

RESEARCH ARTICLE

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No Effect of Note Order on the Response of Coal Tits to Conspecific, Heterospecific and Artificial Mobbing Calls

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ABSTRACT

Most Parid species produce specific, order-constrained mobbing calls. These calls elicit responses from both conspecifics and heterospecifics, with evidence indicating that such responses occur only when the calls are organised in this specific order. One notable exception is the coal tit (*Parus ater*), a species that employs similar types of notes, yet does not exhibit clear order constraints within its mobbing sequences. Despite this apparent absence of order constraints, a recent experiment has demonstrated that coal tits may be sensitive to the order of notes in heterospecific calls. Therefore, the relative significance of note order in conspecific and heterospecific communication among coal tits remains unclear. We conducted a playback experiment to examine the effects of note order (natural coal tit order, typical Parid order and reversed order) and species identity (conspecific, familiar heterospecific—the great tit, *Parus major*, or artificial notes) on coal tit mobbing responses. Our findings indicate that coal tits exhibited a strong response to conspecific calls, regardless of the order of the notes; conversely, they displayed little to no response to heterospecific calls and artificial notes, irrespective of note order. A similar pattern was observed when assessing the general community response. This unexpectedly low response to familiar heterospecific calls may be attributable to a reduced density of great tits in the area we tested: ecological factors, such as community composition, may influence heterospecific mobbing behaviours and the subsequent biological interpretations of playback experiments. This study also underscores the necessity of conducting comparative research on closely related species to evaluate the potential generality of findings, such as strong order constraints recently observed in great tits and Japanese tits.

1 | Introduction

Acoustic exchanges of information between different species are common, with impressive examples such as the

collaborative foraging for honey between wild honeyguide birds and several human groups around the Swahili Coast region of Africa (Spottiswoode et al. 2016). Another well studied example of an apparently cooperative, acoustic heterospecific

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communication is mobbing. Mobbing is an aggressive behaviour, often collective, in which individuals (often prey) harass a threat (often a predator), with the final goal of deterring the threat (Carlson and Griesser 2022; Curio 1978). In birds, heterospecific communication when mobbing is common and efficient in discouraging predators from future hunting attempts (Carlson et al. 2020; Dutour et al. 2017). While usually thought to be a by-product of conspecific communication, some evidence suggests that the heterospecific part of mobbing behaviour strongly influences the fitness of birds and involve complex audience effects (e.g., Krama et al. 2012; Coppinger et al. 2020). Even more notable is the ability of certain species to exchange precise information, such as the size of predators (Hetrick and Sieving 2012; Templeton and Greene 2007). The processes at play are beginning to be experimentally tested and understood. While some species learn species-specific calls and therefore adapt their responses to the local community (Carlson et al. 2017), other examples show that similarities in acoustic features (Fallow et al. 2011) and/or in syntactic organisation (Salis et al. 2023; Suzuki et al. 2017) can be used as strong cues for birds when responding to unknown calls. This order-based recognition system has also led to interesting parallels with syntactic abilities in birds and humans (Suzuki et al. 2019).

In tits and chickadees (Paridae family), heterospecific communication when mobbing is common and well-studied. Most Parids produce a specific mobbing call with a similar organisation: high frequency notes (hereafter F notes) followed by broadband, noisy elements (hereafter D notes, Hailman 1989). This organisation is so conserved that the FD call has been argued to date back to the ancestor of the whole clade, more than 20 million years ago (Salis et al., under review). There are only a few exceptions, such as the Coal tit, *Pariparus ater*, that also produce F and D notes, for the same purpose, but with varied orders (Hailman 1989; Salis et al. 2023). Recent studies have shown that the great tit and the Japanese tit, that produce FD calls, are able to respond to unfamiliar calls if these calls share the FD organisation (Dutour, Lengagne, and Léna 2019; Salis et al. 2021; Salis et al. 2023, Suzuki et al. 2017), but it is unclear if species that have not conserved the FD syntax, such as coal tits, would also be sensitive to this specific call organisation. It is possible that coal tits have lost the ability to recognise the organisation of the FD call, and consequently do not use order as a decoding feature when responding to mobbing calls. Yet, a recent experiment shows that when responding to great tits, *Parus major*, coal tits will respond to the FD call, but not to reversed D-F calls (Dutour, Lengagne, and Léna 2019). Coal tits may therefore recognise and focus on the FD call order, but this may be true only for heterospecific communication. If true, this would show that there is a decorrelation between production and conspecific perception (no order constraint), and heterospecific reception (responding only to FD calls) in coal tits. This could be because coal tits are sensitive to order constraints specifically on great tit calls (because they learned to recognise this specific call), or because there is something inherently more natural to the most common and ancient FD order, such that unfamiliar calls should a priori adhere to it. To disentangle these hypotheses, we should differentiate the response to (i) conspecific calls, (ii) familiar heterospecific

calls, and (iii) unfamiliar calls. Indeed, different processes may be at play when responding to different call origins. The recognition of conspecific calls is based on very fine-grained acoustic parameters, with decreased responses to small acoustic differences (Charrier and Sturdy 2005), while the response to heterospecific calls can often be triggered with unfamiliar, dissimilar calls (Hurd 1996; Salis et al. 2024). This suggests that the rules used when responding to conspecific or heterospecific calls may be different.

To explore this, we organised a playback experiment on wild, free-ranging coal tits. We created playbacks with different types of notes: coal tit notes (conspecific treatment), great tit notes (familiar heterospecific treatment) and artificial notes (synthetic notes that are known to trigger a mobbing response in other Paridae species). We organised these notes in three different orders, to test the importance of syntactic constraints on coal tits: the coal tits' natural organisation, the great tits' natural organisation (FD calls), and the unnatural DF organisation (reversed order, produced by none of the Parids, and, to our knowledge, none of the birds present in the community of coal tits) leading to 9 playback treatments (3 origins $\times$ 3 orders; Figure 1), to which we added a negative control with no mobbing calls.

Given our current knowledge regarding call syntax in Paridae, our predictions are that coal tits have different syntactic constraints depending on the origin of the calls. In conspecific interactions, we expect coal tits to respond to any type of note order, given that no ordering pattern has been found in their own natural note combinations. With familiar heterospecific calls, we expect them to respond to only the known and learned FD call. Finally, when confronted with unfamiliar, artificial combinations, we consider two hypotheses. First, coal tits may interpret artificial FD calls as calls from an unknown Parid, since this call organisation is present in all French Parid species (Salis 2022). They therefore may favour the typical Parid organisation compared to other call ordering. This would align with the response exhibited by great tits under the same protocol (Salis et al. 2024). Alternatively, coal tits may adhere to the decoding rules employed in conspecific contexts and thus respond to any combinations that contain F and D notes, with no preference for the FD syntax.

2 | Material and Methods

2.1 | Study Site and Species

The playback experiment detailed below was conducted in the Ariège department of France, characterised by mountainous terrain (tests were carried out at a minimum of 800m of altitude) and mixed deciduous-coniferous forests. The area boasts high densities of tits, particularly coal tits, which are, in some parts, monitored by a long-term scientific project since 2011. At one of the sites surveyed for our study, indicative of the environments selected for our fieldwork, about half of the occupied nest boxes were used by coal tits in 2023 (19 out of 35 occupied nest boxes) and about a third by great tits (13/35 occupied nest boxes). In this region, small birds often fall prey to various predators, including the Eurasian sparrowhawk (*Accipiter nisus*). During

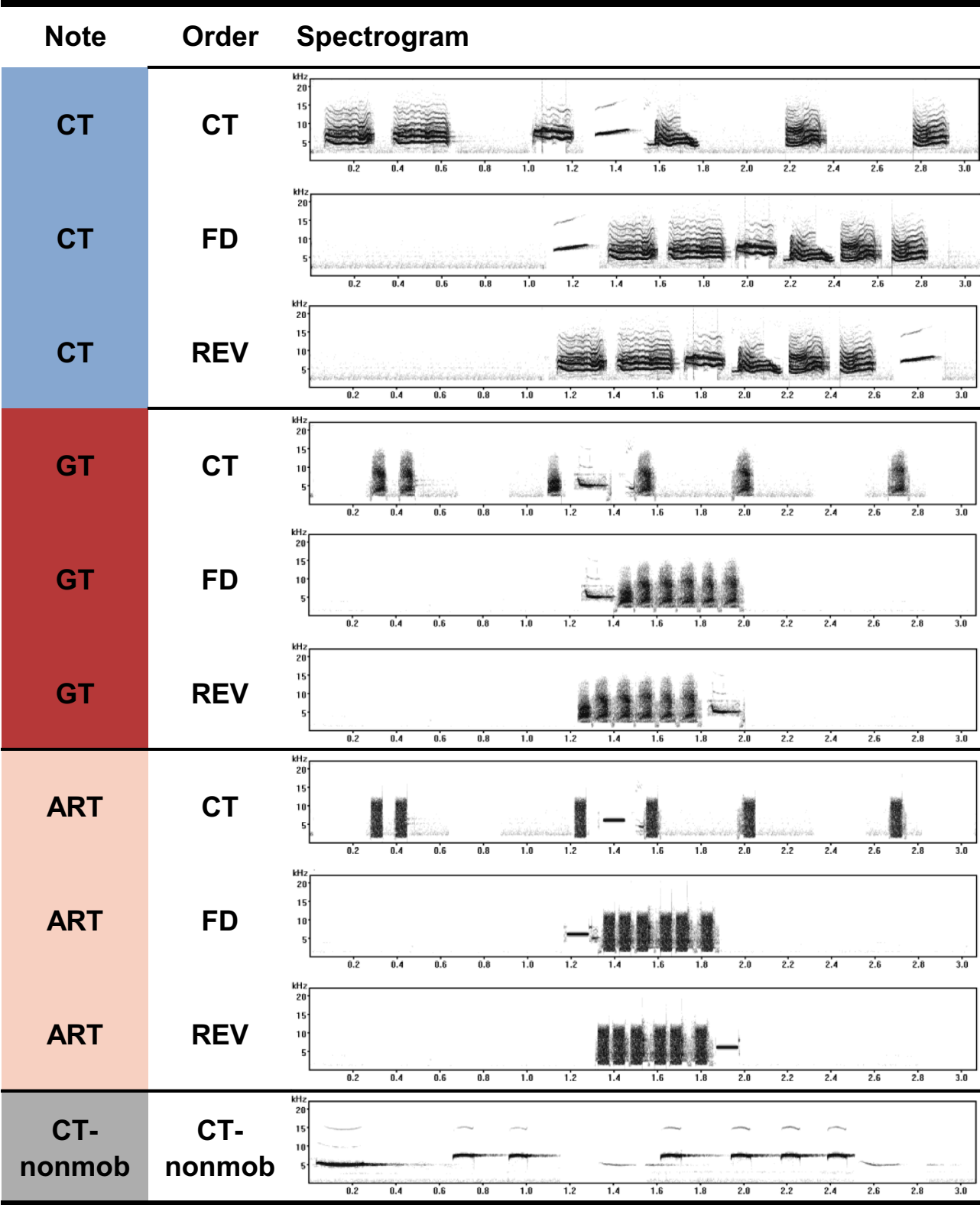


FIGURE 1 | An example of the 10 treatments used in the playback experiment. The nine first treatments are organised as follows: They either possess coal tit notes (CT), great tit notes (GT) or artificially created notes (ART). They are then organised either with coal tit note order (CT), great tit note order (FD) or with the reversed order (D-F syntax referred to as 'REV'). The last treatment is added to control for the importance of D notes in the mobbing response of coal tits (coal tit notes produced in a non-mobbing context).

mobbing, tits typically approach the predator while emitting specific calls and aggressive behaviours such as wing flicks and numerous hops (Carlson and Griesser 2022). Coal tits and great

tits are well-known to respond to each other's anti-predator calls (Carlson et al. 2020), sometimes with predator specific responses (e.g., anti-snake calls, Suzuki 2020).

2.2 | General Organisation

We aimed at testing the mobbing response of free-ranging birds to different soundtracks. To achieve this, we designated 750 spots ($n = 75$ for each playback treatment) for conducting playback tests within a 743 km² area (see Data S1 for the complete map). Each location was chosen alongside an existing trail or roadside, close to a tree to allow for bird approach and experimenter concealment. Distances between spots were designed to be at approximately 200 m, with a resulting average distance between spots in the same zone being 219.72 ± 1.56 m (mean $\pm$ SE). Previous protocols show that birds do not follow the observer between successive spots in tests distanced by 100 m (Parker 2023; Salis et al. 2022), and a distance of 200 m is sufficient to ensure that the sound is not heard between subsequent locations, thereby avoiding any priming effects. Although birds can cover considerable distances in a short span, we therefore consider it to be improbable that we tested the same birds in consecutive trials during this experiment.

We implemented a factorial design, with one of our 10 distinct treatments (varying emitter species and call structures, detailed in the Playbacks section) at each spot. These experiments took place at the onset of the reproductive season, when coal tits began to sing frequently in the region (28 February 2024) and stopped when the first eggs were detected in the monitored population (12 April 2024). All 750 tests were conducted within a short timeframe (7 weeks) early in the season to minimise potential intra-seasonal effects and to capitalise on the minimal leaf coverage that makes it easier to spot the birds in the trees. Furthermore, conducting the experiment when tits were singing allowed us to confirm their presence on the sites. This singing behaviour further supports the case for not testing the same bird twice, as birds typically remain very close to their territory during the spring season. Each spot received only one playback test, and we never used the same path twice. On average, we did 25 ± 10 tests each day, depending on the quality of the trails and the weather of the day. To avoid spatial and temporal autocorrelation, we alternated between treatments at consecutive spots with at least 3 min between each consecutive test. Testing occurred exclusively on clear days. This type of protocol is unable to distinguish between instances where no bird responses occurred due to a lack of birds present versus a lack of willingness to respond. However, our large sample size was specifically designed to mitigate this potential confounding effect. By testing all treatments numerous times across various locations and times, we randomised the risk of having differing numbers of birds in proximity just before a test commenced.

We made no exclusion of data points (original and final sample size = 75 tests for each of the 10 playback treatments). As the birds tested were wild, free-ranging, and not ringed, we could not control for the age, sex, personality trait, body condition, or social status of the birds responding to the playbacks. As always with playback experiments on free-ranging birds, we may have recorded the response of the bolder individuals. However, our tests accurately represented a potential bird mobbing in its natural habitat, and the overall community response is therefore ecologically relevant and interpretable in this regard. In a subset (~100 points) of the dataset,

coal tits may have been previously in contact with researchers in ecology, as part of the forest has been monitored with nest boxes since 2011.

2.3 | Playbacks

We created 10 different acoustic treatments (Figure 1), each treatment having five replicas (calls from five different individuals) to avoid pseudoreplication issues (Kroodsma 1989). The first nine treatments were created by crossing two ternary factors: notes, and order. First, *notes* were picked from coal tits (CT), great tits (GT) or artificial versions of these (ART). Second, they were organised in the same *order* as coal tits (CT), great tits (FD) or in the reversed order of great tits (REV). The additional 10th treatment was made with call sequences from coal tits produced in a non-mobbing context.

To create the playbacks, we first looked for pairs of call sequences from coal tits and great tits that possessed similar numbers of F and D notes. This allowed us to have ‘templates’ in which we then replaced the notes with either coal tits, great tits, or artificial notes. In practice, we took advantage of the previously published information (Salis et al. 2023) that great tits and coal tits have overall a very similar rate of note production (100 notes/min), as well as similar proportions of F and D notes (15% F, 85% D) in mobbing calls, making it possible to find similar recordings to compare. We chose recordings previously obtained by AS between 2019 and 2022 from great tits and coal tits responding to a heterospecific chorus of mobbing calls in the East of France (bird alone when mobbing, confirmed identification of the calling bird, ~10 m distance). We looked for pairs of similar recordings with one coal tit and one great tit matching for both their note rate and percentage of F and D notes. We found five pairs with an average of 123 notes/min (range 112–144) and 15% of F notes (range 9.8%, 22%), corresponding to the average of what great tits and coal tits produce when mobbing (Salis et al. 2023). To exactly match the number of F and D notes of the coal tits sequences in each pair, we then deleted a few D notes (between 2 and 12) of the great tit call sequences. We chose to modify slightly the great tit sequences rather than the coal tit sequences as we know more about their mobbing call coding strategy (adding or suppressing a few D notes does not strongly impact the level of response from great tits, see for example the comparable mobbing response to different number of notes in Salis et al. 2022, 2023). This exact match between coal tits sequences and great tits sequences was made to greatly facilitate the creation of the other playback treatments (by replacing the original notes with notes from another origin), and to facilitate the discussion of our results. Finally, we replaced the actual notes of our selected recordings with notes from 10 other recordings. We did that because they had the best signal-to-noise ratio, ensuring the best quality for playback. Notes were replaced in their natural order. Therefore, for each recording, we used the specific call organisation from one individual, but with the F notes from another individual, and the D notes of a third individual to control for any individual-specific effect. The amplitude of F notes is naturally higher than that of D notes (Salis et al. 2024). We kept this difference by normalising the amplitude (change volume: normalise in Avisoft) of F notes at 75%

and the amplitude of D notes at 35% (thus, it also suppresses potential amplitude variations that may arise from different recordings). With this first step, we therefore obtained the two natural treatments: CT-CT and GT-FD (Figure 1).

To create playback treatments of great tit notes with coal tit call organisation (GT-CT), we replaced the natural call sequences (CT-CT) with the great tits' notes of the great tit recording that matched the coal tit's call, while keeping the same intra-call interval between notes. Because the coal tit notes are longer, this led to inter-call intervals slightly larger in treatments with great tit notes. The same method was applied to create the playback treatments with great tit FD call organisation and coal tit notes (CT-FD, Figure 1). Finally, to create the Reverse treatments (CT-REV and GT-REV, Figure 1), we used the natural great tit call sequences (that only possessed FD calls) and artificially placed the F notes after the D notes (while keeping the same inter-note temporal interval). To create the artificial treatments (ART-CT, ART-GT, ART-REV, Figure 1), we replaced the F and D notes from coal tit natural sequences or great tit natural sequences by artificial F and D notes previously created. The method for the creation of artificial notes is described in detail in Salis et al. (2024), where mobbing recordings were studied to then recreate F and D notes using the Avisoft SASLab graphical synthesiser. The notes were simplified while maintaining the key features of F and D notes in Parids: F notes were synthesised as flat pure tones lasting 0.10 s, with a peak frequency of 6000 Hz. D notes were crafted in two steps: first, 0.05 s note with flat harmonics with the same amplitude, spaced from 400 Hz (1500–11,000 Hz), then superimposing these with white noise to add some non-linearities to the notes.

For our last treatment, we used five sequences of coal tit non-mobbing calls. The recordings were obtained on [Xeno-canto.org](https://xeno-canto.org) and selected following the information provided by the recorder, mentioning either a feeding context or intra-pair communication. These sequences were not song sequences, since songs could have triggered an approach and potential aggressive response from conspecifics. We selected recordings with a similar note rate (115 ± 15 notes/min) as our other treatments and a high signal to noise ratio. We also confirm that no D notes were present in these recordings, as we wanted our control to test for the importance of D notes to trigger mobbing in coal tits (Carlson et al. 2017; Figure 1).

2.4 | Test Procedure

One trial consisted of playing 60 s of a mobbing call sequence at each spot with a Bose Soundlink Revolve loudspeaker mounted on a tripod (H: 1 m), positioned near a tree. A 60-s sequence is sufficient to elicit a mobbing response from nearby birds (Salis et al. 2022), who can then approach and call as a response, sometimes with additional aggressive behaviour (e.g., wing flicking, Salis et al. 2021). Such a short stimulation also limits the influence of the first birds to call on the following birds recruited. Once the loudspeaker was set, two observers positioned themselves at vantage points 10 m away from the tripod before initiating the soundtrack via a smartphone. Using binoculars, we monitored the area for any birds exhibiting either calling and/or

approaching behaviour from the beginning of the test to 20 s after the end of the soundtrack. A bird was considered as approaching if it entered in the 10 m radius around the tripod (Dutour et al. 2017). If a bird displayed the complete sequence of mobbing behaviour (i.e., simultaneously producing mobbing calls and approaching the loudspeaker), it was considered as giving a mobbing response. The two observers then agreed on the highest number of birds observed simultaneously by both of them.

2.5 | Statistical Analyses

First, we wanted to test whether our treatments led to different responses specifically from coal tits, to understand their decoding processes. Secondly, we were interested in the overall efficiency of our treatments in triggering a mobbing event in the community. We therefore focused on two types of response variables: (1) whether at least one coal tit mobbed to our acoustic treatment (dichotomous variable), and (2) the total number of mobbing birds (whether coal tits or others). In Data S2, we also analysed the number of birds excluding the coal tits, to confirm that the overall community response is not driven by the coal tits' response. For both variables, we split our analyses in two steps.

First, we tested which treatments triggered a different response from the CT-NonMob treatment, as we expected this treatment to be our reference in terms of non-mobbing responses. To do this, we ran generalised linear mixed models (package *lme4*, Bates et al. 2015) with the soundtrack replica as a random variable, and the playback treatment as an explanatory variable. For the presence of coal tits, we used a binomial family distribution; while for the number of birds, we used a negative binomial distribution (overdispersion detected with a Poisson distribution, function `check_overdispersion` from package *performance*, Lüdtke et al. 2021). We then calculated the contrasts between the CT-NonMob treatment and each of the other treatments with package *emmeans* (Lenth 2021) and a Sidak correction.

Secondly, we discarded the CT-NonMob treatment and tested the relative importance of the order of the notes and the origin of the notes in the response of coal tits and the bird community. We therefore ran similar models as above, but this time with finer-grained explanatory variables including separately the origin of the notes (CT, GT, ART) and the order of the notes (CT, FD, REV), and their interaction. We used the function `Anova` (package *car*, Fox and Weisberg 2019) to estimate the weight of these variables on birds' responses. We also tested the overall difference between the three note origins by running the contrasts (with *emmeans*) between the CT, GT and ART treatments. For the model with the occurrence of coal tits, adding soundtrack as a random effect led to convergence issues; we therefore used a simple GLM model instead. We calculated contrasts with package *emmeans* and a Tukey correction.

3 | Results

When notes from mobbing coal tits were present in our treatments, coal tits approached and produced mobbing calls in

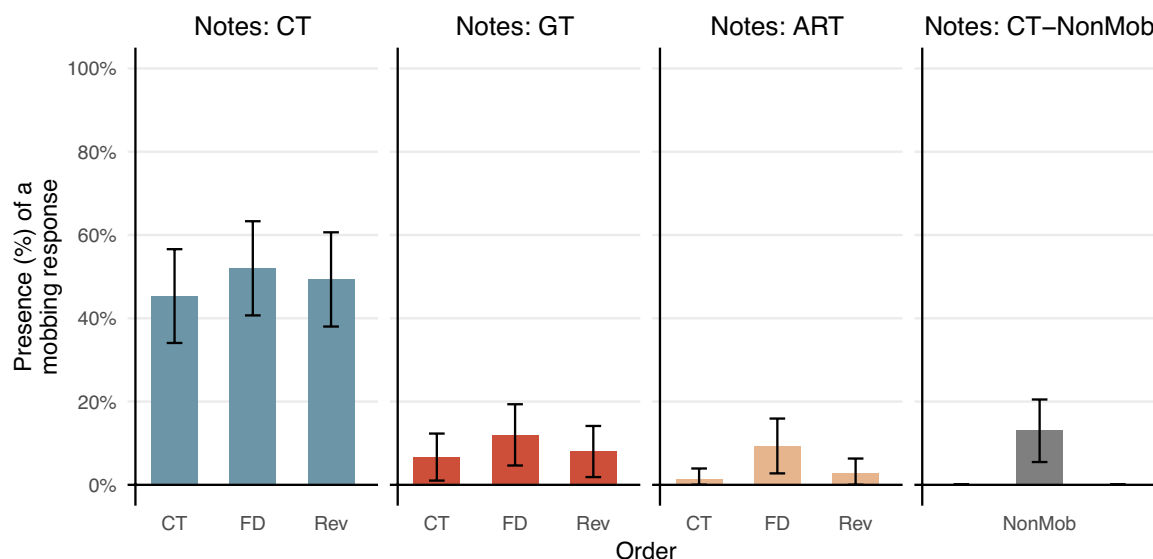


FIGURE 2 | Percentages of tests in which at least one coal tit responded with mobbing behaviour (with 95% confidence intervals, $n = 75$ in each treatment). The nine first treatments are organised as follows: The first three panels correspond to playbacks with notes from different species (ART, Artificial notes; CT, Coal tit notes; GT, Great tit notes) presented in three different orders within each of these panels (CT: Coal tit order, FD: Great tit order, in a typical F-then-D call, REV: D-then-F order, never produced in natural settings). The last treatment, in the far-right panel, is used as a control (sequences from coal tits produced in a non-mobbing situation).

about 50% of the trials (Figure 2). This was significantly higher than when the playback contained notes from coal tits but from a non-mobbing context (13% of responses to the CT-NonMob treatment). In contrast, treatments using great tit or artificial notes did not trigger a statistically higher response than the CT-NonMob treatment (Table 1A). When discarding this control treatment to test the relative importance of note origin and order in the response of coal tits, we find that only note origin has an impact (Table 2A). Indeed, the largest response was given to the coal tit notes and a significantly lower response to the great tit and artificial notes (Table 3A). In sum, coal tits responded to coal tit mobbing notes, regardless of the order of notes, and did not respond strongly to great tit notes, nor artificial notes, whatever order was proposed (Figure 2).

When focusing on the number of birds, regardless of species, that mobbing playbacks recruited, we found that treatments with notes from coal tit mobbing calls triggered higher responses than non-mobbing coal tit notes (Figure 3; Table 1B). Mobbing call playbacks with great tit and artificial calls showed no difference in recruitment of tits relative to non-mobbing coal tit calls, except artificial calls with a coal tit sequence which recruited significantly fewer birds than non-mobbing coal tit calls (Table 1B). When testing the effect of note order and note origin on the general community response, we found an interaction between the two factors (Table 2B): in the artificial treatments, notes with an FD order triggered more response than notes with a coal tit call organisation (ART-FD vs. ART-CT: ratio = 5.75, SE = 3.24, $p = 0.05$). This difference is not present for treatments with coal tit notes (CT-FD vs. CT-CT: ratio = 1.14, SE = 0.23, $p = 0.99$) or with great tit notes (GT-FD vs. GT-CT: ratio = 1.43, SE = 0.39, $p = 0.93$). When merging the three orders and testing the overall response to each type of note (Table 3B), we find that the highest response was given to the coal tit notes, an intermediate response to great tit notes, and the smallest response to the

artificial notes. Overall, the number of birds recruited to mob was highly dependent on the origin of the notes, with a lesser impact from the order of the notes.

4 | Discussion

Coal tits responded with mobbing behaviour when hearing notes from their own species, even when these notes were re-ordered in very unusual sequences. This observation suggests that, in conspecific settings, coal tits exhibit a weaker syntactic constraint compared to closely related species such as the great tit and Japanese tit (Dutour, Lengagne, and Léna 2019; Suzuki et al. 2016). While the quantity of notes in a sequence has been shown to increase mobbing in several Parid species (Courter and Ritchison 2010; Soard and Ritchison 2009; Wilson and Mennill 2011), we show that it is not a sufficient parameter to trigger mobbing response in birds. Indeed, the CT-NonMob treatments possessed the same call rhythm and similar note durations than the CT-CT treatment yet triggered significantly less responses from coal tits. The main difference was in the properties of the notes, as the CT-NonMob treatment did not contain any D notes. Thus, the key factor in triggering a mobbing behaviour in coal tits appears to be the recognition of specific notes (such as the presence of D notes), rather than the organisation of these notes within the sequence. However, due to the non-mobbing sequences originating from xeno-canto files with less precise contexts and potentially varying recording quality, the specific feature responsible for triggering mobbing behaviour in coal tits requires further investigation. For instance, it remains plausible that certain D notes may be produced outside of a mobbing context, akin to the call system of North American chickadees (e.g., in food contexts, Mahurin and Freeberg 2009). There may be subtle acoustic features in the D notes that help

TABLE 1 | Contrasts obtained when comparing our control treatment to our nine other treatments. The responses are (A) the presence of at least one coal tit mobbing, and (B) the total number of birds mobbing. Generalised mixed models are used (A, binomial distribution; B, negative binomial distribution), with a Sidak correction for multiple testing. We provide the ratio (effect size), with standard error (SE), the Z ratio and the associated *p*-value.

Contrast of CT-NonMob vs. ...					
Notes	Order	Ratio	SE	Z ratio	<i>p</i>
A. Occurrences with at least one mobbing coal tit					
CT	CT	5.57	2.31	4.14	<0.001
CT	FD	7.33	3.04	4.80	<0.001
CT	REV	6.57	2.72	4.54	<0.001
GT	CT	0.46	0.27	-1.34	0.83
GT	FD	0.89	0.44	-0.25	1
GT	REV	0.56	0.31	-1.05	0.96
ART	CT	0.09	0.09	-2.30	0.18
ART	FD	0.67	0.35	-0.77	0.99
ART	REV	0.18	0.14	-2.19	0.23
B. Number of mobbing birds					
CT	CT	4.49	1.14	5.89	<0.001
CT	FD	3.96	1.02	5.35	<0.001
CT	REV	4.78	1.21	6.14	<0.001
GT	CT	1.12	0.33	0.38	1
GT	FD	1.60	0.45	1.68	0.58
GT	REV	1.65	0.46	1.79	0.50
ART	CT	0.13	0.07	-3.62	0.002
ART	FD	0.77	0.25	-0.81	0.99
ART	REV	0.47	0.17	-2.11	0.28

Note: Our significance threshold is $p < 0.05$ (in bold).

distinguish a mobbing situation from a non-mobbing one. In this experiment, our control was specifically designed to avoid eliciting a mobbing response, enabling us to compare the levels of response across the various treatments. Designing a specific experiment that gradually reduces the effectiveness of a complete mobbing sequence through the modification of specific features will be essential to identify the additional factors required for recognising a mobbing sequence in coal tits.

Coal tits did not respond much to the great tit call even when it was in the natural FD order. This is unexpected, as previous results have shown that coal tits often respond to great tit mobbing calls (Dutour, Lengagne, and Léna 2019). A lower mobbing response may be explained by differences in the environment affecting birds' behaviour. For example, anti-predator responses in Parids are affected by the perceived predator pressure (Tilgar and Moks 2015) which may be attributed to altitudinal

TABLE 2 | Outputs of our model testing the relative importance of the order of the notes, the origin of the notes, and their interaction (function Anova, Type II test).

Variable	LR	<i>p</i>
A. Occurrence of coal tits		
Order	4.15	0.13
Notes	161.60	<0.001
Order×Notes	4.14	0.39
B. Number of birds		
Order	2.05	0.36
Notes	153.99	<0.001
Order×Notes	11.12	0.03

Note: Our significance threshold is $p < 0.05$ (in bold).

Abbreviations: LR, Likelihood ratio; *p*, *p*-value.

TABLE 3 | Contrasts between the three types of notes (CT: Coal tit notes, GT, great tit notes, ART: Artificial notes). We provide the ratio (effect size), with standard error (SE), the Z ratio and the associated *p*-value.

Contrast	Ratio	SE	Z ratio	<i>P</i>
A. Occurrence of coal tits				
GT-ART	2.81	1.39	2.09	0.09
CT-ART	28.42	12.86	7.40	<0.001
CT-GT	10.11	2.78	8.40	<0.001
B. Number of birds				
GT-ART	3.93	0.94	5.75	<0.001
CT-ART	12.06	2.73	11.00	<0.001
CT-GT	3.07	0.42	8.20	<0.001

Note: Our significance threshold is $p < 0.05$ (in bold).

variations (Crouchet et al. 2024). In our study, birds were tested at territories from 800 to 1200 m. While we do not possess the precise altitudes of the tests done by Dutour et al., we suspect this may have been done in territories with a slightly lower altitude (Rhône department, max. altitude 1012 m). Yet, we still recorded a relatively high mobbing response to coal tits' calls, hinting that the overall anti-predator response of the community is not the central factor explaining our difference. Rather, it was specifically the response to great tits that was lower than expected. Birds are known to adjust their mobbing investment to the local community, for example by rapidly learning to associate a novel sound to a mobbing situation (e.g., Dutour, Léna, et al. 2019). Our results suggest that the density of heterospecifics is another factor that affects the overall attentiveness and responsiveness of the community to a specific call. Indeed, while we confirmed during fieldwork that great tits could be found in our test areas, as they sometimes responded to our treatments, the overall density of this species may have been lower than sites used in previous work: we detected great tits prior to our tests in only 13.5% of cases, whereas we detected coal tits before our

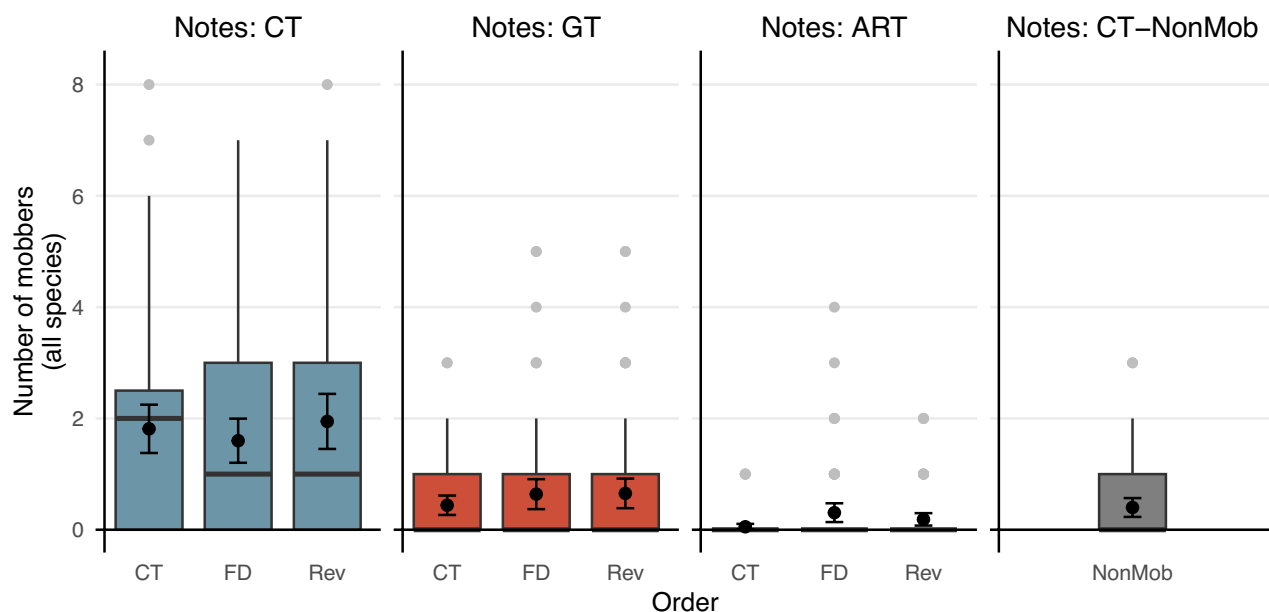


FIGURE 3 | Number of birds that mobbed in response to our 10 different treatments. The nine first treatments include mobbing calls and are organised as follows: The first three panels correspond to playbacks with notes from different species (ART, Artificial notes; CT, Coal tit notes; GT, Great tit notes) presented in three different orders within each of these panels (CT: Coal tit order, FD: Great tit order, in a typical F-then-D call, REV: D-then-F order, never produced in natural settings). The last treatment is used as a control (sequences from coal tits not produced in a mobbing situation). Boxplots are shown (horizontal line: Median value; box ends: Upper and lower quartiles; whiskers: Variation range of values.) with mean (black point) and 95% confidence intervals.

tests in 35.4% of cases. Our results follow a general pattern of exposure: this community, and particularly the coal tit, respond to the sounds they meet more often. This strongly suggests that the density of specific species is a crucial factor explaining how the community will consider responding to a given mobbing call. This also emphasises the importance of replicating experiments in different populations to fully understand the decoding processes of a species.

Coal tits rarely responded to our artificial calls. These artificially created notes triggered a mobbing response from great tits in another study, but only when ordered into FD sequences (Salis et al. 2024). Great tits and coal tits therefore may use different decoding processes when responding to mobbing calls. While several results point towards a general recognition of the FD pattern in great tits (Dutour, Lengagne, and Léna 2019; Salis et al. 2021, 2023, 2024), it seems more probable that coal tits learn to recognise the species-specific notes present in mobbing situations. Indeed, we show that the artificial notes in the coal tit order triggered almost no response from coal tits: placing notes with features shared by Parids in their natural order is not sufficient to trigger a mobbing response from coal tits. These artificial calls were however sufficient to trigger a mobbing response from great tits (Salis et al. 2024). The resemblance between the artificial notes and those of great tits may be a contributing factor to this observation; however, a Principal Component Analysis (PCA) with notes from all French Paridae species and our artificial notes, strongly suggests that the artificial notes were distinctly different from all species (Data S3). More work on coal tits and closely related species is needed to fully understand how Parids decode novel signals. In addition to testing other types of playbacks, testing coal tit responses in winter or later in the reproductive season may also bring different results. Seasonal

effects in mobbing responses have been shown (Shedd 1983) and appear to interact with the origin of the calls (higher responses to heterospecific calls outside the reproductive season, Dutour, Cordonnier, et al. 2019). Therefore, the results obtained in this experiment may be influenced by the specific timeframe during which the birds were tested. At the onset of the reproductive season, there may be a greater tendency to rely less on heterospecific and novel calls compared to other times of the year. Consequently, the lack of difference between the different orders for heterospecific calls and artificial calls may have been simply hindered by their overall low response, and do not strictly indicate that coal tits do not pay attention to order in heterospecific calls. Gaining a comprehensive understanding of the decoding processes in several closely related species, at different times of the year, will ultimately enhance our insight into how ordering rules may have evolved on a larger scale.

In conclusion, coal tits exhibit a pronounced response to conspecific notes, regardless of the order in which these notes are presented; and show a minimal response to heterospecific and artificial notes that share essential acoustic features, regardless of their order. This strongly indicates that precise note recognition, as opposed to order recognition—a mechanism observed in closely related species such as the great tit and the Japanese tit—plays a significant role in this species. Our study highlights the need for comparative research on closely related species to evaluate the potential applicability of specific hypotheses to entire clades. We demonstrate that order constraints in conspecific mobbing calls are absent in at least one Paridae species, the coal tit. This is striking given that the FD call organisation is well conserved in most Parids. We now need to explore what pressure led to coal tits to lose this shared coding mechanism which appears to be beneficial for enhancing heterospecific

communication about threats. More generally, this observation raises questions about the evolutionary pressures that may contribute to the constrained order in bird call sequences. Moreover, the unexpectedly low response to familiar heterospecific calls underscores the considerable influence of community composition and/or season on the attentiveness and responsiveness to natural calls. Future investigations should examine the impact of heterospecific density on the responses of coal tits, and other Paridae species, to both natural and unnatural call orders.

Author Contributions

Ambre Salis: conceptualization, methodology, investigation, formal analysis, visualization, project administration, resources, writing – original draft, writing – review and editing. **Axel Molina:** formal analysis, investigation, methodology, writing – original draft, writing – review and editing. **Laura Mephane-Montel:** methodology, writing – review and editing. **Alexis Chaine:** funding acquisition, project administration, resources, writing – review and editing. **Philippe Schlenker:** conceptualization, funding acquisition, supervision, writing – review and editing. **Emmanuel Chemla:** conceptualization, funding acquisition, methodology, supervision, visualization, writing – review and editing.

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Ethics Statement

This work did not necessitate any specific permit to be conducted and followed the ASAB guidelines for research on animals.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

R code & dataset are on Zenodo: Salis, A. (2025). Data, Code & Supporting Information for “No effect of note order on the response of coal tits to conspecific, heterospecific and artificial mobbing calls” [Data set]. Zenodo. <https://doi.org/10.5281/zenodo.14801449>.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.