interspecific variation in hissing calls of tit species (Sibley 1995; Krams et al. 2014; Koosa and Tilgar 2016; Zub et al. 2017). An early report suggested that the hissing calls of tits were an acoustic form of Batesian mimicry and provided a brief description of the hissing calls of different species (Sibley 1995). Here, we recorded in detail the hissing calls of five tit species located in the same area and investigated interspecific differences.

We found that 60% of individual cinereous tits gave a hissing call; this is comparable to the 70% reported in a previous study in which hissing calls of great tits were found to substantially reduce the predation rate of breeding females (Krams et al. 2014). The hissing call of tits was also shown to discourage the nest exploration behavior of yellow-necked mouse (*Apodemus flavicollis*) (Zub et al. 2017). However, hissing call behavior did not reduce the rate of nest predation in tits. In SHB, the artificial nest boxes were mainly distributed in plantations located in areas where there were few snakes and few Swinhoe's striped squirrels (*Tamiops swinhoei*)—two of the natural predators of tits—due to the high altitude and low temperature. On the other hand, in XRD, nest predation rates of cinereous, varied, and marsh tits did not differ despite variation in the proportion of individuals with hissing calls. This is because the

main nest predators in this area were Korean and steppe rat-snakes, which are not influenced by the hissing calls of tits.

An earlier study found that the nest predation rate is markedly lower for great tits compared to the two species without hissing calls (Krams et al. 2014). A possible reason for the discrepancy between our study and this earlier study is the small sample size (85 cinereous and 89 varied tits vs. 477 samples in the study by Krams et al. 2014). Additionally, the predators in their study were mainly European pine marten (*Martes martes*), least weasel (*Mustela nivalis*), and great spotted woodpecker (*Dendrocopos major*), which are more likely scared off by the snake-like hissing calls of great tits than the predators in our study area (mainly snakes) (Weatherhead and Blouin-Demers 2004).

Hissing calls have been reported not to be related to the breeding performance of female great tits (Koosa and Tilgar 2016) in that females with and without hissing calls were found to lay a similar clutch size. Our results support this observation. Furthermore, we also determined that the hissing calls of all five tit species are unrelated to nest predation risk.

In conclusion, our results showed that hissing calls are common among sympatrically breeding tit species such as cinereous, willow, coal, marsh, and varied tits. In addition, the hissing calls of cinereous and varied tits were unrelated to nest predation risk or life history traits such as date of the first egg,

clutch size and nesting success. The present study made a contribution to intra- and inter-specific differences in vocal behavior in closely related species.

Ethics The experiments reported here comply with the current laws of China. Fieldwork was carried out under the permission from Saihanba National Forest Park and Xianrendong National Nature Reserve, China. Experimental procedures were in agreement with the Animal Research Ethics Committee of Hainan Provincial Education Centre for Ecology and Environment, Hainan Normal University (permit no. HNECEE-2011-001).

Data accessibility Data used in this study are available in Electronic Supplementary Materials.

Competing interests The authors declare that they have no competing interests.

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References

Apel KM, Weise CM. 1986. The hiss-display of nesting black-capped chickadees in captivity. Wilson Bulletin, 98: 320–321.

Broughton RK. 2005. Hissing display of incubating marsh tit and antipredator response of the young. British Birds, 98: 267–268.

Broughton RK. 2012. Nest defence behaviour by marsh tits in response to a stuffed weasel. British Birds, 105: 39–40.

Burger SJ. 1992. Differential responses of tropical roseate terns to aerial intruders throughout the nesting cycle. Condor, 94: 712–719.

Cox AHM. 1930. Nestling wood-warblers "hissing". British Birds, 23: 219–220.

Cramp S, Perrins CM. 1994. The birds of the western Palearctic. Oxford University Press, New York.

Crisologo TL, Bonter DN. 2017. Defending the weak: Parental defense peaks when chick vulnerability is greatest in the herring gull (*Larus argentatus*). Ethology, 123: 113–122.

Czeszczewik D. 2004. Breeding success and timing of the pied flycatcher *Ficedula hyploeuca* nesting in natural holes and nest-boxes in the Białowieża forest, Poland. Acta Ornithology, 39: 15–20.

Daniela C, Sealy SG. 2018. Evolutionary significance of antiparasite, antipredator and learning phenotypes of avian nest defence. Scientific Reports, 8: 10569.

306 Du Y, Huo Y, Wan D, Sun J, Lv Y, Cao J. 2010. Moss selection as nest
307 materials by *Parus varius*. Chinese Journal of Zoology, 45: 144–149.
308 Fasanella M, Fernández GJ. 2009. Alarm calls of the southern house wren
309 *Troglodytes musculus*: Variation with nesting stage and predator model.
310 Journal of Ornithology, 150: 853–863.
311 Fontaine JJ, Martin TE. 2006. Parent birds assess nest predation risk and adjust
312 their reproductive strategies. Ecology Letters, 9: 428–434.
313 Forstmeier W, Weiss I. 2004. Adaptive plasticity in nest-site selection in
314 response to changing predation risk. Oikos, 104: 487–499.
315 Fu Y, Chen B, Dowell SD, Zhang Z. 2016. Nest predators, nest-site selection
316 and nest success of the Emei Shan liocichla (*Liocichla omeiensis*), a
317 vulnerable babbler endemic to southwestern China. Avian Research, 7: 18.
318 Fuong H, Keeley KN, Bulut Y, Blumstein DT. 2014. Heterospecific alarm
319 calleavesdropping in nonvocal, white-bellied copper-striped skinks, *Emoia*
320 *cyanura*. Animal Behaviour, 95: 129–35.
321 Gaul AT. 1952. Audio mimicry: An adjunct to color mimicry. Psyche, 59: 82–
322 83.
323 Gill SA, Bierema AMK. 2013. On the meaning of alarm calls: A review of
324 functional reference in avian alarm calling. Ethology, 119: 449–461.
325 Gosler A, Clement P. 2007. Family Paridae (tits and chickadees). In del Hoyo J,
326 Elliott A, Christie D (eds). Handbook of the birds of the world. Vol. 12:
327 Picathartes to tits and chickadees. Pp. 662–709. Lynx Edicions, Barcelona.

328 Guppy M, Guppy S, Marchant R, Priddel D, Carlile N, Fullagar P. 2017. Nest
329 predation of woodland birds in south-east Australia: Importance of
330 unexpected predators. Emu, 117: 92–96.
331 Hinde RA. 1952. The behaviour of the great tit (*Parus major*) and some other
332 related species. Behaviour, 2 (Suppl.): 1–201.
333 Kelly LA, Coe RL, Madden JR, Healy SD. 2008. Vocal mimicry in songbirds.
334 Animal Behaviour, 76: 521–528.
335 Kitchen D, Bergman T, Cheney D, Nicholson J. 2010. Comparing responses of
336 four ungulate species to playbacks of baboon alarm calls. Animal Cognition,
337 13: 861–870.
338 Klump GM, Shalter MD. 1984. Acoustic behaviour of birds and mammals in
339 the predator context; I. Factors affecting the structure of alarm signals. II.
340 The functional significance and evolution of alarm signals. Zeitschrift für
341 Tierpsychologie, 66: 189–226.
342 Koosa K, Tilgar V. 2016. Is hissing behaviour of incubating great tits related to
343 reproductive investment in the wild? Acta Ethologica, 19: 173–180.
344 Krams I, Krama T, Berzins A, Rantala MJ. 2010. The risk of predation favors
345 cooperation among breeding prey. Communicative and Integrative Biology,
346 3: 243–244.
347 Krams I, Vrublevska J, Koosa K, Krama T, Mierauskas P, Rantala MJ, Tilgar V.
348 2014. Hissing calls improve survival in incubating female great tits (*Parus*
349 *major*). Acta Ethologica, 17: 83–88.

Lima SL. 2009. Predators and the breeding bird: behavioral and reproductive flexibility under the risk of predation. Biological Reviews, 84: 485–513.

Lima SL, Rattenborg NS, Lesku JA, Amlaner CJ. 2005. Sleeping under the risk of predation. Animal Behaviour, 70: 723–736.

Liu J, Ma L, Zhang Z, Gu D, Wang J, Li J, Gao L, Hou J. 2017. Maximum frequency of songs reflects body size among male dusky warblers *Phylloscopus fuscatus* (Passeriformes: Phylloscopidae). European Zoological Journal, 84: 186–192.

Martin TE. 1995. Avian life history evolution in relation to nest sites, nest predation, and food. Ecological Monograph, 65: 101–127.

Maziarz M, Piggott C, Burgess M. 2018. Predator recognition and differential behavioural responses of adult wood warblers *Phylloscopus sibilatrix*. Acta Ethologica, 21: 13–20.

Maziarz M, Wesołowski T, Hebda G, Cholewa M, Broughton RK. 2016. Breeding success of the great tit *Parus major* in relation to attributes of natural nest cavities in a primeval forest. Journal of Ornithology, 157: 343–354.

Odum EP. 1942. Annual cycle of the black-capped chickadee. Auk, 59: 499–531.

Owings DH, Rowe MP, Rundus AS. 2002. The rattling sound of rattlesnakes (*Crotalus viridis*) as a communicative resource for ground squirrels

371 (*Spermophilus beecheyi*) and burrowing owls (*Athene cunicularia*). Journal
372 of Comparative Psychology, 116: 197–205.
373 Parejo D, Avilés JM, Peña A, Sánchez L, Ruano F, Zamora-Muñoz C, Martín-
374 Vivaldi M. 2013. Armed rollers: Does nestling's vomit function as a
375 defence against predators? PLoS One, 8: e68862.
376 Päckert M, Martens J, Eck S, Nazarenko AA, Valchuk OP, Petri B, Veith M.
377 2005. The great tit (*Parus major*): A misclassified ring species. Biological
378 Journal of the Linnean Society, 86: 153–174.
379 Perrins CM. 1979. British tits. Collins, London.
380 Pickens AL. 1928. Auditory protective mimicry of the chicadee. Auk, 45: 302–
381 304.
382 Pipia A, Ciuti S, Grignolio S, Luchetti S, Madau R, Apollonio M. 2009. Effect
383 of predation risk on grouping pattern and whistling behaviour in a wild
384 mouflon *Ovis aries* population. Acta Theriologica, 54: 77–86.
385 Ricklefs RE. 1969. An analysis of nesting mortality in birds. Smithsonian
386 Contributions to Zoology, 9: 1–48.
387 Ritchison SG. 1993. The nest defense behavior of eastern screech-owls: Effects
388 of nest stage, sex, nest type and predator location. Condor, 95: 288–296.
389 Rowe MP, Coss RG, Owings DH. 1986. Rattlesnake rattles and burrowing owl
390 hisses: A case of acoustic Batesian mimicry. Ethology, 72: 53–71.
391 Sherman PW. 1977. Nepotism and the evolution of alarm calls. Science, 197:
392 1246-53.

393 Sibley CG. 1955. Behavioral mimicry in the titmice (Paridae) and certain other
394 birds. Wilson Bulletin, 67: 128–132.
395 Suzuki TN. 2011. Parental alarm calls warn nestlings about different predatory
396 threats. Current Biology, 21: R15–R16.
397 Suzuki TN. 2014. Communication about predator type by a bird using discrete,
398 graded and combinatorial variation in alarm calls. Animal Behaviour, 87:
399 59–65.
400 Suzuki TN. 2015. Assessment of predation risk through referential
401 communication in incubating birds. Scientific Reports, 5: 10239.
402 Tilgar V, Moks K. 2015. Increased risk of predation increases mobbing
403 intensity in tropical birds of French Guiana. Journal of Tropical Ecology, 31:
404 243–250.
405 Townsend SW, Charlton BD, Manser MB. 2014. Acoustic cues to identity and
406 predator context in meerkat barks. Animal Behaviour, 94: 143–149.
407 Townsend SW, Manser MB. 2013. Functionally referential communication in
408 mammals: The past, present and the future. Ethology, 119: 1–11.
409 Walankiewicz W. 2002. Nest predation as a limiting factor to the breeding
410 population size of the collared flycatcher *Ficedula albicollis* in the
411 Białowieża National Park (NE Poland). Acta Ornithology, 37: 91–106.

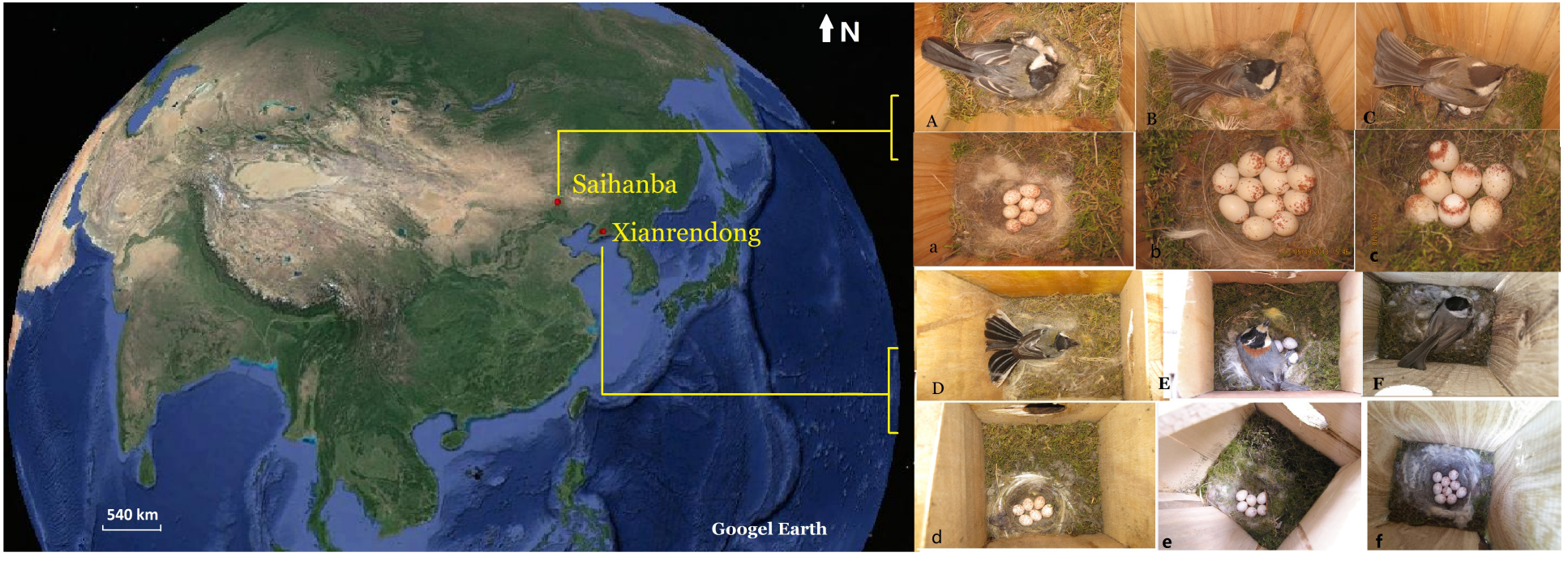
412 Weatherhead PJ, Blouin-Demers G. 2004. Understanding avian nest predation:
413 Why ornithologists should study snakes. Journal of Avian Biology, 35:
414 185–190.
415 Wesołowski T. 2002. Antipredator adaptations in nesting marsh tits *Parus*
416 *palustris*: the role of nest site security. Ibis, 144: 593–601.
417 Wesołowski T, Rowiński P. 2012. The breeding performance of blue tits
418 *Cyanistes caeruleus* in relation to the attributes of natural holes in a
419 primeval forest. Bird Study, 59: 437–448.
420 Yorzinski JL, Platt ML. 2012. The difference between night and day:
421 antipredator behavior in birds. Journal of Ethology, 30: 211–218.
422 Yorzinski JL, Vehrencamp SL. 2009. The effect of predator type and danger
423 level on the mob calls of the American crow. Condor, 111: 159–168.
424 Zub K, Czeszczewik D, Ruczyński I, Kapusta A, Walankiewicz W. 2017.
425 Silence is not golden: The hissing calls of tits affect the behaviour of a nest
426 predator. Behavioral Ecology and Sociobiology, 71: 79.
427 Zuberbühler K. 2009. Survivor signals: The biology and psychology of animal
428 alarm calling. Advance in the Study of Behaviour, 40: 277–322.
429 Zheng G. 2017. A checklist on the classification and distribution of the birds of
430 China (Third Edition). Science Press, Beijing.

Legends to figures

Figure 1. Study areas and study species in this study. Capital letters (A-F) refer to bird species and lowercase letters (a-f) refer to its nest and eggs. A and D refer to cinereous tit; B coal tit; C willow tit; E varied tit; and F marsh tit.

Figure 2. Proportion of hissing individuals in five tit species at two study sites.

Figure 3. Comparison of nesting success (% eggs that resulted in fledged young) between individuals with and without hissing call in cinereous and varied tits.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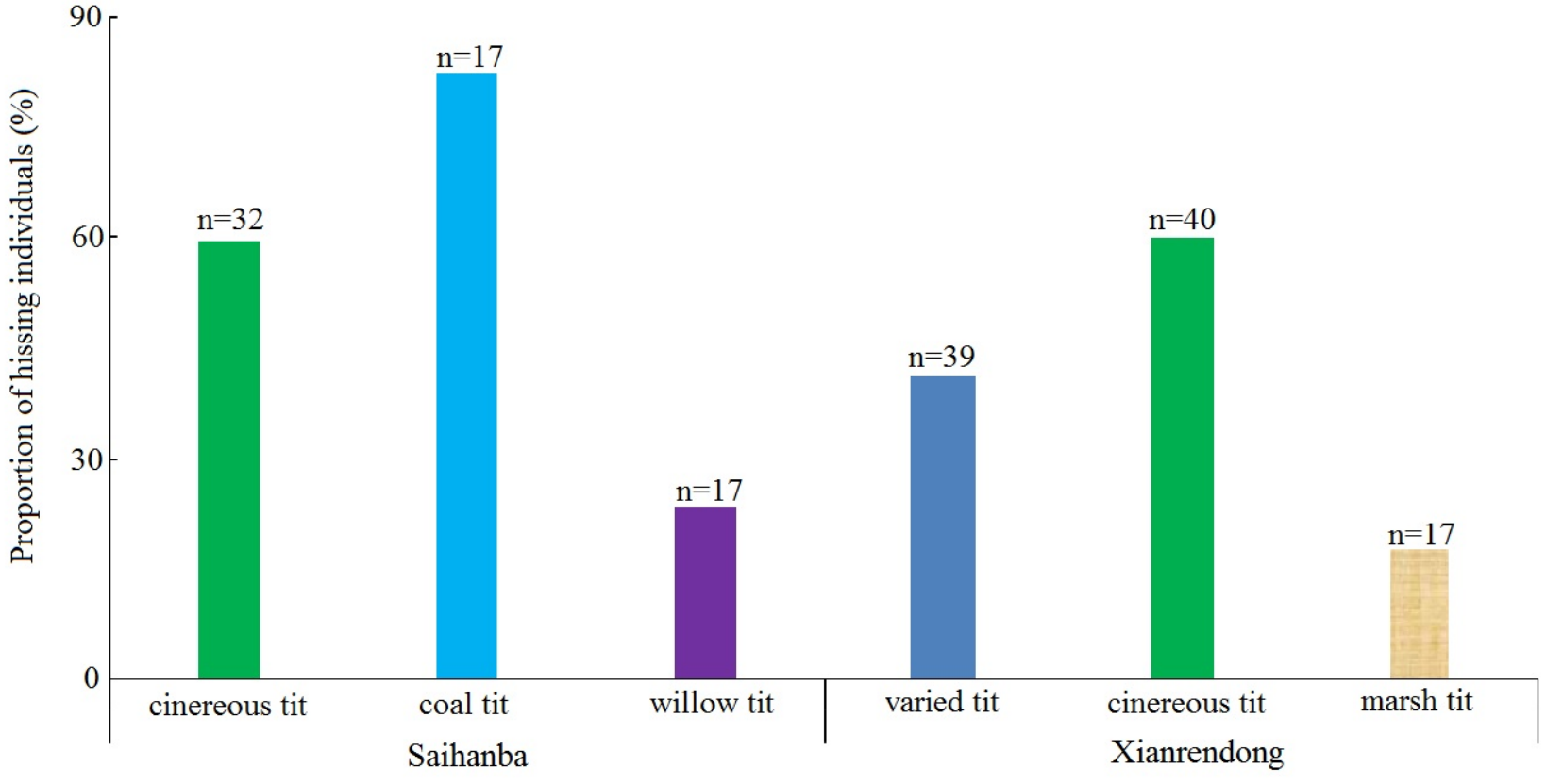
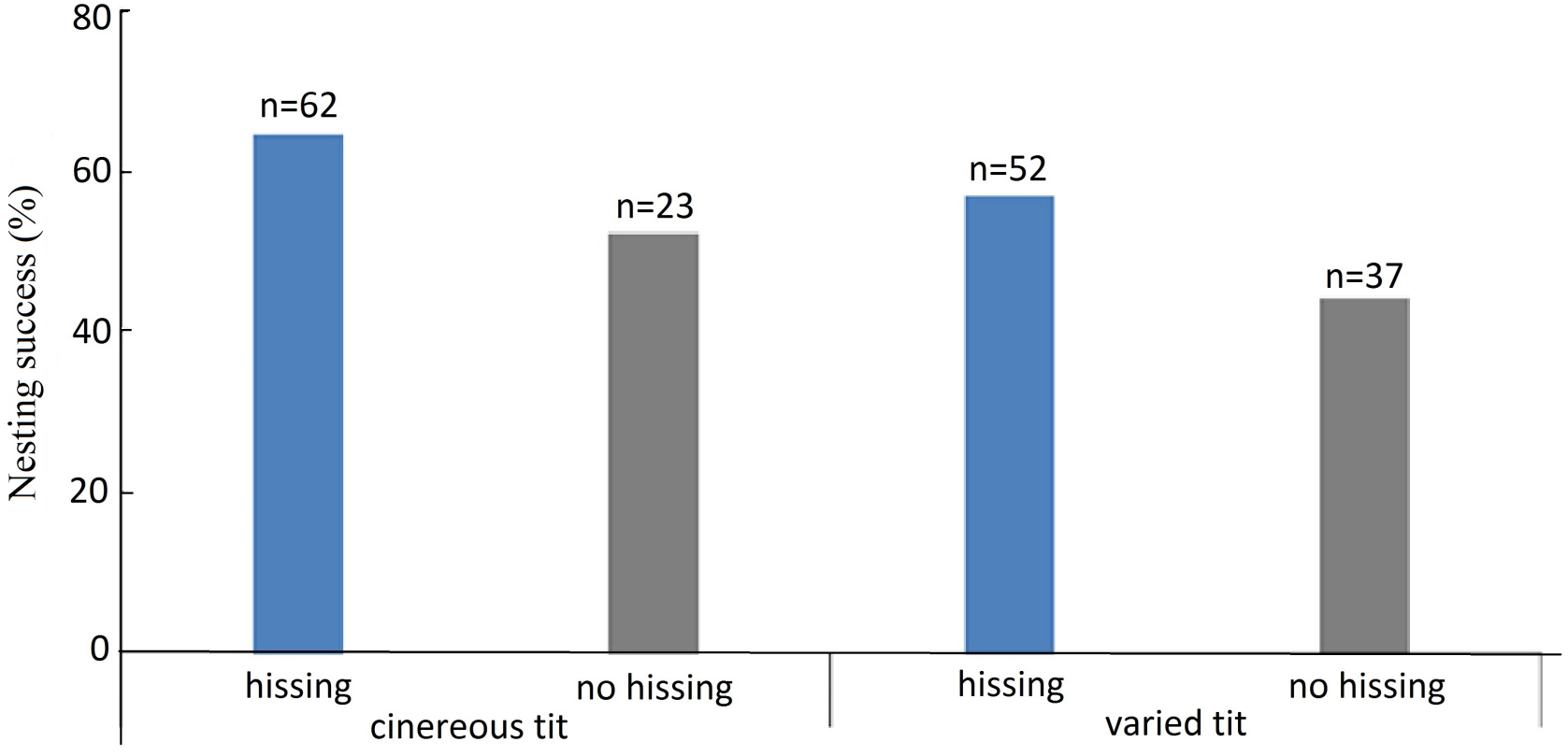

Proportion of hissing individuals (%)
90
60
30
0
n=32
n=17
n=17
n=39
n=40
n=17
cinereous tit
coal tit
willow tit
varied tit
cinereous tit
marsh tit
Saihanba
Xianrendong


80
60
40
20
0
Nesting success (%)
n=62
n=23
n=52
n=37
hissing
no hissing
hissing
no hissing
cinereous tit
varied tit

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Anders Pape Møller Conceptualization, Methodology, Writing - Review & Editing.

Comparative analysis of hissing calls in five tit species

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Running headline: Hissing calls in tit species

Abstract

Nest predation often leads to breeding failure and is an important component of natural selection that affects the evolution of nest defense behavior in birds. Many tit species give a hissing call as nest defense, but there are few studies of interspecific variation in hissing calls, and whether these are related to nest predation and nesting success. In this study, we compared the hissing calls of five tit species including cinereous tits (*Parus cinereus*), marsh tits (*Poecile palustris*), varied tits (*Sittiparus varius*), willow tits (*Poecile montanus*), and coal tits (*Periparus ater*) in Saihanba National Forest Park in Hebei and Xianrendong National Nature Reserve in Liaoning. In Saihanba of Hebei, the proportion of cinereous, willow, and coal tit individuals giving a hissing call differed significantly but the rate of nest predation was similar. It was also true for the three tit species (cinereous, varied, and marsh tits) in Xianrendong of Liaoning. Cinereous and varied tits showed no differences in clutch size, date of the first egg, nest predation and nesting success between individuals that gave and those that did not give a hissing call. These results indicate that for tit species that breed in nest boxes distributed within the same area, there is interspecific variation in hissing calls but this variation is not significantly correlated with nest predation risk.

Keywords: hissing call; nest defense; nest predation; repeatability; Paridae.

Introduction

Nest predation often leads to reproductive failure and is a major selective force that affects nest defense behavior in birds (Ricklefs 1969; Martin 1995; Forstmeier and Weiss 2004; Fontaine and Martin 2006; Lima 2009; Tilgar and Moks 2015; Fu et al. 2016; Guppy et al. 2017). Birds have evolved complex anti-predation strategies to protect their nests and demonstrate specific behaviors when facing predators of different types and risk levels (Lima et al. 2005; Yorzinski and Vehrencamp 2009; Krams et al. 2010; Yorzinski and Platt 2012; Suzuki 2011, 2015; Daniela et al. 2018; Maziarz et al. 2018). Nest stage, sex, nest type and predator location may also have an effect on anti-predator behaviour of birds (Burger 1992; Ritchison 1993; Crisologo and Bonter 2017). For example, when a blue peacock (*Pavo cristatus*) encounters a raccoon during the day, it calls loudly, stretches its neck, opens its wings, and strikes a flying pose as it approaches the predator. However, at night it will instead remain silent and give a soft hissing call (Yorzinski and Platt 2012).

In animals, sound is often used to transmit predator information (Zuberbühler 2009; Fasanella and Fernández 2009; Suzuki 2011, 2014, 2015; Fuong et al. 2014; Townsend et al. 2014). Individuals of the same or different species use this acoustic information to evade predation (Sherman 1977; Pipia et al. 2009; Kitchen et al. 2010; Suzuki 2011, 2015; Gill and Bierema 2013; Townsend and Manser 2013). For example, Japanese tits (*Parus minor*) give different warning sounds to indicate the predator type: the “jar” sound is used to

indicate a Japanese rat snake (*Elaphe climacophora*) whereas the "chicka" sound indicates a jungle crow (*Corvus macrorhynchos*) (Suzuki 2014). When chicks close to fledging hear the "jar" sound coming from the female they jump out of the nest to evade predation by the snake, but upon hearing the "chicka" sound they instead huddle in the nest to avoid being preyed upon (Suzuki 2011).

Some birds have also evolved acoustic Batesian mimicry that simulates the sound of a toxic, inedible, or more dangerous species so as to gain security benefits (Gaul 1952; Sibley 1955; Klump and Shalter 1984; Apel and Weise 1986; Rowe et al. 1986; Owings et al. 2002; Kelly et al. 2008; Zub et al 2017). When a female bird like a great tit (*Parus major*) senses the presence of a predator, the bird stretches its wings forward and down rapidly in a curve, raises and extends its tail, and gives a spontaneous hissing call (Perrins 1979; Cramp and Perrins 1994) that leads to its misidentification as a snake by the predator, which is then discouraged from approaching (Cox 1930; Sibley 1955; Rowe et al. 1986; Perrins 1979; Krams et al. 2014). As an additional example, the hissing call of burrowing owls (*Athene cunicularia*) simulates the crackling sound of alerted Prairie rattlesnakes (*Crotalus viridis*) and is used to scare off its predator, the California ground squirrel (*Spermophilus beecheyi*) (Rowe et al. 1986).

Hissing calls are common in cavity breeding birds including tits (Odum 1942; Hinde 1952; Sibley 1955; Apel and Weise 1986; Broughton 2005, 2012). In many tit species, females incubate the eggs while males scarcely engage in

89 alerting behavior or assist with nest defense (Perrins 1979). A recent study
90 showed that hissing females of great tits survive better than silent females
91 (Krams et al. 2014). Playing recordings of the hissing calls of the great tit,
92 Eurasian blue tit (*Cyanistes caeruleus*), and marsh tit (*Poecile palustris*) could
93 lead to lower levels of nest exploration by their predators, the yellow-necked
94 mouse (*Apodemus flavicollis*) (Zub et al. 2017). Among different tit species
95 breeding in the same area, rates of nest predation are lower for those that exhibit
96 hissing behavior than for those that do not (Walankiewicz 2002; Wesołowski
97 2002; Czeszczewik 2004; Wesołowski and Rowiński 2012; Maziarz et al. 2016).
98 However, hissing calls of many tit species have not yet been investigated.
99 Moreover, there is little information on whether there is interspecific variation
100 in hissing calls of tit species located in the same area, and whether the hissing
101 call of different species is related to their life history traits.
102 To address these questions, in this study we compared the hissing calls of
103 five tit species located in the same area. For sympatrically breeding cinereous
104 and varied tits, we also investigated whether there were differences in the
105 breeding parameters of individuals with or without a hissing call, such as date of
106 the first egg, clutch size, nest predation rate and nesting success in order to
107 determine the relationship between hissing call and breeding performance.
108

109 **Materials and methods**

110 **Study area and study species**

The Saihanba National Forest Park (SHB) is located in Weichang, 240 km from Chengde City, Hebei Province (42°02'–42°36' N, 116°51'–117°39' E) at an altitude of 1,350–1,650 m. The park has a semi-arid/semi-humid cold-temperature continental monsoon climate and is the main natural secondary forest and plantation forest area in Hebei. Within the park there are plateaus, mountains, forests, and grasslands (Fig. 1; Liu et al. 2017). In 2017, 195 wooden nestboxes were put up at a height of 3–4 m on Mongolia scotch pines (*Pinus sylvestris var. mongolica*) and telegraph poles. These nestboxes have an entrance-hole diameter of 4.5 cm and a large chamber (14 cm × 17 cm × 34 cm).

The Xianrendong National Nature Reserve (XRD) is located in Zhuanghe, Liaoning Province (39°54'–40°03' N, 122°53'–123°03' E) at an altitude of 200–600 m. It is adjacent to the Yellow Sea and is located in a warm, temperate, humid monsoon climate zone (Fig. 1; Du et al. 2010). A total of 332 and 426 wooden nestboxes were put up in 2016 and 2018, with a height of 1.3–4.4 m in pine trees (*Pinus densiflora*), poplar trees (*Alnus japonica*) and Chinese ashes (*Pterocarya stenoptera*). These nestboxes have an entrance-hole diameter of 3.5-4.5 cm and a large chamber (outside 14 cm × 16 cm × 34 cm, inside 10 cm × 12 cm × 32 cm).

Tits belong to the Paridae family, which comprises small passerine birds that are mainly distributed in the Northern hemisphere and Africa. These small, stocky, woodland species have short bills and a length of 10–22 cm (Gosler and Peter, 2007). Great tits, which were originally distributed in Eurasia, are now

133 classified as three separate species: great tits from Europe to Northwestern Asia,
134 the cinereous tit (*Parus cinereus*) of South Asia, and the Japanese tit of East
135 Asia (Päckert et al. 2005).

136 In 2018, the birds attracted to artificial nest boxes hung in SHB were
137 mainly cinereous tits, willow tits (*Poecile montanus*), and coal tits (*Periparus*
138 *ater*) (Fig. 1, A-C). Between 2016 and 2018, the birds that were attracted to nest
139 boxes hung in XRD were mainly cinereous, marsh, and varied tits (*Sittiparus*
140 *varius*) (Fig. 1, D-F).

141

142 **Field data collection**

143 The nest boxes, particularly those used by tits, were routinely examined during
144 the breeding season. In SHB, we performed our first nest inspection on May 10,
145 2018, and the last nest inspection on August 2, 2018. In XRD, in 2016 our first
146 nest inspection was conducted on April 10, and the last nest inspection was
147 conducted on July 3; in 2018 our first nest inspection was performed on April 8,
148 and our last nest inspection was performed on July 1. During a day, we checked
149 nest boxes between 09:30 a.m. and 16:00 p.m. Hatching status was determined
150 according to clutch size and date of the first egg. The date at which the female
151 laid the last egg was defined as day 0 of the incubation period. In this study, the
152 incubation period of each of the five tit species was approximately 12 days. We
153 divided the incubation period into three stages: early, mid and late incubation.
154 The nest boxes were inspected once during each stage. When we opened the lid

of the nest box during inspection, some tits gave a hissing call instead of escaping from the nest. Depending on the response of the female upon opening the nest box, we divided birds into those with or without hissing calls. During field work, we found that the hissing call of the five tit species was highly repeatable—i.e., individuals that did not give a hissing call at the start of the study also did not give any hissing calls later during the breeding period.

Nest predation rate was defined as the proportion of depredated nests to the total nests monitored (Krams et al. 2014). Nesting success was defined as the proportion of successful nests (success to fledge at least one young), and it was a dichotomous variable for measuring predation intensity (Pribil 1998).

Statistical analysis

Statistical analysis was performed using SPSS v.16.0 for Windows (IBM, Armonk, NY, USA). The one-sample Kolmogorov-Smirnov test was used to analyze the normality of the data. When the data normality condition was met, the t-test or one-way analysis of variance was used to compare mean values. Otherwise, non-parametric tests—i.e., the Mann-Whitney U test and Kruskal-Wallis test—were used. All tests were two-tailed, with a significance level of $P < 0.05$. Data are expressed as mean ± standard deviation (mean ± SD).

Results

During 2018 and 2019 in SHB, 19 out of 32 (59.4%) cinereous tit, 14 out of 17 (82.3%) coal tit, and 4 out of 17 (23.5%) willow tit nests contained females that gave hissing calls. There were significant differences among hissing calls of the three tit species located in the same area (Fig. 2; $P = 0.002$, Fisher's exact test).

During 2018 in XRD, 16 out of 39 (41.0%) varied tits, 24 out of 40 (60.0%) cinereous tits, and 3 out of 17 (17.6%) marsh tits gave hissing calls. Significant differences were observed in the frequency of hissing calls of the three tit species located in the same area (Fig. 2; $P = 0.011$, Fisher's exact test).

In SHB, none of the 66 cinereous tits and 45 coal tits and only one of the 31 willow tit nests were depredated. There were no significant differences in predation rates among the three tit species ($P > 0.05$, Fisher's exact test).

In XRD, 18 out of 39 (46.2%) varied tits, 14 out of 40 (35.0%) cinereous tits, and 8 out of 19 (42.1%) marsh tit nests were targeted by predators. There were no significant differences in predation rates among the three tit species ($P = 0.597$, Fisher's exact test). For a total of 51 depredated nests, 30 nests were confirmed to be depredated by mice (10%; 3 out of 30 nests) or snakes (90%; including Korean rat-snakes *Elaphe anomala* and steppe rat-snakes *E. dione*).

For cinereous tits, 62 tits gave hissing calls whereas 23 tits did not. The date of the first egg and clutch size did not differ significantly between the two groups of tits [9.75 ± 1.31 (n = 60) vs. 9.77 ± 1.23 (n = 22)] ($P = 0.880$, Mann-Whitney U test). Similar rates of nest predation (19.4% vs. 30.4%) and nesting

197 success (64.5% vs. 52.2%) were also observed between the two groups (Fig. 3;
198 nest predation: P =0.379; nesting success: P =0.326, Fisher's exact test).
199 For varied tits, 52 tits gave hissing calls whereas 37 tits did not. The two
200 groups were similar in terms of date of the first egg and clutch size (7.31 ± 1.08
201 vs. 7.10 ± 1.12) (P = 0.264, Mann-Whitney U test) as well as nest predation
202 (21.6% vs. 30.8%) and nesting success (56.8% vs. 44.2%) (Fig. 3; nest
203 predation: P =0.468; nesting success: P =0.286, Fisher's exact test).
204
205 **Discussion**
206 Nest predation is a major cause of death in birds and has led to the evolution of
207 morphological, physiological, and behavioral strategies to avoid predation
208 (Lima 2009; Parejo et al. 2013; Fu et al. 2016). Previous work showed that
209 cavity breeding tits give hissing calls to frighten approaching predators (Rowe
210 et al. 1986; Zub et al. 2017) and thereby increase the survival rate of female
211 birds and fledglings (Krams et al. 2014). Our study indicated that in SHB, there
212 were significant differences in the proportions of individuals giving a hissing
213 call among cinereous, willow and marsh tits. Furthermore, in XRD, differences
214 were also observed in the proportions of cinereous, varied, and marsh tits giving
215 hissing calls. However, in both SHB of Hebei and XRD of Liaoning, nest
216 predation rates of tits breeding in nest boxes located in the same area did not
217 differ. Cinereous and varied tits showed no differences in clutch size, date of the

first egg, nest predation rate and nesting success between birds with and without hissing calls.

Tit species in the wild are known to give hissing calls (Pickens 1928; Sibley 1995). However, there have been few studies investigating interspecific variation in hissing calls of tit species (Sibley 1995; Krams et al. 2014; Koosa and Tilgar 2016; Zub et al. 2017). An early report suggested that the hissing calls of tits were an acoustic form of Batesian mimicry and provided a brief description of the hissing calls of different species (Sibley 1995). Here, we recorded in detail the hissing calls of five tit species located in the same area and investigated interspecific differences.

We found that 60% of individual cinereous tits gave a hissing call; this is comparable to the 70% reported in a previous study in which hissing calls of great tits were found to substantially reduce the predation rate of breeding females (Krams et al. 2014). The hissing call of tits was also shown to discourage the nest exploration behavior of yellow-necked mouse (*Apodemus flavicollis*) (Zub et al. 2017). However, hissing call behavior did not reduce the rate of nest predation in tits. In SHB, the artificial nest boxes were mainly distributed in plantations located in areas where there were few snakes and few Swinhoe's striped squirrels (*Tamiops swinhoei*)—two of the natural predators of tits—due to the high altitude and low temperature. On the other hand, in XRD, nest predation rates of cinereous, varied, and marsh tits did not differ despite variation in the proportion of individuals with hissing calls. This is because the

main nest predators in this area were Korean and steppe rat-snakes, which are not influenced by the hissing calls of tits.

An earlier study found that the nest predation rate is markedly lower for great tits compared to the two species without hissing calls (Krams et al. 2014). A possible reason for the discrepancy between our study and this earlier study is the small sample size (85 cinereous and 89 varied tits vs. 477 samples in the study by Krams et al. 2014). Additionally, the predators in their study were mainly European pine marten (*Martes martes*), least weasel (*Mustela nivalis*), and great spotted woodpecker (*Dendrocopos major*), which are more likely scared off by the snake-like hissing calls of great tits than the predators in our study area (mainly snakes) (Weatherhead and Blouin-Demers 2004).

Hissing calls have been reported not to be related to the breeding performance of female great tits~~birds~~ (Koosa and Tilgar 2016) in that ~~For example,~~ females with and without hissing calls were found to lay a similar clutch size. Our results support this observation. Furthermore, we also determined that the hissing calls of all five tit species are unrelated to nest predation risk.

In conclusion, our results showed that hissing calls are common among sympatrically breeding tit species such as cinereous, willow, coal, marsh, and varied tits. In addition, the hissing calls of cinereous and varied tits were unrelated to nest predation risk or life history traits such as date of the first egg,

clutch size and nesting success. The present study made a contribution to intra- and inter-specific differences in vocal behavior in closely related species.

Ethics The experiments reported here comply with the current laws of China. Fieldwork was carried out under the permission from Saihanba National Forest Park and Xianrendong National Nature Reserve, China. Experimental procedures were in agreement with the Animal Research Ethics Committee of Hainan Provincial Education Centre for Ecology and Environment, Hainan Normal University (permit no. HNECEE-2011-001).

Data accessibility Data used in this study are available in Electronic Supplementary Materials.

Competing interests The authors declare that they have no competing interests.

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286 **References**

287 Apel KM, Weise CM. 1986. The hiss-display of nesting black-capped
288 chickadees in captivity. Wilson Bulletin, 98: 320–321.
289 Broughton RK. 2005. Hissing display of incubating marsh tit and antipredator
290 response of the young. British Birds, 98: 267–268.
291 Broughton RK. 2012. Nest defence behaviour by marsh tits in response to a
292 stuffed weasel. British Birds, 105: 39–40.
293 Burger SJ. 1992. Differential responses of tropical roseate terns to aerial
294 intruders throughout the nesting cycle. Condor, 94: 712–719.
295 Cox AHM. 1930. Nestling wood-warblers "hissing". British Birds, 23: 219–220.
296 Cramp S, Perrins CM. 1994. The birds of the western Palearctic. Oxford
297 University Press, New York.
298 Crisologo TL, Bonter DN. 2017. Defending the weak: Parental defense peaks
299 when chick vulnerability is greatest in the herring gull (*Larus argentatus*).
300 Ethology, 123: 113–122.
301 Czeszczewik D. 2004. Breeding success and timing of the pied flycatcher
302 *Ficedula hyploeuca* nesting in natural holes and nest-boxes in the
303 Białowieża forest, Poland. Acta Ornithology, 39: 15–20.
304 Daniela C, Sealy SG. 2018. Evolutionary significance of antiparasite,
305 antipredator and learning phenotypes of avian nest defence. Scientific
306 Reports, 8: 10569.

307 Du Y, Huo Y, Wan D, Sun J, Lv Y, Cao J. 2010. Moss selection as nest
308 materials by *Parus varius*. Chinese Journal of Zoology, 45: 144–149.
309 Fasanella M, Fernández GJ. 2009. Alarm calls of the southern house wren
310 *Troglodytes musculus*: Variation with nesting stage and predator model.
311 Journal of Ornithology, 150: 853–863.
312 Fontaine JJ, Martin TE. 2006. Parent birds assess nest predation risk and adjust
313 their reproductive strategies. Ecology Letters, 9: 428–434.
314 Forstmeier W, Weiss I. 2004. Adaptive plasticity in nest-site selection in
315 response to changing predation risk. Oikos, 104: 487–499.
316 Fu Y, Chen B, Dowell SD, Zhang Z. 2016. Nest predators, nest-site selection
317 and nest success of the Emei Shan liocichla (*Liocichla omeiensis*), a
318 vulnerable babbler endemic to southwestern China. Avian Research, 7: 18.
319 Fuong H, Keeley KN, Bulut Y, Blumstein DT. 2014. Heterospecific alarm
320 calleavesdropping in nonvocal, white-bellied copper-striped skinks, *Emoia*
321 *cyanura*. Animal Behaviour, 95: 129–35.
322 Gaul AT. 1952. Audio mimicry: An adjunct to color mimicry. Psyche, 59: 82–
323 83.
324 Gill SA, Bierema AMK. 2013. On the meaning of alarm calls: A review of
325 functional reference in avian alarm calling. Ethology, 119: 449–461.
326 Gosler A, Clement P. 2007. Family Paridae (tits and chickadees). In del Hoyo J,
327 Elliott A, Christie D (eds). Handbook of the birds of the world. Vol. 12:
328 Picathartes to tits and chickadees. Pp. 662–709. Lynx Edicions, Barcelona.

329 Guppy M, Guppy S, Marchant R, Priddel D, Carlile N, Fullagar P. 2017. Nest
330 predation of woodland birds in south-east Australia: Importance of
331 unexpected predators. Emu, 117: 92–96.
332 Hinde RA. 1952. The behaviour of the great tit (*Parus major*) and some other
333 related species. Behaviour, 2 (Suppl.): 1–201.
334 Kelly LA, Coe RL, Madden JR, Healy SD. 2008. Vocal mimicry in songbirds.
335 Animal Behaviour, 76: 521–528.
336 Kitchen D, Bergman T, Cheney D, Nicholson J. 2010. Comparing responses of
337 four ungulate species to playbacks of baboon alarm calls. Animal Cognition,
338 13: 861–870.
339 Klump GM, Shalter MD. 1984. Acoustic behaviour of birds and mammals in
340 the predator context; I. Factors affecting the structure of alarm signals. II.
341 The functional significance and evolution of alarm signals. Zeitschrift für
342 Tierpsychologie, 66: 189–226.
343 Koosa K, Tilgar V. 2016. Is hissing behaviour of incubating great tits related to
344 reproductive investment in the wild? Acta Ethologica, 19: 173–180.
345 Krams I, Krama T, Berzins A, Rantala MJ. 2010. The risk of predation favors
346 cooperation among breeding prey. Communicative and Integrative Biology,
347 3: 243–244.
348 Krams I, Vrublevska J, Koosa K, Krama T, Mierauskas P, Rantala MJ, Tilgar V.
349 2014. Hissing calls improve survival in incubating female great tits (*Parus*
350 *major*). Acta Ethologica, 17: 83–88.

Lima SL. 2009. Predators and the breeding bird: behavioral and reproductive flexibility under the risk of predation. Biological Reviews, 84: 485–513.

Lima SL, Rattenborg NS, Lesku JA, Amlaner CJ. 2005. Sleeping under the risk of predation. Animal Behaviour, 70: 723–736.

Liu J, Ma L, Zhang Z, Gu D, Wang J, Li J, Gao L, Hou J. 2017. Maximum frequency of songs reflects body size among male dusky warblers *Phylloscopus fuscatus* (Passeriformes: Phylloscopidae). European Zoological Journal, 84: 186–192.

Martin TE. 1995. Avian life history evolution in relation to nest sites, nest predation, and food. Ecological Monograph, 65: 101–127.

Maziarz M, Piggott C, Burgess M. 2018. Predator recognition and differential behavioural responses of adult wood warblers *Phylloscopus sibilatrix*. Acta Ethologica, 21: 13–20.

Maziarz M, Wesołowski T, Hebda G, Cholewa M, Broughton RK. 2016. Breeding success of the great tit *Parus major* in relation to attributes of natural nest cavities in a primeval forest. Journal of Ornithology, 157: 343–354.

Odum EP. 1942. Annual cycle of the black-capped chickadee. Auk, 59: 499–531.

Owings DH, Rowe MP, Rundus AS. 2002. The rattling sound of rattlesnakes (*Crotalus viridis*) as a communicative resource for ground squirrels

(*Spermophilus beecheyi*) and burrowing owls (*Athene cunicularia*). Journal of Comparative Psychology, 116: 197–205.

Parejo D, Avilés JM, Peña A, Sánchez L, Ruano F, Zamora-Muñoz C, Martín-Vivaldi M. 2013. Armed rollers: Does nestling's vomit function as a defence against predators? PLoS One, 8: e68862.

Päckert M, Martens J, Eck S, Nazarenko AA, Valchuk OP, Petri B, Veith M. 2005. The great tit (*Parus major*): A misclassified ring species. Biological Journal of the Linnean Society, 86: 153–174.

Perrins CM. 1979. British tits. Collins, London.

Pickens AL. 1928. Auditory protective mimicry of the chicadee. Auk, 45: 302–304.

Pipia A, Ciuti S, Grignolio S, Luchetti S, Madau R, Apollonio M. 2009. Effect of predation risk on grouping pattern and whistling behaviour in a wild mouflon *Ovis aries* population. Acta Theriologica, 54: 77–86.

Ricklefs RE. 1969. An analysis of nesting mortality in birds. Smithsonian Contributions to Zoology, 9: 1–48.

Ritchison SG. 1993. The nest defense behavior of eastern screech-owls: Effects of nest stage, sex, nest type and predator location. Condor, 95: 288–296.

Rowe MP, Coss RG, Owings DH. 1986. Rattlesnake rattles and burrowing owl hisses: A case of acoustic Batesian mimicry. Ethology, 72: 53–71.

Sherman PW. 1977. Nepotism and the evolution of alarm calls. Science, 197: 1246-53.

394 Sibley CG. 1955. Behavioral mimicry in the titmice (Paridae) and certain other
395 birds. Wilson Bulletin, 67: 128–132.
396 Suzuki TN. 2011. Parental alarm calls warn nestlings about different predatory
397 threats. Current Biology, 21: R15–R16.
398 Suzuki TN. 2014. Communication about predator type by a bird using discrete,
399 graded and combinatorial variation in alarm calls. Animal Behaviour, 87:
400 59–65.
401 Suzuki TN. 2015. Assessment of predation risk through referential
402 communication in incubating birds. Scientific Reports, 5: 10239.
403 Tilgar V, Moks K. 2015. Increased risk of predation increases mobbing
404 intensity in tropical birds of French Guiana. Journal of Tropical Ecology, 31:
405 243–250.
406 Townsend SW, Charlton BD, Manser MB. 2014. Acoustic cues to identity and
407 predator context in meerkat barks. Animal Behaviour, 94: 143–149.
408 Townsend SW, Manser MB. 2013. Functionally referential communication in
409 mammals: The past, present and the future. Ethology, 119: 1–11.
410 Walankiewicz W. 2002. Nest predation as a limiting factor to the breeding
411 population size of the collared flycatcher *Ficedula albicollis* in the
412 Białowieża National Park (NE Poland). Acta Ornithology, 37: 91–106.

413 Weatherhead PJ, Blouin-Demers G. 2004. Understanding avian nest predation:
414 Why ornithologists should study snakes. Journal of Avian Biology, 35:
415 185–190.
416 Wesołowski T. 2002. Antipredator adaptations in nesting marsh tits *Parus*
417 *palustris*: the role of nest site security. Ibis, 144: 593–601.
418 Wesołowski T, Rowiński P. 2012. The breeding performance of blue tits
419 *Cyanistes caeruleus* in relation to the attributes of natural holes in a
420 primeval forest. Bird Study, 59: 437–448.
421 Yorzinski JL, Platt ML. 2012. The difference between night and day:
422 antipredator behavior in birds. Journal of Ethology, 30: 211–218.
423 Yorzinski JL, Vehrencamp SL. 2009. The effect of predator type and danger
424 level on the mob calls of the American crow. Condor, 111: 159–168.
425 Zub K, Czeszczewik D, Ruczyński I, Kapusta A, Walankiewicz W. 2017.
426 Silence is not golden: The hissing calls of tits affect the behaviour of a nest
427 predator. Behavioral Ecology and Sociobiology, 71: 79.
428 Zuberbühler K. 2009. Survivor signals: The biology and psychology of animal
429 alarm calling. Advance in the Study of Behaviour, 40: 277–322.
430 Zheng G. 2017. A checklist on the classification and distribution of the birds of
431 China (Third Edition). Science Press, Beijing.

Legends to figures

Figure 1. Study areas and study species in this study. Capital letters (A-F) refer to bird species and lowercase letters (a-f) refer to its nest and eggs. A and D refer to cinereous tit; B coal tit; C willow tit; E varied tit; and F marsh tit.

Figure 2. Proportion of hissing individuals in five tit species at two study sites.

Figure 3. Comparison of nesting success (% eggs that resulted in fledged young) between individuals with and without hissing call in cinereous and varied tits.

Highlights

- Many tit species in the Paridae family give a hissing call for nest defense, but there are few studies on inter-specific variation in hissing calls and whether this variation is ~~these are~~ related to nest predation and nesting success.

- This study showed that there is inter-specific variation in hissing calls of five tit species in China that breed in nest boxes distributed within the same area, but this variation~~these~~ is~~are~~ not significantly correlated with nest predation risk and nesting success.