The role of associative learning process on the response of fledgling great tits (*Parus major*) to mobbing calls

Mylène Dutour, Jean-Paul Léna, Adeline Dumet, Vanessa Gardette, Nathalie Mondy, Thierry Lengagne

► To cite this version:

Mylène Dutour, Jean-Paul Léna, Adeline Dumet, Vanessa Gardette, Nathalie Mondy, et al.. The role of associative learning process on the response of fledgling great tits (*Parus major*) to mobbing calls. *Animal Cognition*, 2019, 22 (6), pp.1095-1103. 10.1007/s10071-019-01301-1 . hal-02328411

HAL Id: hal-02328411

<https://univ-lyon1.hal.science/hal-02328411>

Submitted on 16 Nov 2021

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

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

**The role of associative learning process on the response of
fledgling great tits (*Parus major*) to mobbing calls**

**Mylène Dutour¹ · Jean-Paul Léna¹ · Adeline Dumet¹ · Vanessa Gardette¹ · Nathalie
Mondy¹ · Thierry Lengagne¹**

¹ Université de Lyon, UMR5023 Ecologie des Hydrosystèmes Naturels et Anthropisés,
Université Lyon 1, ENTPE, CNRS, 6 rue Raphaël Dubois, 69622 Villeurbanne, France

Author for correspondence:

Mylène Dutour

mylene.dutour@hotmail.com

Abstract

When they detect a predator, many species emit antipredator vocalizations. In some cases, they emit mobbing calls, which are associated with the caller approaching and harassing the predator while attracting others to join it. Surprisingly, although mobbing has been widely reported in adults of numerous species, there has been no test of the role of learning in mobbing call recognition, especially during ontogeny. Here, we exposed wild great tit (*Parus major*) nestlings to playbacks of an unthreatening, novel sound either associated with conspecific mobbing calls (experimental treatment) or with another unthreatening novel sound (control treatment). We then tested them as nestlings and fledglings to see how responses to the novel sound compared to conspecific mobbing calls. Results revealed that fledglings in the experimental treatment behaved similarly to conspecific mobbing calls and the novel sound associated with conspecific mobbing calls. Because mobbing efficiency is often linked to interspecific communication, associative learning should be used by heterospecifics as a mobbing calls recognition mechanism. Regardless of treatment during the nestling phase, fledglings always were sensitive to the playback of conspecific mobbing calls. However, fledglings from the control group were more likely to approach the loudspeaker than those from the experimental group when mobbing calls were played, suggesting that overexposure during the nestling phase altered mobbing learning. Overall, these results suggest that learning could play a role in the recognition of calls, like heterospecific mobbing calls, when paired with conspecific mobbing, and that mobbing is perceived as a threatening stimulus from a very young age.

Key words Alarm call · Associative learning · Birds · Communication · Mobbing

Introduction

In many species of small birds and mammals, two contrasted anti-predator strategies, each associated to a specific alarm call, can be observed according to predator dangerousness: prey may flee or mob the predator (Marler 1957). Mobbing behaviour is usually exhibited when predators do not represent an immediate threat and is characterized by harassment or even attacks on the predator as well as vocalizations (i.e., mobbing calls) that quickly gather neighboring conspecifics and other prey species in the mob (Hartley 1950; Curio 1978). Both the evaluation of the threat associated with the predator and the recognition of mobbing calls, including heterospecific calls, are required to make mobbing behaviour an efficient anti-predator strategy.

Associative learning is a behavioural modification following reinforcement, based on associations between two stimuli, responses and events (Shettleworth 1998; Griffin et al. 2015). This process is essential to the development of vocalizations and several behaviours such as those involved in predator avoidance (Ferrari and Chivers 2011). Animals can learn to flee after associating an unfamiliar sound (i.e., novel sound) with (i) the appearance of a model predator (birds: Magrath et al. 2015a; mammals: Shriner 1999; Wheeler et al. 2019) or (ii) the hearing of alarm calls, without having to see the callers or a predator (Potvin et al. 2018). In mobbing behaviour, there is ample evidence of a process of associative social learning between conspecific mobbing calls and predators (Curio et al. 1978a; Maloney and McLean 1995; McLean et al. 1995; Griffin and Galef 2005; McIvor et al. 2018) but in all cases the training phase was based on visual cues; individuals were trained about predators through pairing model predator presentations with a training stimulus (i.e., live conspecific demonstrator engaging in mobbing, mobbing calls paired with mounts of conspecifics, or mobbing calls alone). However, visual associative learning needs the inexperienced learner to

61 be in the vicinity of the mobbing scene, so that the opportunities for learning while reducing
62 the risk of threat may be limited, especially for species living in dense vegetation. This
63 challenge is overcome in the case of acoustic-acoustic association, which may be efficient
64 even when the tutor and the threat are difficult to observe, possibly resulting in the rapid
65 spread of mobbing call recognition in natural communities.

66 Mobbing calls are usually characterized by loud and repetitive vocalizations, both
67 features that should facilitate associative learning, as there is ample opportunity to hear
68 mobbing calls while observing the threat, with less likelihood of immediate danger (Magrath
69 et al. 2015b). In birds, such association between a predator and the expression of a mobbing
70 behaviour could be achieved after young fledge leave their nest. In this case, young fledglings
71 can learn from their parents and several observations are in agreement with this statement
72 (Curio et al. 1978b; Griesser and Suzuki 2016). If nestlings however are able to associate
73 several acoustic signals from their nest cavities (i.e., they are able to associate the mobbing
74 calls emitted by parents with other vocalizations prevailing on a mobbing scene) they should
75 also be able to respond to mobbing calls appropriately as soon as they leave the nest. To our
76 knowledge, this capacity has never been investigated before. Indeed, until now, associative
77 learning has been examined using the association of several sensory channels, especially
78 visual and acoustic (e.g. Maloney and McLean 1995; McIvor et al. 2018). However, as
79 outlined above, young could also associate unknown vocalizations with conspecific mobbing
80 calls if both are simultaneously emitted in a mobbing scene. Such a mechanism could be
81 particularly relevant in learning to recognize heterospecific mobbing calls.

82 The great tit (*Parus major*) is a well-suited species to study the ontogeny of mobbing
83 behaviour in the wild. It is well known for its learning abilities (Exnerová et al. 2006; Cole et
84 al. 2011; Morand-Ferron et al. 2015) and its tendency to exhibit mobbing behaviour and emit
85 mobbing calls when confronted with a predator (Lind et al. 2005; Dutour et al. 2016; Carlson

et al. 2017a; Kalb et al. 2019). Furthermore, this altricial species provides a good system for studying whether naïve nestlings are able to associate mobbing calls with unfamiliar stimuli in the absence of any visual cues from adults or predators in the nest cavity.

The goal of this study was to determine whether associative learning might serve as a mechanism for the development of mobbing calls recognition for free-living animals. Specifically, we investigated whether experiencing a novel stimulus associated with conspecific mobbing calls during the nestling period led great tit nestlings to associate this novel sound with mobbing during the fledgling period. We designed playback experiments in two groups: (i) an experimental group in which we trained individuals by broadcasting a novel sound (i.e., associative learning stimulus) with mobbing calls (i.e., functional stimulus) and (ii) a control group in which the associative learning stimulus was broadcast with another novel sound (hereafter control stimulus).

In line with previous work showing that nestlings cease begging when they hear playbacks of their parents' alarm calls (Davies et al. 2004; Hollén and Radford 2009; Barati and McDonald 2017), we predict that at the end of the learning period, only nestlings belonging to the experimental group should cease begging when they hear the associative learning stimulus. We also measured responses at the fledgling stage after the playback of the associative learning stimulus. We predict that the playback of the associative learning stimulus should elicit a stronger mobbing response from the experimental group compared to the control group (i.e., increases in scanning, calling and moving) because the experimental group should associate the unfamiliar sound with mobbing and respond to the unfamiliar sound as threatening, while the control group should not. Because it was important to ensure that fledglings were sensitive to mobbing, we also tested whether they responded to the playback of conspecific mobbing calls.

Material and methods

Study sites and species

We chose great tits, a common territorial passerine which breeds in secondary holes and wooden artificial nest boxes throughout Europe and parts of Asia and North Africa (Perrins 1965). The study was conducted in the Pierre Vérots Foundation, a private natural reserve located in the south-east France (4°91'E, 45°95'N). The study area covers approximately 150 ha and contains 120 nest boxes separated at minimum by 50 m and mainly occupied by great tits. This distance corresponds to the minimum distance separating tit territories during the breeding season in our study area and is far enough to ensure that the playbacks are not heard by neighbours (at 50 meters, the amplitude levels fluctuate from 33 to 39 dB, obtained from the loudspeaker, Solo 01dB Metravib, Z weighting, re: 20 μ m Pa; these levels are lower than the natural background noise levels in forest areas, fluctuate from 35 to 45 dB in quiet situations, 52 to 70 dB in noisy situations). The study took place during the breeding season (April-July 2017). We visited nest boxes at least every two days from the beginning of the breeding season onwards to determine the laying and hatching dates. When nestlings were 8 days old, they were ringed with a unique combination of color bands for individual identification. Nestlings spent 19.3 ± 1.4 days in the nest. Nestlings from the same nest all hatched on the same day and subsequently also fledged on the same day.

Experimental procedures: presentation of stimuli

The aim of our experiment was to assess whether chicks were able to associate a novel stimulus with conspecific mobbing calls. In 120 nest boxes, 31 were unoccupied, 14 occupied

by the blue tit (*Cyanistes caeruleus*) and one was occupied by the willow tit (*Poecile montanus*). Each of the remaining 74 nest boxes occupied by great tits was randomly assigned to one of the two treatments (balanced for laying date to ensure equal representation of both treatment groups over the breeding season). In the first treatment (38 nests), the associative learning stimulus was associated with conspecific mobbing calls (hereafter experimental group) while in the second treatment (36 nests) the associative learning stimulus was associated with unfamiliar stimulus (control sequence, hereafter control group). Behavioural tests were conducted both during the development of nestlings and the fledging day. Playbacks were always broadcasted once adults were silent and away from the nest (at least 15 m) or from the focal fledgling (at least 5 m). If parents responded to playback during tests, we stopped the playback and restarted at least 30min after, but such cases were rare (~5% of total tests).

Choice of experimental stimuli and playback materials

Three kinds of stimuli were used during playback experiments. First, we used mobbing calls produced by four great tits in response to the Eurasian pygmy owl (*Glaucidium passerinum*) and to conspecific mobbing calls. Calls were recorded with a Fostex FR2LE digital recorder connected to a Sennheiser ME67-K6P microphone. We also used mobbing calls produced by ten great tits obtained from the Xeno Canto online database (<http://www.xeno-canto.org>) (see online supplement, Appendix 1). For the associative learning and the unfamiliar stimulus, we used two musical instrument sounds (clarinet and trumpet sequences) obtained from an online sound bank (<http://www.universal-soundbank.com>) to ensure the stimuli used had no biological value and were unfamiliar. In order to ensure that control sounds were unfamiliar, we first conducted a pilot study to assess the response of adult great tits ($n = 9$). None of them

161 approached and mobbed the loudspeaker. We matched the frequency characteristics and
162 temporal structure of the associative learning and the unfamiliar stimulus to obtain stimuli of
163 the same duration and the same frequency bandwidth using Avisoft-SASLab Pro. For the
164 experimental treatment, we built playbacks containing the associative learning stimulus (i.e.,
165 clarinet) with mobbing calls. For the control treatment, the associative learning stimulus was
166 associated with the unfamiliar stimulus (i.e., trumpet) (spectrograms of mobbing calls,
167 associative learning stimulus and unfamiliar stimulus are available in supplementary material
168 Appendix 2 Fig. A2). Each playback track consisted of 10 seconds of associative learning
169 stimulus followed by 10 seconds of the mobbing calls or the unfamiliar signal, and this
170 sequence was repeated three times in order to reach one-minute in total, time used in a recent
171 study conducted on nestling passerines in response to calls (Wheatcroft 2015). In order to
172 avoid habituation to playbacks, we constructed 21 one-minute playback samples for each
173 group and each sample was used only once (i.e., as nestlings spent 19.3 ± 1.4 days in the nest,
174 we used one stimulus by day) (Hurlbert 1984; Kroodsma et al. 2001). Because we used
175 mobbing calls produced by 14 great tits in total to construct 21 playback sequences, some
176 soundtracks are therefore only imperfectly independent from other ones, but the risk of
177 pseudoreplication was clearly minimized. We also built two additional 30-second playback
178 samples containing associative learning stimulus or mobbing calls to test the response of
179 nestlings and fledglings. We decided to keep the test duration shorter than was usual in
180 previous studies conducted on adults' great tit (including our own Dutour et al. 2017b; see
181 also Suzuki et al. 2012) in order to limit the risk of interference from parents during tests. The
182 average amplitude used for these playbacks was obtained with a sound level meter placed 1 m
183 from the loudspeaker (83.9 ± 3.51 dB, mean $\pm$ SD, Solo 01dB Metravib, Z weighting, re: 20μ
184 m Pa). We broadcast sound tracks using a Shopinnov 20 W loudspeaker (frequency response
185 100 Hz -15 kHz).

186

187 **Behavioural responses of nestlings**

188

189 Begging calls suppression is described as a good measure of whether nestlings interpret
190 sounds as cues of danger (Magrath et al. 2010). To determine whether playback treatment
191 (i.e., associative learning stimulus with unfamiliar stimulus or associative learning stimulus
192 with mobbing calls) influenced nestling calling activity, every day, between 7am and 1pm,
193 and between hatching day and fledging day, we measured nestling production of begging calls
194 within the nest box during 30 seconds (begging call = 1, no begging call = 0) before and after
195 broadcasting the treatment, 1 minute being time by which passerine nestlings' behaviour
196 return to normal (Wheatcroft 2015). To measure this behaviour, one observer was placed ~
197 50 cm from each nest to listen to the calls of the nestlings, which were clearly heard at short
198 distance. Although there were multiple observers ($n = 8$) to measure begging call propensity,
199 we did not detect an observer effect (GLMM: $P > 0.05$). Additionally, at the end of the
200 nestling phase, between 19 days post hatch and fledging day, begging call propensity was
201 measured before and after playback of the associative learning stimulus. To create as natural a
202 situation as possible, the loudspeaker was placed on the top of the nest box for all trials. The
203 average amplitude used for the playback was obtained with a sound level meter placed in the
204 nest boxes (67.98 ± 0.94 dB, mean $\pm$ SD, Solo 01dB Metravib Z weighting, re: 20 μ m Pa).

205

206 **Behavioural responses of fledglings**

207

208 To test whether associative learning is involved in the development of mobbing calls
209 recognition, we studied fledglings' responses to the playback of either the associative learning
210 stimulus alone (control: $n = 30$ from 22 nests; experimental: $n = 31$ from 19 nests) or the

211 conspecific mobbing calls (control: $n = 19$ from 14 nests; experimental: $n = 26$ from 16 nests).
212 Tests were performed during the day of fledging (except 4 tests conducted the second day
213 after fledging corresponding to 3.8% of total tests). Each test was conducted by two field
214 assistants. One was assigned to the soundtrack preparation and playback operation, while the
215 other was kept unaware of the selected soundtrack (although they could hear it) and assigned
216 to the observation of the focal bird. In all experiments and before the playback, the identity of
217 the focal fledgling was determined using the unique leg color bands fitted to each fledgling
218 using binoculars. Neither of the two field assistants knew whether the fledging was in the
219 control or experimental group. Once a focal fledging was identified, the loudspeaker used to
220 broadcast the acoustic stimulus was placed ~ 20 m from the fledgling at the base of a tree at
221 1.5 meter high. All observations were done using binoculars. Observations were made ~ 10 m
222 from the loudspeaker and the focal fledging, a distance from which the tits' behaviour was not
223 disturbed. Trials were conducted mostly around 12am (range 8am - 5pm) to ensure good light,
224 in calm and dry weather. Playbacks were started when no bird was visible close to the speaker
225 and no bird was calling. To test fledglings' responses, we recorded the following behavioural
226 variables during the 30 s of playbacks: (i) number of horizontal scans (i.e., the number of
227 movements that birds made with their heads from left to right or right to left), (ii) calling (i.e.,
228 producing any mobbing vocalizations; call propensity: mobbing vocalization = 1; no mobbing
229 vocalization = 0), (iii) approaching the loudspeaker (i.e., we recorded whether birds
230 approached within 15 m of the loudspeaker during playback; see Dutour et al. 2017b for more
231 details), and (iv) fleeing (i.e., we recorded whether birds moved away more than 10 m from
232 their initial position). We measured these behaviours because they are good indicators of
233 perceived danger in birds (Curio et al. 1978a) and are common during mobbing events
234 (Suzuki et al. 2016; Carlson et al. 2017a; Carlson et al. 2017b; Dutour et al. 2017b; Suzuki et

al. 2017). There was 10-minute gap between playback of the associative learning stimulus and playback of mobbing calls.

Data analysis

Analyses were done using SAS 9.4 (SAS Institute Inc., Cary, NC, U.S.A.) and R v.2.15.1 softwares (The R Foundation for Statistical Computing, Vienna, Austria, <http://www.r-project.org>). We first examined whether begging call propensity varied between treatment groups during the nestling period. For this purpose, we analyzed call propensity recorded each day before broadcasting the treatment using a logistic linear mixed model (LLMM) for which the call propensity was treated as a repeated dependent binary variable. To this aim, nest was considered as a random effect and the correlation between successive observations recorded at the same nest was specified using an autoregressive covariance structure. The treatment group, the time elapsed since hatch (i.e., nestlings age) as well as their interactive effect, were introduced as fixed effects. The significance of each fixed effect was tested using a non-sequential F test and the Kenward-Roger method was used to approximate the denominator degrees of freedom (SAS 9.4). Non-significant terms were successively dropped to obtain the final model. We used a similar model framework to compare the propensity of nestlings to stop begging after the onset of the broadcasting treatment according to the treatment group and the time elapsed since hatching. For this purpose, all observations for which nestlings were not calling before the onset of the playback treatment were discarded from the analysis. We used a Fisher exact test to test whether the begging calls propensity just after the playback of the associative learning stimulus only when nestlings were between 19 days post hatch and fledging day varied according to the group (experimental or control).

To investigate the behavioural responses of fledglings, we first examined whether the number of horizontal scans varied between treatment groups and playback during the fledgling period ($n = 106$). For this purpose, we ran generalized linear mixed models (GLMM) which included the treatment group, the playback (associative learning stimulus or mobbing calls) and their interactive effect as a fixed terms and nest as a random term. We used a negative binomial error distribution and log-link function for this analysis (*glmer.nb* in the package *lme4*; Bates et al. 2014). To investigate the call propensity, we used a binomial error distribution and logit-link function (*glmer* in the package *lme4*; Bates et al., 2014) (calling response = 1; no calling response = 0). As above, nests were included in the model as a random effect. The treatment group, the playback and their interactive effect were introduced as fixed effects. For both scanning and call propensity, we further conducted pairwise comparisons (*glht* in the package *multcomp*). Finally, because approaching and fleeing behaviours (two behaviours linked to movements) were opposed to immobility behaviour (see online supplement Appendix 3, Fig. A3), all approaching and fleeing individuals were regrouped within a unique category (hereafter moving individuals) and compared to the individuals who stayed still. To compare the propensity of fledglings to move vs individuals who stayed still according to treatment group and playback, we used a binomial error distribution and logit-link function. We then focused our analysis on moving individuals only (i.e., fleeing and approaching individuals; $n = 23$). Because only a small number of fledglings moved, Fisher's exact test was used to investigate the variation in mobbing propensity (i.e., approach) according to the treatment group in response to the mobbing call playbacks ($n = 17$). Because only one of the control fledglings moved when exposed to the playback of the associative learning stimulus, we discarded this group and we used a Fisher's exact test to investigate the variation in mobbing propensity of individuals from the experimental group between the two playbacks ($n = 16$).

Data availability The datasets generated during the current study are available from the corresponding author on request.

Results

Behavioural responses of nestlings

The calling behaviour before the broadcasting treatment did not significantly vary in function to the time elapsed since hatching up to fledging but it was significantly higher for the experimental group than the control group ($n = 1420$, treatment effect: $F_{1,341} = 4.17$; $P = 0.042$; time effect: $F_{1,426} = 0.66$; $P = 0.417$; interaction: $F_{1,425} = 0.40$; $P = 0.529$). The propensity to stop calling after the onset of the broadcasting treatment significantly increased in function to the time elapsed since hatching but did not vary between treatment groups although call suppression tended to increase with time slightly more for the control group than for the experimental group ($n = 353$, treatment effect: $F_{1,156} = 0.11$; $P = 0.745$; time effect: $F_{1,176} = 46.19$; $p < 0.0001$; interaction: $F_{1,170} = 3.69$; $P = 0.057$). We did not detect a treatment group effect on the nestlings responses to the playback of the associative learning stimulus ($n = 11$; $P = 0.608$).

Behavioural responses of fledglings

Scanning was significantly affected by the interaction between the stimuli that were broadcast (associative learning stimulus or mobbing calls) and the treatment group (experimental and control) (Table 1; Fig. 1). In response to mobbing calls playbacks, the rate of horizontal scans

did not differ between the two groups ($P = 0.99$; Fig. 1). It was higher during playback of mobbing calls than during playback of the associative learning stimulus for the control group ($P = 0.004$; Fig. 1) while it did not differ for the experimental group ($P = 0.99$; Fig. 1). Similar variations were found for call propensity as indicated by a significant interaction effect between group and playback (Table 1; Fig. 2), although these variations were not statistically different between both groups in response to the associative learning stimulus playback ($P = 0.95$; proportion of calling: associative learning = 22.5%; control = 6.7%). Furthermore, there was a significant effect of playback on the probability of moving (i.e., approaching and flight behaviours), whereas group had no significant effect (Table 1; Fig. 3). In response to mobbing calls, fledglings were more likely to move than in response to the associative learning stimulus playback. Results indicate that (i) in response to the mobbing call playbacks, individuals from the control group were more prone to approach the loudspeaker than experimental individuals ($n = 17$; Fisher test: $P = 0.049$; Fig. 3) (ii) approaching behaviour of individuals from the experimental group did not differ between the two playbacks ($n = 16$; Fisher test: $P = 0.999$; Fig. 3).

Discussion

We have investigated whether nestlings submitted to a novel stimulus associated with mobbing calls enabled them to recognize this stimulus as an indicator of threat proximity when becoming fledglings. This experiment, involving classical conditioning, showed that fledglings learned to associate a novel stimuli with mobbing calls and exhibited vigilance behaviours in response to the novel stimuli whereas they do not as nestlings.

Effect of the associative learning during the nestling phase

334

335 Several studies have shown that nestlings become silent when they hear playbacks of their
336 parents' alarm calls (Davies et al. 2004; Platzen and Magrath 2004; Madden et al. 2005; Haff
337 and Magrath 2012; Barati and McDonald 2017). However, most of these studies have
338 compared alarm calls to background noise or sympatric species stimuli (i.e., stimuli known).
339 In the present study, nestlings suppressed calling when submitted to associative learning
340 stimulus equally to mobbing call playbacks, suggesting that novelty (i.e., a stimulus never
341 heard before) alone may be used as a signal of danger (Schaller and Emlen 1961; Curio et al.
342 1978b). We found that, contrary to young nestlings, older nestlings reduced begging calls in
343 response to the associative learning stimulus and mobbing calls, suggesting that older
344 nestlings are less prone to recognition errors than younger ones (Davies and Brooke 1988;
345 Davies et al. 2004). This pattern of gradual acquisition is in accordance with previous studies
346 conducted on nestling great tits (Rydén 1978). We did not detect a group effect on nestling'
347 responses to playbacks of the associative learning stimulus at the end of the learning period,
348 but the low sample size may have reduced our ability to detect a significant pattern. A detailed
349 study of nestlings' behaviour would be insightful to actually address this question (e.g., if they
350 crouched down inside their nest cavity, suppressed their movements, increase their heart rate
351 and electromyographic activity after parental alarm calls; Rydén 1978; Ryden 1980; Suzuki
352 2011). Finally, another interesting aspect is related to the sound degradation. As we used hole
353 nesters, a sound degradation may occur that a sound will appear different within and outside
354 the nest box. However, quite low-frequency sounds have been used in this study, with
355 probably low alteration, and the sounds were broadcast using a loudspeaker placed on the top
356 of the nest box for all trials, with the only obstacle being the wall thickness of the nest box.
357 Future work could investigate which sound characteristics of the parent calls could be

perceived by offspring in their nest according to their nestling conditions (hole nesters vs open nesters).

Mobbing calls recognition and associative learning in fledglings

Fledglings exhibited a higher rate of scanning when hearing the associative learning stimulus but only if it was associated with mobbing calls during the nestling phase. A similar trend was also observed for calling activity and the propensity to move. Together, these results indicate that individuals associated the artificial sound with conspecific mobbing calls without having to see the mobbing scene. Scanning rate is a good proxy of vigilance effort (Lendrem 1983; Huang et al. 2012; Creel et al. 2014) suggesting that chicks actually perceived the associative learning stimulus as an indicator of threat proximity only if it was associated with mobbing calls during the learning phase. Whether fledglings associated the artificial stimulus as the source of threat itself or as heterospecific mobbing calls (indicating the presence of a threat) and whether the ontogeny of mobbing behaviour only relies on learning processes, remain open questions. Positive correlation between the rate of response development and the magnitude of exposure to heterospecific alarm calls suggest a learning process. For instance, infant vervet monkeys (*Cercopithecus aethiops pygerythrus*) develop responses to superb starling (*Lamprotornis superbus*) mobbing calls more quickly on territories where these birds are common, suggesting that these young have more opportunities to learn about the calls than young on territories where starlings are less common (Hauser 1988). Concerning adults, they can associate novel sounds with a chorus of conspecific and heterospecific aerial alarm calls (Potvin et al. 2018). One may thus expect that a learning process where individuals associate the threat and/or heterospecific mobbing calls with the conspecific mobbing calls can operate in the wild. Such mechanisms could make the set of recognized heterospecific mobbing calls

adjusted to the local composition of prey communities, which is also congruent with field studies reporting local variations of the rate of responses towards heterospecific mobbing calls (Wheatcroft and Price 2013).

In addition to such associative learning processes, our study also reveals that fledglings responded to conspecific mobbing calls despite the treatment they experienced. Indeed, in both groups, hearing conspecific mobbing calls resulted in increased vigilance, a higher proneness to call as well as to move. Since all these tests were performed on fledgling day, we can safely assume that no fledgling could have had the opportunity to observe a mobbing scene before the tests. Our results therefore indicate that fledglings do not need to observe mobbing events to respond to mobbing calls, suggesting that conspecific mobbing calls are inherently perceived as indicating a threat. However, two hypotheses, not mutually exclusive, could explain the responses to mobbing calls: the response (1) is at least partly innate, this result has been found in adult great tits in response to allopatric mobbing calls (Randler 2012, Dutour et al. 2017b), or (2) is due to the impregnation of acoustic signals heard during the nestling phase. Mobbing calls are composed of frequency modulated elements, which are involved in vigilance, and D notes involved in foraging flocks or to recruit partners during the breeding season, to which receivers respond by approaching the caller (Dutour et al. 2019). In the present study, tests were performed during fledgling when great tits form family groups and often use D notes to maintain group cohesion (Dutour, pers. observation). The responses of fledglings could be related to the context (i.e., presence of family members in the vicinity), or could be due to sensitivity to the D notes. Furthermore, contrary to fledglings belonging to the control group, those belonging to the experimental group were more prone to flee than approach the loudspeaker. It could be from the playbacks which were always presented to fledglings in the same order (i.e., associative learning sound, then mobbing calls), creating biases that differed between treatments. Indeed, this could be a

carry-over from the experimental group hearing the unfamiliar sound, which is threatening to them, before mobbing calls, while the control group heard a non-threatening sound and then the mobbing calls. The situation seemed more dangerous for the experimental group if the two cues had an additive effect. However, this result suggests that reinforcement with mobbing calls during the nestling phase altered mobbing learning. Such impairment could be either due to call overexposure, as previously shown in vocalization learning (Tchernichovski et al. 1999; Brainard and Doupe 2013), or because of the absence of a threat associated with the mobbing call during exposure. An alternative explanation is that maybe fleeing is the adaptive response to mobbing calls for young juveniles. This should be addressed in further studies. It would be feasible in relatively resident birds like great tits where dispersal distances are small. Flee would not be an impairment when expressed by a young, inexperienced, and physically clumsy individual compared with more experienced, agile and older juveniles (~ 120 days post fledging) (Kullberg & Lind, 2002) or adults. In that case, being exposed to additional mobbing via playbacks may have sped up the development of this response.

Conclusion

This study shows that nestling great tits do not discriminate between two different acoustic signals but demonstrates that they associate mobbing calls with a novel sound when exiting the nest cavity as fledglings. Nevertheless, fledglings did not respond with typical mobbing behaviour (i.e., approach and harass), which likely require experience of parental visual response (i.e., cultural transmission) (Kullberg and Lind 2002). The present findings raise questions about how nestlings extract information about the nature of predators and how finely they can discriminate between different acoustic stimuli.

References

- Barati A, McDonald PG (2017) Nestlings reduce their predation risk by attending to predator-information encoded within conspecific alarm calls. *Sci Reps* 7:11736.
<https://doi.org/10.1038/s41598-017-11528-y>
- Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. <http://CRAN.R-project.org/package=lme4>
- Brainard MS, Doupe AJ (2013) Translating birdsong: songbirds as a model for basic and applied medical research. *Annu Rev Neurosci* 36:489–517.
<https://doi.org/10.1146/annurev-neuro-060909-152826>
- Carlson NV, Healy SD, Templeton CN (2017a) A comparative study of how British tits encode predator threat in their mobbing calls. *Anim Behav* 125:77–92.
<https://doi.org/10.1016/j.anbehav.2017.01.011>
- Carlson NV, Pargeter HM, Templeton CN (2017b) Sparrowhawk movement, calling, and presence of dead conspecifics differentially impact blue tit (*Cyanistes caeruleus*) vocal and behavioral mobbing responses. *Behav Ecol Sociol* 71:133.
<https://doi.org/10.1007/s00265-017-2361-x>
- Cole EF, Cram DL, Quinn JL (2011) Individual variation in spontaneous problem-solving performance among wild great tits. *Anim Behav* 81:491–498.
<https://doi.org/10.1016/j.anbehav.2010.11.025>
- Creel S, Schuette P, Christianson D (2014) Effects of predation risk on group size, vigilance, and foraging behavior in an African ungulate community. *Behav Ecol* 25:773–784.
<https://doi.org/10.1093/beheco/aru050>
- Curio E (1978) The adaptive significance of avian mobbing. I. Teleonomic hypotheses and predictions. *Z Tierpsychol* 48:175–183. <https://doi.org/10.1111/j.1439-0310.1978.tb00254.x>

457 Curio E, Ernst U, Vieth W (1978a) Cultural transmission of enemy recognition: one function
 458 of mobbing. *Science* 202:899–901. <https://doi.org/10.1111/j.1439-0310.1978.tb00255.x>
 459 Curio E, Ernst U, Vieth W (1978b) The adaptive significance of avian mobbing. II. Cultural
 460 transmission of enemy recognition in blackbirds: effectiveness and some constraints. *Z*
 461 *Tierpsychol* 48:184–202. <https://doi.org/10.1126/science.202.4370.899>
 462 Davies NB, Brooke MD (1988) Cuckoos versus reed warblers: adaptations and
 463 counteradaptations. *Anim Behav* 36:262–284. [https://doi.org/10.1016/S0003-](https://doi.org/10.1016/S0003-3472(88)80269-0)
 464 [3472\(88\)80269-0](https://doi.org/10.1016/S0003-3472(88)80269-0)
 465 Davies NB, Madden JR, Butchart SHM (2004) Learning fine-tunes a specific response of
 466 nestlings to the parental alarm calls of their own species. *Proc R Soc Lond B Biol Sci*
 467 271:2297–2304. <https://doi.org/10.1098/rspb.2004.2835>
 468 Dutour M, Lena JP, Lengagne T (2016) Mobbing behaviour varies according to predator
 469 dangerousness and occurrence. *Anim Behav* 119:119–124. [http://](http://dx.doi.org/10.1016/j.anbehav.2016.06.024)
 470 dx.doi.org/10.1016/j.anbehav.2016.06.024
 471 Dutour M, Lena JP, Lengagne T (2017a) Mobbing behaviour in a passerine community
 472 increases with prevalence in predator diet. *Ibis* 159:324–330.
 473 <http://dx.doi.org/10.1111/ibi.12461>
 474 Dutour M, Léna JP, Lengagne T (2017b) Mobbing calls: a signal transcending species
 475 boundaries. *Anim Behav* 131:3–11. <https://doi.org/10.1016/j.anbehav.2017.07.004>
 476 Dutour M, Lengagne T, Léna JP (2019) Syntax manipulation changes perception of mobbing
 477 call sequences across passerine species. *Ethology*. <https://doi.org/10.1111/eth.12915>
 478 Exnerová A, Štys P, Fučíková E, Veselá S, Svádová K, Prokopová M, ... Landová E (2006)
 479 Avoidance of aposematic prey in European tits (Paridae): learned or innate?. *Behav*
 480 *Ecol* 18:148–156. <https://doi.org/10.1093/beheco/arl061>

481 Ferrari MCO, Chivers DP (2011) Learning about non-predators and safe places: the forgotten
482 elements of risk assessment. *Anim Cogn* 14:309–316. [https://doi.org/10.1007/s10071-](https://doi.org/10.1007/s10071-010-0363-4)
483 010-0363-4 PMID: 21203793

484 Griesser M, Suzuki TN (2016) Kinship modulates the attention of naïve individuals to the
485 mobbing behaviour of role models. *Anim Behav* 112:83–91.
486 <https://doi.org/10.1016/j.anbehav.2015.11.020>

487 Griffin AS, Galef Jr BG (2005). Social learning about predators: does timing matter?. *Anim*
488 *Behav* 69:669–678.

489 Griffin AS, Guillette LM, Healy SD (2015) Cognition and personality: an analysis of an
490 emerging field. *Trends Ecol Evol* 30:207–214. <https://doi.org/10.1016/j.tree.2015.01.012>

491 Haff TM, Magrath RD (2012) Learning to listen? Nestling response to heterospecific alarm
492 calls. *Anim Behav* 84:1401–1410. <https://doi.org/10.1016/j.anbehav.2012.09.005>

493 Hartley PHT (1950) An experimental analysis of interspecific recognition. *Symp Soc Exp*
494 *Biol* 4:313–336

495 Hauser MD (1988) How infant vervet monkeys learn to recognize starling alarm calls: the
496 role of experience. *Behaviour* 105:187–201. <https://doi.org/10.1163/156853988X00016>

497 Hollen LI, Radford AN (2009) The development of alarm call behaviour in mammals and
498 birds. *Anim Behav* 78:791–800. <https://doi.org/10.1016/j.anbehav.2009.07.021>

499 Huang P, Sieving KE, St Mary CM (2012) Heterospecific information about predation risk
500 influences exploratory behavior. *Behav Ecol* 23:463–472.
501 <https://doi.org/10.1093/beheco/arr212>

502 Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. *Ecol*
503 *Mono* 54:187–211. <https://doi.org/10.2307/1942661>

504 Kalb N, Anger F, Randler C (2019) Subtle variations in mobbing calls are predator-specific in
 505 great tits (*Parus major*). Sci Rep-Uk 9:6572. [https://doi.org/10.1038/s41598-019-](https://doi.org/10.1038/s41598-019-43087-9)
 506 43087-9

507 Kroodsma DE, Byers BE, Goodale E, Johnson S, Liu WC (2001) Pseudoreplication in
 508 playback experiments, revisited a decade later. Anim Behav 61:1029–1033.
 509 <https://doi.org/10.1006/anbe.2000.1676>

510 Kullberg C, Lind J (2002) An experimental study of predator recognition in great tit
 511 fledglings. Ethology 108:429–441. <https://doi.org/10.1046/j.1439-0310.2002.00786.x>

512 Lendrem DW (1983) Predation risk and vigilance in the blue tit (*Parus caeruleus*). Behav
 513 Ecol Sociol 14:9–13

514 Lind L, Jöngren F, Nilsson J, Schönberg Alm D, Strandmark A (2005) Information, predation
 515 risk and foraging decisions during mobbing in great tits *Parus major*. Orn Fennica
 516 82:89–96

517 Madden JR, Kilner RM, Davies NB (2005) Nestling responses to adult food and alarm calls:
 518 1. Species specific responses in two cowbird hosts. Anim Behav 70:619–627.
 519 <https://doi.org/10.1016/j.anbehav.2004.11.019>

520 Magrath RD, Haff TM, Horn AG, Leonard ML (2010) Calling in the face of danger: how
 521 predation risk affects acoustic communication by parent birds and their offspring. Adv
 522 Stud Behav 41:187–253. [https://doi.org/10.1016/S0065-3454\(10\)41006-2](https://doi.org/10.1016/S0065-3454(10)41006-2)

523 Magrath RD, Haff TM, McLachlan JR, Iqic B (2015a) Wild birds learn to eavesdrop on
 524 heterospecific alarm calls. Curr Biol 25:2047–2050.
 525 <https://doi.org/10.1016/j.cub.2015.06.028>

526 Magrath RD, Haff TM, Fallow PM, Radford AN (2015b) Eavesdropping on heterospecific
 527 alarm calls: from mechanisms to consequences. Biol Rev 90:560–586.
 528 <https://doi.org/10.1111/brv.12122>

- Maloney RF, McLean IG (1995) Historical and experimental learned predator recognition in free-living New Zealand robins. *Anim Behav* 50:1193–1201.
[https://doi.org/10.1016/0003-3472\(95\)80036-0](https://doi.org/10.1016/0003-3472(95)80036-0)
- Marler P (1957) Specific distinctiveness in the communication signals of birds. *Behaviour* 11:13–38. <https://doi.org/10.1163/156853956X00066>
- McIvor GE, Lee VE, Thornton A (2018) Testing social learning of anti-predator responses in juvenile jackdaws: the importance of accounting for levels of agitation. *R Soc Open Sci* 5:171571. <https://doi.org/10.1098/rsos.171571>
- McLean IG, Hoelzer C, Studholme BJS (1999) Teaching predator-recognition to a naive bird: implications for management. *Biol Conserv* 87:123–130. [https://doi.org/10.1016/S0006-3207\(98\)00024-X](https://doi.org/10.1016/S0006-3207(98)00024-X)
- Morand-Ferron J, Hamblin S, Cole EF, Aplin LM, Quinn J L (2015) Taking the operant paradigm into the field: associative learning in wild great tits. *PLoS One* 10:e0133821. <https://doi.org/10.1371/journal.pone.0133821>
- Perrins CM (1965) Population fluctuations and clutch-size in the Great Tit, *Parus major* L. *J Anim Ecol* 601–647. <https://doi.org/10.2307/2453>
- Platzen D, Magrath RD (2004) Parental alarm calls suppress nestling vocalization. *Proc R Soc Lond B Biol Sci* 271:1271–1276. <https://doi.org/10.1098/rspb.2004.2716>
- Potvin DA, Ratnayake CP, Radford AN, Magrath RD (2018) Birds learn socially to recognize heterospecific alarm calls by acoustic association. *Cur Biol* 28:2632–2637. <https://doi.org/10.1016/j.cub.2018.06.013>
- Randler C (2012) A possible phylogenetically conserved urgency response of great tits (*Parus major*) towards allopatric mobbing calls. *Behav Ecol Sociobiol* 66:675–681. <https://doi.org/10.1007/s00265-011-1315-y>

554 Rydén O (1978) Differential responsiveness of great tit nestlings, *Parus major*, to natural
 555 auditory stimuli. *Ethology* 47:236–253. [https://doi.org/10.1111/j.1439-](https://doi.org/10.1111/j.1439-0310.1978.tb01834.x)
 556 [0310.1978.tb01834.x](https://doi.org/10.1111/j.1439-0310.1978.tb01834.x)
 557 Rydén O (1980) Heart rate response in great tit nestlings (*Parus major*) to an alarm call.
 558 *Journal of comparative and physiological psychology*, 94:426.
 559 <http://dx.doi.org/10.1037/h0077680>
 560 Schaller GB, Emlen JT (1961) The development of visual discrimination patterns in the
 561 crouching reactions of nestling grackles. *The Auk* 73:125–137
 562 Shettleworth SJ (2010) Cognition, evolution, and behavior. Oxford University Press.
 563 Shriner WM (1999) Antipredator responses to a previously neutral sound by free-living adult
 564 golden-mantled ground squirrels, *Spermophilus lateralis* (Sciuridae). *Ethology*
 565 105:747–757. <https://doi.org/10.1046/j.1439-0310.1999.00454.x>
 566 Suzuki TN (2011) Parental alarm calls warn nestlings about different predatory threats.
 567 *Current Biology* 21: R15-R16. <https://doi.org/10.1016/j.cub.2010.11.027>
 568 Suzuki TN (2012) Referential mobbing calls elicit different predator-searching behaviours in
 569 Japanese great tits. *Animal Behaviour*, 84(1), 53–57.
 570 <https://doi.org/10.1016/j.anbehav.2012.03.030>
 571 Suzuki TN, Wheatcroft D, Griesser M (2016) Experimental evidence for compositional
 572 syntax in bird calls. *Nat Commun* 7:10986. <https://doi.org/10.1038/ncomms10986>
 573 Suzuki TN, Wheatcroft D, Griesser M (2017) Wild Birds Use an Ordering Rule to Decode
 574 Novel Call Sequences. *Curr Biol* 27:2331–2336. <https://doi.org/10.17632/r7v96zf5pp.1>
 575 Tchernichovski O, Lints T, Mitra PP, Nottebohm F (1999) Vocal imitation in zebra finches is
 576 inversely related to model abundance. *Proc Natl Acad Sci U S A* 96:12901–12904.
 577 <https://doi.org/10.1073/pnas.96.22.12901>

578 Wheatcroft D, Price TD (2013) Learning and signal copying facilitate communication among
579 bird species. *Proc R Soc Lond B Biol Sci* 280:20123070.
580 <https://doi.org/10.1098/rspb.2012.3070>

581 Wheatcroft, D (2015) Repetition rate of calls used in multiple contexts communicates
582 presence of predators to nestlings and adult birds. *Anim Behav*, 103, 35–44.
583 <https://doi.org/10.1016/j.anbehav.2015.02.009>

584 Wheeler BC, FahyM, Barbara Tiddi (2019) Experimental evidence for heterospecific alarm
585 signal recognition via associative learning in wild capuchin monkeys. *Anim Cogn*, 1–9.
586 <https://doi.org/10.1007/s10071-019-01264-3>

587

Acknowledgments We warmly thank the “Fondation Pierre Vérots” (PVF) for giving us access to the field site. We are indebted to JP Rabatel (PVF) for his assistance. We express our gratitude to several students who assisted with the field work. We are grateful to Bernard Kaufmann for English corrections. We thank the editor, Christoph Randler, and anonymous reviewers for constructive comments on an earlier version of this manuscript.

Funding This work was supported by French Ministry of Research and Higher Education funding (to M.D. PhD grants 2015-2018).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Our work was carried out under permission from the Prefecture du Rhône (Ref 2015-13), Prefecture de l’Ain (DDPP01-15-230) and with the approval of the ethics committee at Lyon 1 University, France (permit number: 2017012410184917). All authors are accredited for performing experiments with living animals (French diploma “Experimentation animale” first level for researchers). After ringing, all nestlings were readily accepted back by their parents.

Human and animal rights This article does not contain any studies with human participants performed by any of the authors.

Table 1

Generalized linear mixed models (GLMM) results for playback (associative learning stimulus or mobbing calls) and group (experimental or control) as predictors of variation in fledglings response behaviours (scanning, calling, approaching + fleeing). The “*” character indicates the interaction between the explanatory terms. Significant P values are indicated in bold.

Behaviours	Fixed effects	df	χ^2	<i>P</i>
scanning	playback	1	5.8243	0.0158
	group	1	6.1785	0.0129
	playback*group	1	5.4487	0.0196
calling	playback	1	0.9227	0.3368
	group	1	0.0247	0.8752
	playback*group	1	4.8979	0.0269
approaching + fleeing	playback	1	8.9545	0.0028
	group	1	1.6161	0.2036
	playback*group	1	0.8797	0.3483

FIGURE CAPTIONS

Fig. 1 Number of horizontal scans made by experimental and control fledglings during the playbacks of associative learning stimulus and mobbing calls (mean $\pm$ SE)

Fig. 2 Experimental and control fledglings' probability of calling during playbacks of associative learning stimulus and mobbing calls

Fig. 3 Response of experimental and control fledglings to playbacks of associative learning stimulus (white) and mobbing calls (grey)