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Ambre Salis, Thierry Lengagne, Jean-Paul Léna, Mylène Dutour. Biological conclusions about importance of order in mobbing calls vary with the reproductive context in Great Tits ( *Parus major* ). *Ibis*, 2021, 163 (3), pp.834-844. 10.1111/ibi.12940 . hal-03250443

**HAL Id: hal-03250443**

**<https://univ-lyon1.hal.science/hal-03250443>**

Submitted on 26 Nov 2021

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**Biological conclusions about importance of order in mobbing calls vary  
with the reproductive context in Great Tits *Parus major***

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Currently, there is considerable debate surrounding the presence of some human language specific characteristics in non-human animals, such as the use of compositional syntax (i.e., meaning of a sequence determined both by meaning of its individual parts and in the way they are combined). Compositional syntax has been investigated in mobbing calls of two closely related tit species, the Japanese Tit *Parus minor* and the Great Tit *Parus major*, but with one contrasting result: hearing calls in the reversed order diminished the behavioural responses of Japanese Tits, however only partially those of Great Tits. This difference may have been due to an external factor such as the season in which the experiment was undertaken, as the Japanese Tits were tested in winter and Great Tits in spring. Here, we studied the responses of Great Tits towards natural and reversed mobbing sequences during spring and winter by investigating two behaviours: approaching and vigilance behaviours. We found that sensitivity to syntax reversal was impacted by the season. The birds were vigilant but less likely to approach reversed calls in winter. However the opposite occurred in spring, with the birds scanning less but still approaching. This study suggests that the perception of combinatorial calls in Great Tits is influenced by the season, emphasizing the importance of context in studies investigating complex cognitive processing in animals.

**Keywords:** Acoustic communication, Alarm call, Compositionality, Mobbing Behaviour,

*Paridae*

Animal communication is one of the most prolific subjects in animal behaviour studies (Bradbury & Vehrencamp 2011), with one major question being the extent to which we can compare it to human language. Some defining characteristics of human language are classically presented as intentionality, referentiality, vocal learning, and syntax (Hauser *et al.* 2002). Numerous comparative studies conducted in animal systems have focused on the first three criteria - intentionality (Seyfarth & Cheney 2003, Graham *et al.* 2019), referentiality (e.g., in Siberian Jays *Perisoreus infaustus*, Griesser 2008), and vocal learning (e.g., song in oscines, Wilbrecht & Nottebohm 2003, Tyack 2019) - and have revealed that these abilities are present in diverse animal species, emphasizing the importance of studying animal communication in our understanding of the evolution of language (Fishbein *et al.* 2019). Indeed, finding such characteristics in our closest relatives may indicate a gradual emergence of language features (Searcy 2019). In contrast, the presence of analogous traits in distant species suggests convergence: the independent rise of the same evolutionary strategy (Searcy 2019).

Birds are one great example of distant taxa possessing characteristics similar to human language, especially regarding complex combinatorial rules (i.e., a set of principles by which meaning-bearing units can be combined into well-formed complexes, Zuberbühler 2019a). However, previous findings have focused on sequences lacking direct semantic content, with many combinatorial forms being phonocoding (i.e., sound combinations whose individual parts do not possess proper meaning, Engesser & Townsend 2019). Numerous examples of such combinatorial rules can be found in bird songs. For instance, while information about male quality is extracted from the global song, the individual syllables that make up the song do not carry specific information (Catchpole & Slater 1995).

Recently, studies have begun to discover various forms of semantic combinatoriality in animals, with some recent research focusing on compositional syntax (Engesser &

Townsend 2019, Suzuki *et al.* 2019b, Zuberbühler 2019b). This is observed when, in opposition to phonocoding, the meaning of the whole depends on the meanings of the component parts, and the way they are organized (Hurford 2011). Cases where animals use such compositional structuring have been reported in birds when mobbing predators (Engesser *et al.* 2016, Suzuki *et al.* 2016, Suzuki *et al.* 2017).

Mobbing - when a prey moves towards and harasses a predator to chase it away (Carlson *et al.* 2018) - is a suitable behaviour to study compositional syntax. Indeed, prey produce calls to recruit mobbers from a variety of species (Hurd 1996, Randler & Vollmer 2013, Dutour *et al.* 2016). In such risky situations (Curio & Regelman 1986), where callers and receivers have a level of shared interest, and communication should be clear and unambiguous to co-ordinate a response that combines several behaviours, it has been suggested that compositional syntax could emerge (Griesser *et al.* 2018). Experimental studies in the Japanese Tit *Parus minor* provide the strongest case of compositional syntax to date (Suzuki *et al.* 2016, Suzuki *et al.* 2017, but see also Engesser *et al.* 2016 on Southern Pied Babblers *Turdoides bicolor*). Indeed, Suzuki and colleagues demonstrated that the mobbing sequences of Japanese Tits are made through the concatenation of two types of notes - Frequency modulated elements (FME) and D notes (FME-D calls), respectively triggering a vigilance and an approach behaviour - and that order has importance (Suzuki *et al.* 2016). Debates about these results have been profuse (Bolhuis *et al.* 2018a, 2018b, Suzuki *et al.* 2018, Townsend *et al.* 2018), and there is a need for broader data on potential compositionality in birds.

Dutour *et al.* (2019b) replicated the same experiment on a French population of Great Tits *Parus major*, during the breeding season. This species is the closest relative of the Japanese Tit (Päckert *et al.* 2005, Johansson *et al.* 2013) and produces similarly structured mobbing calls (FME-D calls, Fig. 1). For both species, the FME motif appears to be used in

79 vigilance situations and serves as an alarm signal to which receivers respond by scanning for  
80 danger (Suzuki *et al.* 2016, Dutour *et al.* 2019b). The D notes are produced within foraging  
81 flocks and by nest mates to recruit social partners and elicit an approach by receivers  
82 (recruitment calls, Suzuki *et al.* 2016, Dutour *et al.* 2019b). Therefore, these acoustically  
83 distinct calls fulfil the first characteristic of compositionality (i.e., each different part has  
84 meaning when produced in isolation). In addition, Great Tits showed excitement signs (wing  
85 flicking and body swinging) only toward the combined FME-D calls, and never toward the  
86 isolated parts, indicating a meaning more complex than the simple sum of the isolated parts  
87 (Salis *et al.* 2020). The second characteristic of compositional structuring reflects the  
88 importance of organization, tested through measuring the Great Tits' response when the  
89 sequence is reversed (D-FME, demonstrated in the Japanese Tit, Suzuki *et al.* 2016). Here  
90 however, almost as many Great Tits approached the speaker to natural sequence (FME-D)  
91 than to reversed sequence (D-FME, Dutour *et al.* 2019b). Therefore, the conclusion on syntax  
92 use in Great Tits suggested it may not be as clear-cut as for the Japanese Tit and may globally  
93 weaken a generalization of syntax results to other species in the genus.

94         One crucial distinction between the studies of Japanese Tits and Great Tits, however,  
95 is that the response of Japanese Tits to the inverted sequence was tested during the non-  
96 breeding season in winter flocks (Suzuki *et al.* 2016), while these tests were done in a  
97 reproductive context at the onset of the breeding season for the Great Tits (Dutour *et al.*  
98 2019b). The behaviour of Parids is known to vary markedly between seasons. When breeding,  
99 they show enhanced individual territoriality and aggressive behaviour (Shedd 1983, Krams  
100 and Krama 2002, Samplonius 2018). In contrast, they join other individuals and form flocks  
101 in winter (Hinde 1952, Marra *et al.* 2015). In addition, Great tits approached more  
102 heterospecific calls in winter than in the breeding season (Dutour *et al.* 2019a). The attention  
103 paid to the same auditory stimuli therefore seems to be dependent on the reproductive state of

the birds. We can hypothesize that increased aggressiveness toward intruders in spring may directly affect the behavioural response recorded during syntax-related studies (i.e., approach and scanning, Suzuki *et al.* 2016). In addition, sensibility to conspecific D notes may increase in spring, as pairs use them frequently, and fledglings use similar notes when calling their parents (M.D. pers.obs). In the same way, an increased response toward heterospecific calls (even towards unknown ones, Dutour *et al.* 2017b) in winter potentially indicates an increased attention towards new calls. As a consequence, different biological conclusions could result from the same experiment done in different parts of the life cycle, even if the general syntax-processing ability of the bird remain the same. On a more global scale, determining whether context can impact conclusions on syntax use in Great Tits could be of value in influencing future studies investigating complex language features in animals.

Here, we used field playback experiments to examine whether season has an impact on sensitivity to syntax reversion in Great Tits, allowing us to understand the differences found in the two studies investigating the use of syntax in the family Paridae. Specifically, we recorded the behavioural response of Great tits to either naturally ordered calls, reversed calls, or to a control (background noise) in two seasons: in spring, when territoriality is increased, compared to winter, when individuals join other species in flocks.

## **METHODS**

### **Study sites and experimental design**

Data were collected at the start of the breeding season when individual territories are established (February/ March 2018, territories are established in February and defended until June in the study population) and during the winter when tits often form mixed-species flocks (November 2018/ January 2019, Hinde 1952), in the north of Lyon, France.

The ecological context of the two seasons is different. In spring, Great Tits invest mainly in breeding (i.e., defending a territory, building a nest, rearing a brood of nestlings, Cramp *et al.* 1993). The defence of the nest against any intruder is intense and well documented (Hinde 1952, Hollander *et al.* 2008). As winter arrives, Great Tits join small conspecific groups and often heterospecific flocks (Carlson *et al.* 2020). Even if some dominance relationship remains (Oberski & Wilson 1991), flocks can combine, and space is divided into undefended overlapping home ranges rather than discrete territories (Ekman 1989). Flocks are thought to be an efficient behavioural response toward predator and foraging pressures being different in winter (Sridhar *et al.* 2009). Indeed, increased food storage to counteract the loss of food supply increases predation risk (increase of body mass being negatively correlated with manoeuvrability, Gosler *et al.* 1995), while a decrease in individual territory defence whilst not breeding allows birds to gather without strong competition (Morse 1970).

In order to examine whether the response of Great Tits was dependent upon season, we conducted three playback types. First, we examined whether tits hearing the combined motifs in the natural order (FME-D calls, natural mobbing call sequence) displayed a combination of the behaviours classically monitored in mobbing studies (approach and scan, see below for more details), and we tested whether this was also the case when the combined motifs were presented in the reverse order (D-FME calls, artificially reversed mobbing call sequence). Finally, we also performed control tests for which the playback contained only background noise (hereafter referred as BN tests). We conducted these tests on 120 adult Great Tits (20 individuals for each call sequence, at two different seasons).

## Field test procedure

Each test was conducted by two field assistants. One was assigned to the soundtrack preparation and playback operation, while the other was assigned to the observation of the focal bird. After the localization of a focal bird, the loudspeaker was placed 30 m away from the bird at the bottom of a tree. Both field assistants were positioned opposite each other at vantage points at least 15 m from the loudspeaker and the focal bird to avoid any perturbation during the test. Prior to playback, the baseline behaviour of the focal bird was observed for at least a one-minute pre-trial period. The playback was started when there was no other passerine observed near the focal individual. If the bird showed alarm behaviour before the test (e.g., gave mobbing calls), or if other birds also responded to the playback, the test was abandoned. The sequence was broadcast using a remotely controlled Shopinnov 20 W loudspeaker with an amplitude of  $\sim 80 \pm 3$  dB(A) (measured at 1 m from the loudspeaker using Lutron SL-4001, Taipei, Taiwan; C weighting, slow settings, re: 20  $\mu$ Pa), as this is the natural amplitude of Great Tit mobbing calls (from 77 to 80 dB, Templeton *et al.* 2016). The background noise observed in the field (mean  $\pm$  sd =  $46.1 \pm 2.1$  dB(C),  $n = 20$  measurements) associated to the spherical spread and excess attenuation of sound energy radiating from a source in a deciduous forest (Lengagne & Slater 2002) allowed us to estimate that the broadcast signal reached background noise level at 60-70 m distance.

Subsequently, during one minute of playback treatment, two behavioural variables were recorded, respectively, (1) the vigilance effort with the number of horizontal scans (we counted the number of obvious movements that birds made with their heads from left to right or right to left ( $\sim 180^\circ$  turn, Suzuki *et al.* 2016) and (2) approach within 15 m of the loudspeaker (Dutour *et al.* 2017b). Scanning for threats is a stereotyped agitation behaviour (Curio 1975, 1978) commonly measured in studies of mobbing response of tits (e.g., Suzuki *et al.* 2016, Carlson *et al.* 2017, Suzuki *et al.* 2017). Both dense vegetation and the small size

of these passerine birds prevented a detailed measurement of scanning behaviour using video-recording. These behaviours were thus directly counted an observer using binoculars and reported on a digital audio recorder. To minimize the measurement errors and to make scanning as objective a behaviour as is possible, we considered an individual to scan only if the head movements of the bird from right to left or left to right were obvious. These methods were unable to completely eliminate the potential for unconscious observer bias and we acknowledge that future studies using similar techniques should ideally employ fully blinded designs.

All trials were conducted between 06:00 and 13:00 h during calm and dry weather. On average 10 tests were done each day. Selection of all focal animals was based on location, ensuring successively tested animals were always separated by more than 100 m to minimize pseudoreplication risk and ensure that tested birds had not heard a previous test, given that the active space of a sound emitted at 80 dBA with a 46 dB background noise level was less than 100 m (Brenowitz 1982, Lengagne & Slater 2002).

In addition, we never returned to the same forest path, the size of the research area was large (i.e.  $\sim 43 \text{ km}^2$ ), and population density in this area is high (M.D. unpubl. data). No bird was seen following us. Hence, although birds were not individually ringed, the probability of testing the same individual twice was low and we are confident that our observations were performed on different individuals.

### **Playback stimuli and materials**

We used mobbing calls produced by three Great Tits, previously recorded in response to intraspecific mobbing calls (Dutour *et al.* 2017b). Calls were recorded with a Fostex FR2LE digital recorder (Tokyo, Japan) connected to a Sennheiser ME67-K6 microphone (Wedemark, Germany; see Dutour *et al.* 2017b for more details). We also used mobbing calls obtained

from the Xeno Canto online database ([www.xeno-canto.org](http://www.xeno-canto.org)) recorded in different European countries ( $N = 7$ ). By controlling several factors when constructing our playbacks (see below), we homogenised our stimuli and reduced potential variation between different geographical areas. We recorded or selected good quality (sampling rate: 44.1 kHz; sample size: 16-bits) recordings, and all playback files were saved as .wav files.

From these recording files, we built 20 unique soundtracks of natural mobbing calls (i.e., 20 soundtracks with one individual per soundtrack) using Avisoft-SASLab software (Avisoft Bioacoustics, Glienicke, Germany, one sample of spectrogram and audio file available in Sup.Mat.). These soundtracks were then used to construct the D-FME (i.e. reversed) calls by reversing the motifs order of the original FME-D calls of Great Tits. The same donor individuals were therefore used for both the natural and reversed call sequences. Within each soundtrack, calls were repeated at a rate of 26 calls per minute (characteristics within the range of the natural repetition rates, Dutour *et al.* 2019b). We used a series of five to eight D motifs to construct playback (mean  $\pm$  se =  $7.11 \pm 0.06$ ). In each track, D notes were placed  $82 \pm 40$  ms before FME (no difference with time between FME and D notes in the natural sequence;  $t$ -test = 1.79,  $P > 0.05$ ). All playback sequences were arranged to control the ratio of the D notes over the FME notes in a mobbing call sequence, as note composition is known to alter Great Tits' response (Kalb & Randler 2019). We also constructed 20 control soundtracks using sections where no birds were calling in the same recordings as natural mobbing calls (BN). In order to avoid pseudoreplication (Kroodsma *et al.* 1989), each soundtrack was played back only once.

## Statistical analysis

All analyses were carried out in R Studio 1.2.5033 (R core development team 2018) using generalized linear mixed models (GLMM, *glmer* package *lme4*). Because the same

soundtracks were used in both seasons, soundtrack ID was included as a random effect. To analyse the number of horizontal scans, we used a Poisson error distribution, and a log-link function as no overdispersion was detected (*overdisp.glmer*, package *RVAideMemoire*). We also included the actual time that the bird had been seen during the one-minute trial as offset ( $57.48 \pm 6.36$  sec, mean  $\pm$  sd) and discarded three individuals (all in the spring-natural order call sequence) for which the time was under 20 sec (i.e., sample size for each treatment is 20, except for the spring-natural call sequences with  $n = 17$ ). Approaching behaviour (approaching vs. not approaching, where 1 = response, 0 = no response) was analysed using a binomial distribution for the error term.

For both behavioural variables, the same three steps of analysis were carried out: (1) we first ran a model with the three treatments (BN, Natural, and Reverse) at each season. We then extracted the estimated marginal means and pairwise comparisons with related effect sizes (package *emmeans*, function *emmeans*, and package *multcomp*, function *multcomp::cld*). We only focused on five comparisons: in each season, the BN treatment versus the two other treatments, and the difference between the BN treatment in spring and in winter. We therefore used Bonferroni correction with  $\alpha = 0.05/5 = 0.01$ . (2) Secondly, we discarded the BN tests and ran a model only with Natural and Reversed treatments in order to test the significance of the interaction term (Season\* Treatment, *Anova* function, package *car*). This term indicates whether the difference between the two treatments is different between seasons. (3) Finally, we reported the effect sizes of the difference between Natural and Reversed treatments for the two seasons (two comparisons:  $\alpha = 0.025$ ). The pairwise comparisons and the related effect sizes of the differences were assessed using odds ratio (hereafter OR, *odds.ratio*, package *questionr*) for the approach behaviour, and with the exponential effect size for the scanning behaviour (package *emmeans* and *multcompview*).

Finally, we also verified that approaching behaviour was not correlated to scanning behaviour using Wilcoxon tests for each season and each playback type. As revealed by these analyses, no association between these two behaviours was detected (all  $P > 0.05$ ).

## RESULTS

### Scanning Behaviour

In both seasons, Great Tits scanned less toward the control treatment (BN = background noise) than toward the two other call sequences (all  $P < 0.001$ , Fig. 2, Table 1a), except for the reversed call sequences in spring ( $P = 0.89$ , Table 1a). There was no significant difference in scan number between seasons for BN soundtracks ( $P = 0.03$ ,  $\alpha = 0.01$ , Table 1a).

The interaction between treatment and season was statistically significant when the control tests were discarded (Table 1b), with a substantially larger difference between acoustic treatments in spring than in winter. Indeed, Great Tits produced 2.29 more scans toward natural calls than toward reversed calls in spring (Table 1c, Fig. 2), while there was no such difference between treatments in winter (ratio of 1.3, Table 1c, Fig. 2). The treatment term was also statistically significant, indicating an overall reduced response to reversed calls ( $P < 0.001$ , Table 1b).

### Approaching behaviour

Respectively 50% and 65% of Great Tits approached natural calls in spring and winter. In contrast, only 5% of Great tits in spring, and none of them in winter, approached toward the controls (Fig 3). The difference between natural calls and controls was statistically significant in both seasons (Table 1a). In contrast, no statically significant difference in probability of approach was detected between reversed calls and controls (35% in spring, 25% in winter, Fig 3, Table 1a). There was almost no approach exhibited to control playbacks and

so the difference between controls in spring and in winter was not significant ( $P = 0.49$ , Table 1a).

The interaction between treatment and season was not statistically significant in our model ( $P = 0.25$ , Fig 3, Table 1b), but the treatment term was statistically significant (more approach to the natural treatment,  $P = 0.02$ , Fig 3). Nonetheless, even though no interaction was detected, we found a statistically significant difference between reversed and natural playback in winter (OR = 5.57, Table 1c), but not in spring (OR = 1.86), indicating that Great Tits reduced their response toward reversed calls in winter, but approached both treatments similarly in spring.

## DISCUSSION

Great Tits reduced their overall behavioural response toward reversed calls compared to natural ones. However, season affected the strength of that effect. Great Tits strongly reduced their scanning behaviour when hearing reversed calls compared to natural calls in spring, but such a reduction was not statistically significant in winter. In contrast, for the approach behaviour, the difference between natural and reversed call sequences was statistically significant in winter, but not in spring. The compositional syntax hypothesis requires a decrease in behavioural responses (i.e., scanning and approaching) toward reversed call sequences. Here, we demonstrate that testing birds in different seasons would have resulted in different biological conclusions about syntax-use ability in tits.

For the approach behaviour, the difference between natural and reversed calls was significant in winter, but not in spring. Importantly however, the interaction in our model was not statistically significant. This may be due to the response variable being binary, thus limiting power to detect effects with the available sample sizes. Nonetheless, the fact that this

difference was strong in spring but not in winter is some evidence of an effect of season on the response to different treatments by Great Tits. The absence of variation in the probability of approach in spring is consistent with the results of Dutour *et al.* (2019b) in which the same protocol was used, and could therefore explain the difference with the study of Suzuki *et al.* (2016), in which birds were tested in winter. These results therefore suggest that season does affect Great Tit behavioural responses to natural and reversed playback.

Seasonal effects on responses to different syntactic forms has previously been demonstrated in birds. For example, a similar result was found by Clucas *et al.* (2004) investigating the vocal response of Carolina chickadees *Poecile carolinensis* when confronted with familiar (AAADDD) and unfamiliar order calls (ADADAD): birds discriminated the familiar from the unfamiliar calls in winter, but not in summer. Both Carolina Chickadees and Great Tits live in mixed species flocks during winter (Hinde 1952), which are thought to increase both foraging efficiency and defence against predators (Sridhar *et al.* 2009). Vigilance toward heterospecific and potentially unfamiliar calls may therefore increase in winter, as contact with heterospecifics with respect to food and alarm calls is common (Morse 1970, Clay *et al.* 2012). Corroborating this hypothesis, Great Tits increased their response toward heterospecifics in winter compared to spring (Dutour *et al.* 2019a). We can therefore hypothesize that in winter, birds are highly vigilant and more efficient in discriminating unfamiliar calls, consequently leading to high scanning behaviour and low approach behaviour toward artificial sequences. In contrast, during the breeding season, the response of Great Tits is probably linked to strong competition and reproductive investment expressed through increased aggression (Wingfield & Ramenofsky 1985). This leads to a more risk prone behaviour: approaching toward any calls similar to recruitment calls (i.e., calls with D notes, Kalb *et al.* 2019) without necessarily scanning. Further research is clearly required to test this hypothesis and to understand why the response to reversed ordering of the mobbing

call sequence varies according to the season. In particular, comparisons of the response to the isolated recruitment call (FME notes) and alarm call (D notes) according to the season is still lacking. Such experimental analysis is required to fully appreciate the underlying reason for scanning and approaching behaviours varying according to the season. Moreover, testing such a hypothesis could help understand whether during the breeding season, Great Tits are indeed more sensitive to recruitment calls, irrespective of the syntactic form.

Our findings reveal that in spring, we could not conclude that Great Tits reduced their approach behaviour when hearing reversed calls. On the opposite, the reduced scanning response was statistically significant in spring, but not in winter. Consequently, the same experiment done in different seasons would not have led to the same biological conclusions. Indeed, approaching and scanning more toward natural mobbing call sequences than toward reversed call sequences is an essential prerequisite for compositional syntax in birds (Suzuki *et al.* 2016). Concluding whether Great Tits do truly use syntax is consequently difficult: it is unlikely that high mental ability such as syntax use vary with the seasons; but we show that the external proof of it (the bird's behaviour) does. This may reflect a change of priorities in birds between seasons (heterospecific co-operation in winter versus high territorial defence in spring). In fact, such a problem may be common in language-related studies of animal behaviour (Graham *et al.* 2019). For example, deciphering whether chimpanzees *Pan troglodytes* possess a zero or second order intentionality level (i.e., when the sender does or does not intend to signal and attribute mental state to the receiver; Townsend *et al.* 2017) is dependent on aspects of predator stimuli (Crockford *et al.* 2012, Schel *et al.* 2013, Graham *et al.* 2019).

Even if the effect sizes of the differences varied between the two seasons, we found an overall reduced response in both scanning and approaching behaviours in response to the reversed mobbing calls. This is consistent with previous studies of bird syntax (Suzuki *et al.*

2016, 2017, Dutour *et al.* 2019b). Discussion of whether this reduced response does support the presence of compositional syntax in birds has been intense (see Bolhuis *et al.* 2018a, 2018b, Suzuki *et al.* 2018). Indeed, active use of compositional syntax by birds or simpler mechanisms such as the masking hypothesis (D notes put in the first place acoustically hiding the FME notes, Dutour *et al.* 2019b, Dutour *et al.* 2020) are still to be tested. We advocate for the monitoring of other fine behavioural cues such as wing flicking, tail flit or calling in order to provide a more reliable overview of the bird's internal state, (Cully & Ligon 1976, Carlson *et al.* 2017, Salis *et al.* 2020). Other lines of inquiry, for example in neurobiology, may complete field data and allow a broader view of birds' abilities (Marler 2004). Experiments on Black-capped Chickadees *Poecile atricapillus* brains have shown, for example, that the neural response (i.e., protein expression important for processing and categorizing conspecific vocalizations) increased with the level of threat conveyed in the calls (Avey *et al.* 2011). Consequently, going one step further by studying neural responses to different syntactic forms and thus, the neural mechanisms underlying the production and perception of call combinations could be investigated (Suzuki *et al.* 2019a).

In conclusion, context can affect the behavioural cues used to assess syntax understanding by Great Tits, and thus affect biological conclusions about syntax use in this species. Thorough work on the impact of context on animal behaviour could turn out to be of critical importance and directly impact future studies investigating high cognitive processing in non-human animals.

This work was supported by French Ministry of Research and Higher Education funding (to M.D. PhD grants 2015-2018). We are thankful to the Fondation Vérots for the authorization of fieldwork on their protected grounds. We are grateful to Sarah Walsh for English language checking. We also thank the three anonymous reviewers for helpful comments on our manuscript. Authors comply with The European Code of Conduct for Research Integrity. We have no conflict of interest to declare. The raw data used for the statistical analysis can be provided by A.S. upon request.

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## 536 **SUPPORTING INFORMATION**

537 **Figure 1.** Spectrograms of call treatment played to Great Tits. (a) FME-D calls and (b) D-  
538 FME calls, which are a reversed combination of FME and D calls. These calls were digitally  
539 edited using AvisoftSASLab Pro software.

540 **Table 1.** Overview of the playback stimuli used for the study. XC is the acronym for xeno-  
541 canto.

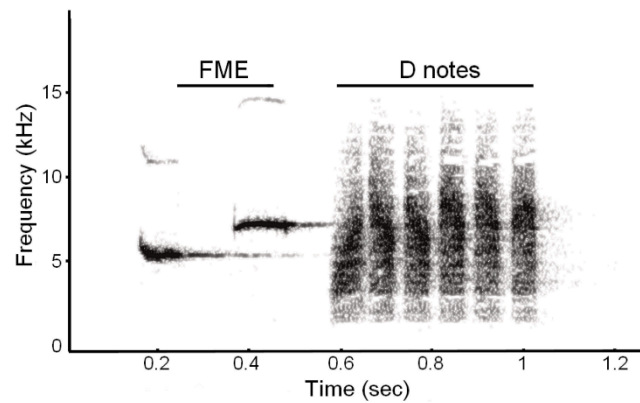
542

# TABLE

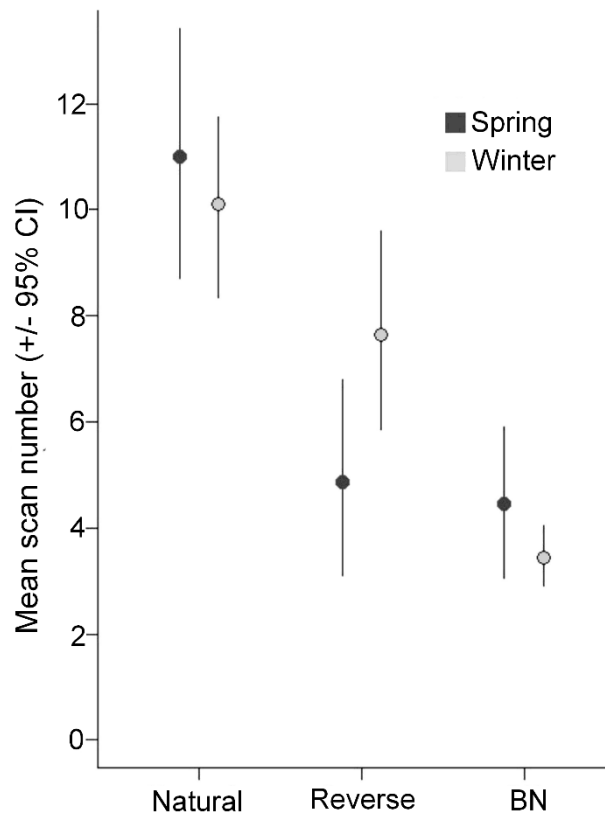
**Table 1.** Details of the pairwise comparisons (a, c) and model output (b). (a) Refers to the comparisons with the control (BN), (b) to the model in which control tests were discarded, and (c) to the comparison between natural and reversed calls in the two seasons studied. For scanning behaviour, the ratio (effect size) indicates the strength of the difference between the first and the second term (e.g., Great Tits scanned on average 1.44 times more toward background noise in spring than in winter). The same reasoning applies for the odds ratio (OR) for the approach behaviour. A GLMM with a Poisson error distribution was used for the number of scans (~180° horizontal head turns) during 1min. A GLMM with a binomial error distribution was used for approach behaviour. The pairwise comparisons were extracted from the model and *P*-values were corrected for multiple comparisons (Bonferroni correction:  $\alpha = 0.01$  for (a),  $\alpha = 0.05$  for (b), and  $\alpha = 0.025$  for (c) due to correction for multiple comparisons). se, standard error; *z*, *z* statistic; *P*, *p*-value (bold= statistically significant);  $\chi^2$ , Chi-squared test; df, degree of freedom; 95%CI, 95% confidence intervals.

| (a) Differences with control BN       | SCAN     |      |                   |                   | APPROACH |                 |             |              |
|---------------------------------------|----------|------|-------------------|-------------------|----------|-----------------|-------------|--------------|
|                                       | Ratio    | se   | <i>z</i>          | <i>P</i>          | OR       | 95%CI           | <i>z</i>    | <i>P</i>     |
| BN Winter- BN Spring                  | 1.44     | 0.24 | 2.2               | 0.03              | 3.15     | [0.12; 82.17]   | 0.69        | 0.49         |
| BN Winter- Natural Winter             | 3.13     | 0.53 | 6.8               | <b>&lt; 0.001</b> | 73.8     | [3.89; 1401.64] | 2.86        | <b>0.004</b> |
| BN Winter- Reversed Winter            | 2.41     | 0.41 | 5.11              | <b>&lt; 0.001</b> | 14.54    | [0.75; 283.38]  | 1.77        | 0.08         |
| BN Spring - Natural Spring            | 2.34     | 0.36 | 5.46              | <b>&lt; 0.001</b> | 19       | [2.12; 170.39]  | 2.63        | <b>0.008</b> |
| BN Spring- Reversed Spring            | 1.02     | 0.17 | 0.13              | 0.89              | 10.23    | [1.12; 93.35]   | 2.06        | 0.04         |
| (b) Model discarding BN tests         | $\chi^2$ | df   | <i>P</i>          |                   | $\chi^2$ | df              | <i>P</i>    |              |
| Season (Spring or Winter)             | 3.52     | 1    | 0.06              |                   | 0.05     | 1               | 0.82        |              |
| Treatment (Natural or Reversed)       | 18.26    | 1    | <b>&lt; 0.001</b> |                   | 5.64     | 1               | <b>0.02</b> |              |
| Season* Treatment                     | 11.49    | 1    | <b>&lt; 0.001</b> |                   | 1.33     | 1               | 0.25        |              |
| (c) Difference Natural-Reversed calls | Ratio    | se   | <i>z</i>          | <i>P</i>          | OR       | 95%CI           | <i>z</i>    | <i>P</i>     |
| Natural Winter- Reversed Winter       | 1.3      | 0.18 | 1.92              | 0.05              | 5.57     | [1.42; 21.86]   | 2.46        | <b>0.01</b>  |
| Natural Spring- Reversed Spring       | 2.29     | 0.35 | 5.45              | <b>&lt; 0.001</b> | 1.86     | [0.52; 6.61]    | 0.96        | 0.33         |

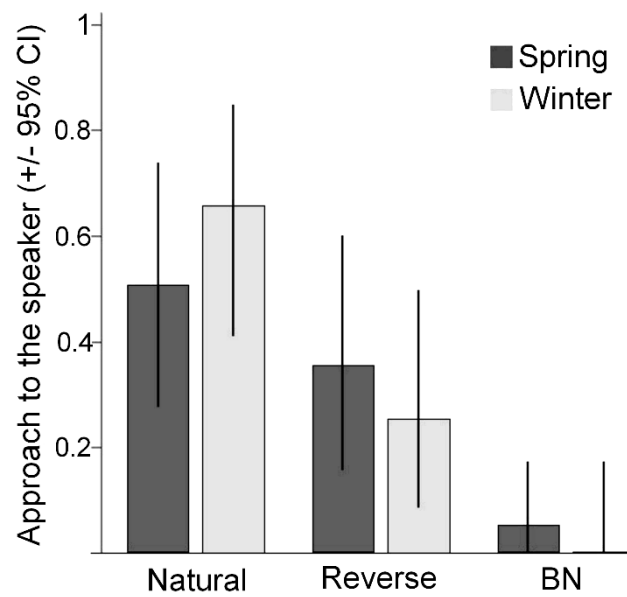
## FIGURES



**Figure 1.** Sonogram of a Great Tit mobbing call, comprising two distinct calls: (i) alert call (composed of Frequency Modulated Elements, FME) and (ii) recruitment call (a string of notes with broadband frequencies, D notes). This mobbing call was produced with Avisoft-SASLab.



**Figure 2.** Mean number of scans (horizontal head movement of  $\sim 180^\circ$ ) produced in 1 min by Great Tits as a function of the call sequence used and the Season ( $\pm 95\%$  boot-strapped Confidence Intervals). Great Tit mobbing calls in their natural order (FME-D) are compared to reversed calls (D-FME) and to Background Noise (BN). Statistical inference was made using confidence intervals: difference is statistically significant when less than half of the CI overlap (Cumming & Finch 2005, Cumming 2007).



**Figure 3.** Percentage of individuals ( $\pm$  95% CI) approaching the speaker as a function of the call sequence and the season. Great Tit mobbing calls in their natural order (FME-D) are compared to reversed calls (D-FME) and to Background Noise (BN). Statistical inference can be made using the confidence intervals: the difference is statistically significant when less than half of the CI overlap (Cumming & Finch 2005, Cumming 2007).