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Geographic variation in marmots' alarm calls causes different responses

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Abstract

Geographic variation in acoustic signals has been investigated for five decades to better understand the evolution of communication. When receivers are able to discriminate among signals and to react accordingly, geographic variation can have major impacts on the ability of conspecifics to communicate. Surprisingly, geographic variation in alarm calls and its consequences for the communication process have been so far neglected despite their crucial role on individual survival. Working with four wild populations of Alpine marmots (*Marmota marmota*), we found differences in the acoustic structure of their alarm calls. These differences cannot be explained by geographic or genetic distances but more likely by other mechanisms including random processes. Moreover, playback experiments provided evidence that receivers discriminate between alarm calls from their own *versus* other populations, with responses at lower intensity when the alarm calls played back originated from their own population. Research on the mechanistic causes of geographic variation and on the relationship between alarm call variation, familiarity and intelligibility of signal and behavioral responses is now required to better understand how predation pressure, and more widely natural selection, could drive the evolution of communication.

Significance statement

Dialects (*i.e.* geographic variation) can have major impacts on the ability of conspecifics to communicate. Surprisingly, dialects in alarm calls have been neglected despite their crucial role on survival of individuals. Alpine marmots have dialects in alarm calls and discriminate their own dialects from others, being more frightened by alarm calls from another population than by those from their own. Confronted with an unknown dialect, marmots may adopt a self-preserving strategy and choose to run away before assessing the danger.

Keywords: accent, acoustic communication; dialect; genetic differentiation; geographic variation; alarm call

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Introduction

Considerable geographic variation among populations in acoustic signals, usually termed dialect or accent (Conner 1982), has been documented across the whole animal kingdom (in mammals (Lameira et al. 2010), in birds (Krebs and Kroodsma 1980), in anurans (Velásquez 2014), in fish (Parmentier et al. 2005) and in invertebrates (Zuk et al. 2001), for reviews, see Wilczynski and Ryan 2001, Podos and Warren 2007). Many factors can influence geographic variation in acoustic signals, such as geographic barriers (Thomas et al. 1988; Cleator et al. 1989; Thomas et al. 1992; Perry and Terhune 1999), that may imply environmental differences (habitat structure, background noise, Hunter and Krebs 1979; Wiley and Richards 1982; van Parijs et al. 2003; Nicholls and Goldizen 2006) or variation in selection pressures, notably through sexual selection (*e.g.*, female preferences, Slater 1986). Various processes, such as genetic and/or cultural drifts between isolated populations (Baker 1982; Mundinger 1982; Davidson and Wilkinson 2001; Janik and Slater 2003; Irwin et al. 2008) are involved in geographic variation in acoustic signals, and studying this variation is particularly relevant to understanding divergent evolution in communication systems (Wilczynski and Ryan 2001; Campbell et al. 2010).

Geographic variation of signals implies divergences in the structure of the vocalizations but must also involve detection and discrimination processes by the receiver. For instance, extensive literature on songbirds shows that geographic variation can strongly affect breeding behavior, especially mate attraction and intra-sexual competition (Searcy et al. 2002). Many studies suggest that individuals are able to discriminate among songs from different populations, preferring the signal of their local population (Baker 1982; Searcy et al. 2002; Gray 2005; Boul et al. 2007; Podos 2007; Nicholls 2008; Uy et al. 2009; Bradley et al. 2013; Mortega et al. 2014; Lin et al. 2016); even over geographically close populations (Leader et al. 2002; but see Colbeck et al.

2010; Danner et al. 2011). As such, dialects can act as prezygotic barriers and play an important role in speciation (Baker and Cunningham 1985; Slabbekoorn and Smith 2002; Price 2008; Wilkins et al. 2013). Similar patterns and processes have also been suggested in mammals, although evidence is much more limited (Maeda and Masataka 2010).

To date, geographic variation has been evidenced primarily in social calls and songs, while knowledge about geographic variation in alarm calls and perception of this variation by the receiver remains scarce, even though alarm calls could be as relevant to speciation as acoustic sexual signals because of their direct consequences on the survival of individuals. Alarm calls are often a repetition of a single call unit (Randler et al. 2011; but see for instance complex syntax in mobbing calls, Suzuki et al. (2016) and the complex signal structure in monkey screams, Zuberbühler 2009). Both the structure of the note and its repetition rate can be used to encode information (Manser 2001, but see Rendall et al. 2009). Geographic variation in alarm calls have been documented in several mammalian species (Slobodchikoff et al. 1998; Francescoli 2002; Eiler and Banack 2004; Matrosova et al. 2012; Schlenker et al. 2014; Loughry et al. 2019) while they were absent in others (e.g. in yellow-bellied marmots, *Marmota flaviventris,* Blumstein and Armitage 1997). When present, their perceptual salience remains unknown (Zuberbühler 2009). Hence, the importance of alarm calls variation for the communication process is currently unknown and playback experiments are necessary to determine their biological relevance.

Alpine marmots are cooperatively breeding ground-dwelling territorial squirrels living in family groups. Territory surveillance is undertaken by all the individuals of the family group. Once a predator is detected, marmots can produce alarm calls to warn other individuals before hiding in their burrows (Perrin et al. 1993). Marmot alarm calls are usually composed of one stereotyped and frequency-modulated single note (Perrin et al. 1993). Alpine marmots are naturally distributed in the Alpine arc and the Carpathian mountains and were successfully reintroduced in the Pyrenees from 1948 to 1988.

We investigated the existence of geographic variation in alarm calls produced by Alpine marmots (*Marmota marmota*) from two native populations of French Alps (Vanoise) and two reintroduced in the Pyrenees. One of the reintroduced population originated from the Vanoise and the other from the Mercantour mountain range (Bichet et al. 2016). The comparison between the native populations in the Alps and the reintroduced populations in the Pyrenees is of particular interest to investigate the relationship between signal variation and genetic differentiation, as well as their consequences in terms of between-population recognition. In this study, we investigated whether geographic variation is encoded in the acoustic structure of alarm calls produced by marmots living in the four studied populations. Although dynamic interplay between song learning mechanisms and geographic isolation have been evidenced to be at the origin of geographic variation in bird song (Podos and Warren 2007), the processes underlying the evolution of geographic variation in alarm calls remain to be described. While oscines songs are usually learned (Kroodsma 2004), and display geographic variation likely to result from short-term, cultural, or ecological processes (*e.g.* Ruegg et al. 2006; Halfwerk and Slabbekoorn 2009), rodents alarm call structures are suspected to have a substantial genetic basis (Blumstein 2007; Blumstein et al. 2013) rather than being affected by cultural drift. Therefore, we expected to observe a strong relationship between alarm call variation and genetic differentiation.

We also investigated whether receivers would discriminate among the alarm calls, depending on the population they originated from. We conducted playback experiments to determine whether receivers perceived alarm call variation produced by different populations and changed their antipredatory response. Given that antipredatory response has direct consequences on the survival of individuals but could be costly in terms of time and energy, animals should respond to an alarm call only when relevant. We thus expected marmots to discriminate between alarm calls originating from their own population and alarm calls originating from foreign populations, and to increase their antipredatory response to alarm calls of their population.

Methods

Study populations

We studied Alpine marmots from four populations (Supplementary Material S1): Sassière and Tignes were native populations of the Western Alps (Vanoise mountain range) located 10 km apart, while Cerdanya and Ripollès, located 40 km apart and 500 km away from the native ones, are reintroduced populations from the South-eastern Pyrenees (Couturier 1955; Ramousse et al. 1992). Although all these populations showed a significant genetic differentiation, the source population of Ripollès originated from the Vanoise mountain range and was therefore genetically closer to the native populations (Sassière and Tignes) than Cerdanya, the other Pyrenean population, which was genetically closer to native populations from the Mercantour mountain range (Bichet et al. 2016). All four populations were characterized by short vegetation typical of alpine meadows. In all populations, foxes were present, and hikers were common, sometimes accompanied by dogs on leash. Hunting was forbidden in all populations and no marmot killed by dogs were reported.

Within each population, intensive behavioral observations allowed us to precisely identify family groups and to locate main burrows and territory borders. To conduct genetic analyses, 151 unrelated individuals were captured in these four populations (all the details about the sampling and the genetic analyses are given in the Supplementary Material S2).

Does the acoustic structure of alarm calls differ between populations?

From 2011 to 2014, alarm calls were recorded between mid-April and mid-July from 8:00 am to 6:00 pm, during the main activity period of the marmots. Alarm calls from Sassière were recorded in 2011 and 2012, those from Tignes and Cerdanya in 2013 and those from Ripollès in 2013 and 2014. An omnidirectional microphone (Sennheiser ME62-K6P) connected to a Fostex FR2LE recorder (frequency sampling: 44.1 kHz, resolution: 16 bits) was placed approximately 2 m from the main burrow entrance in order to maximize the chances of recording alarm calls. Once a recording was obtained, we moved to another territory not adjacent to the previous one to record another individual. Marmots typically retreated into their burrows during the setup of the recording material but re-emerged within minutes. Once individuals had resumed their foraging activity, they were exposed to a threatening situation in order to trigger the emission of alarm calls by a focal individual: a human appeared promptly from a hide situated 60 to 80 m away from the burrows and ran in the direction of the marmot. This protocol triggered alarm calls in almost all cases. To avoid recording the same individuals several times, we did not record several individuals within the same territory. Because body size and age might impact frequencies of vocalizations (Tubaro and Mahler 1998; Blumstein and Munos 2005; but see Matrosova et al. 2007), alarm emitters included in the study were all adults, of 3 years old or more (when they reach adult size and stop growing). Given that Alpine marmots are monomorphic, we did not expect sex variation in alarm calls, although sex variation in alarm calls has been found in a dimorphic and closely related species, the yellow-bellied marmot (Blumstein and Munos 2005). If sex variation is present in the alarm call of Alpine marmot, this would add unexplained variance to our analysis and reduce our capacity to detect the effect of geographic variation.

Alarm call records were analysed by the same person (CS) using Avisoft SASlab Pro (version 5.2.09). They were high-pass filtered to remove all noise corresponding to low frequency (*i.e.* less than 1 kHz, mainly wind noise, Hamming windows, FFT 1024pts). Each individual emitting a single calling bout composed of a variable number of alarms calls was recorded. A maximum of 6 alarm calls were processed per calling bout (median: 6, range: 1-6, mean: 4.61, standard deviation: 1.85). From the 37 calling bouts from Sassière, the 23 from Tignes, the 55 from Cerdanya and the 35 from Ripollès, 135, 110, 275 and 145 alarm calls were respectively selected and the modulation of their fundamental frequency was described. For each call, a spectrogram (FFT 1024pts, overlap 93.75%, Hamming windows, frequency resolution, 47Hz) was performed and the frequency modulation pattern was described using a zero crossing analysis (Staddon et al. 1978; Richard 1991). This gives a measure of a full cycle period and thus the instantaneous frequency of the signal. Then, different acoustic parameters that describe the structure of a frequency modulation (see for instance Lengagne 2001) were measured with the same settings (*i.e.* same temporal and spectral windows): the initial, maximum and final frequencies (in kHz) and durations (in ms) of the ascendant (AD), stationary (SD) and descendant (DD) phases of the fundamental frequency (Fig. 1). By doing so, we avoided measuring parameters such as harmonics which are highly altered during the signal propagation in the transmission channel. The maximum and final frequencies were discarded in subsequent analyses due to their strong correlation with the other variables (see Table 1).

Statistical analyses

To test for differences in the acoustic structure of alarm calls between the four populations, we fitted four linear mixed-effect models with either the ascendant phase (AD), the stationary phase (SD), the descendant phase (DD) duration or the initial frequency (F1) as the response variable, the population as a fixed effect and the calling bout (confounded with individual identity in our study) as a random effect on the intercept.

We then tested for potential differences in the acoustic structure (AD, SD, DD and F1) of alarm calls among the four populations using a Linear Discriminant Analysis (LDA). To further compare each pair of populations, we built six other LDAs. To overcome bias due to uneven sample size between individuals and populations, we randomly selected *N* calls per population without replacement with *N* equal to the number of alarm calls of the population with the smallest sample size involved in the comparison. Reclassification rates using cross validation were then calculated with half the data set – the training sample – randomly selected to build the model and the other half – the test sample – used to calculate the percentage of correct classification. The entire procedure, including the random selection of *N* calls/population, was repeated 1,000 times. We calculated the mean of all obtained percentages of correct classification and their 95% confidence intervals. We assumed that the acoustic structure differed between the populations if the percentage of correct classification corresponding to a random classification (%R) was not included in the estimated 95% confidence intervals.

Then, we tested whether the acoustic distance (log-transformed pairwise differences of the x-coordinates of the barycenter for each population in the linear discriminant analysis with the four populations) between populations positively correlated with either the genetic distance (pairwise F_{ST} values linearized as $F_{ST}=F_{ST}/(1-F_{ST})$; Weir and Cockerham 1984) or the geographic distance (log-transformed linear distance in kilometres, see the Supplementary Material S2) between the populations using one-tailed Mantel tests (all permutations, Mantel 1967).

Do marmot responses to alarm calls vary depending on the population of origin of the emitter?

Preparation of acoustic stimuli

Alarm calls previously recorded from Tignes, Sassière and Cerdanya were used to carry out a playback experiment on marmots from Sassière and Tignes in 2015. To limit pseudoreplication, we used 7 different alarm call bouts from 7 different individuals for each of the three populations. Hence, we used 21 different alarm call bouts during our playback experiments to avoid behavioral responses of marmots to be due to the uniqueness of one soundtrack. We selected bouts with the best signal to noise ratio to ensure an efficient propagation of the signal during playback tests. Each bout was composed of 5 randomly chosen alarm calls emitted by the same individuals and separated on average by 0.5s of silence (range 0.46 - 0.58s). The silence duration between calls was adjusted to obtain a bout duration of 3s thus mimicking the average bout duration previously observed in our study.

Electroacoustic material and playback test

To our knowledge the amplitude of alarm calls of Alpine marmots has never been measured accurately (*i.e.* by taking into account the precise distance between the exact position of the head of the emitter and the sound level meter). Hence, we decided to match by ear the amplitude of the calls broadcast to a natural call amplitude during a pilot study, with the estimation of the call amplitude at 20m from the speaker. This amplitude was then measured with a sound level meter Lutron SL-4001, (C weighting, slow settings at 1m in front of the loudspeaker) and corresponded to an average value of 100 dB(C). This amplitude is close to the value measured on yellow-bellied marmot (95-100 dB, Lea and Blumstein 2011). To perform playback tests, we used a speaker (SMC8060 Beyma amplified loudspeaker) connected to a Fostex FR2LE. The speaker was placed on the ground in the upper part of the focal territory at 20-30m of the main burrow entrance. Marmots typically retreated into their burrows during the installation but they re-emerged within minutes and usually went back to the same place. Once marmot displayed a normal activity such as foraging between 5 m and 10 m away from any burrow entrance and at 10-25m from the loud-speaker, an observer placed outside of the focal family group triggered the alarm call playback (beginning of the trial). Once the alarm call played, observers filmed the focal adult individual with a digital video camera (Sony® Handycam model DCR-DVD650 or JVC® digital video model GZ-E 209). The trial was considered completed when the focal individual entered into a burrow, resumed a normal activity (*i.e.* foraging) or 5 minutes after the beginning of the trial.

Between mid-May and mid-July, 2015, from 8:00 am to 6:00 pm, 43 tests were performed in Sassière (16, 17 and 10 tests with alarm calls from Sassière, Tignes and Cerdanya respectively) and 34 tests were done in Tignes (12, 8 and 13 tests with alarm calls from Tignes, Sassière and Cerdanya respectively). To avoid habituation as well as pseudoreplication, each territory was tested once with a playback bout from Cerdanya, Sassière or Tignes (*i.e.* a given marmot could never be tested more than once under the same experimental setting) and two neighboring territories were never tested successively. Moreover, to avoid a neighbor-stranger effect (e.g. Wei et al. 2011; Hyman 2005, reviewed in Stoddard 1996) and to use unfamiliar calls to the receiver we chose carefully the soundtrack used for each test to ensure that it was recorded in another part of the population, at least 500m away from the tested marmot (*i.e.* receiver).

Video processing

All video records were displayed in AVS Video Editor (version 7.1) in slow motion (x0.25 resulting in 6 frames per second) by a unique observer (CS), blind to playback treatment, to ensure an accurate identification of behaviors as well as to record their duration with an accuracy of 0.01s. In one case out of 77, more than a single animal was present, we thus conducted the statistical analyses with and without this trial but given that the results were qualitatively identical and quantitatively similar, we chose to keep this trial in the analyses. We collected the occurrences (coded as a binary outcome) of vigilance, the occurrences of flight/running, the occurrences of entrance into a burrow, the time (in s) spent vigilant and the time (in s) until the focal individual resumed foraging whenever possible. Vigilance behavior was defined as any posture where marmots were standing on their rear feet, or standing on their four feet but suddenly putting their head up and maintaining it above the horizontal plane of their body. Flight/running was defined as an escape-related behavior towards a burrow entrance and was considered to be a more extreme response than any vigilance posture.

Statistical analyses

To test whether the response to alarm calls depends on the population of the emitter, the occurrence of flight and of the entry to a burrow were entered as response variables in two generalized linear models (GLMs) with a logit link and the variance given by a binomial distribution. Since in nearly all playback trials (72 of 77) individuals became vigilant, the frequency of this behavior could not be considered. The time spent vigilant and the time before resuming a normal activity were further entered as response variables in two other GLMs with a logarithmic link and the variance given by a Gamma distribution. In the last model, we categorized the intensity of the focal individual response in four categories: no response, vigilance, flight and entry in a burrow. We entered this ordinal variable as the response variable in an ordered logistic regression model. In each of these five models, we first tested whether the responses were different when the playback alarm calls originated from the population of the focal individual or from another population by entering the origin of the playback (two-modalities factor: same or other population) in interaction with the population of the focal individual (Tignes or Sassière) as explanatory fixed variables. Second, in five other models, we tested whether the geographic distance between the population of the focal individual and the population of the signaler could further impact the responses by entering the origin of the playback (three-modalities factor: same, close, remote) in interaction with the population of the focal individual (Tignes or Sassière) as explanatory fixed variables. Since interactions were not significant, only additive effects are presented in the results. The absence of interactive effects indicates that Tignes and Sassière (focal individuals) behave similarly when they are faced towards the same and other populations (five first models) or towards the same, close and remote populations (five other models).

All statistical analyses conducted in this study were performed using the R software version 3.4.4 (R Core Team 2018) and the packages 'vegan' (Oksanen et al. 2018), 'nlme' (Pinheiro et al. 2018), 'multcomp' (Hothom et al. 2008), 'ade4' (Dray and Dufour 2007) and 'MASS' (Venables and Ripley 2002).

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Results

Does the acoustic structure of alarm calls differ among populations?

Marmot alarm calls from the four populations differed significantly in their initial frequencies ($F_{3, 146} = 2.63$, $P = 0.05$), ascendant phase durations ($F_{3, 146} = 11.33$, $P < 0.001$), stationary phase durations ($F_{3, 146} = 7.27$, $P < 0.001$) and in their descendant phase durations ($F_{3, 146} = 3.49$, $P = 0.02$).

Despite a strong overlap among the four populations when compared altogether (Supplementary Material S3), alarm calls were always assigned more often to the population their emitter originated from than to any other population. Calls were correctly classified in 44.14 [41.14; 47.05] % of the cases (while the percentage of random classification would have been 25%). When populations were compared two by two, alarm calls were once again always better attributed to the population their emitters originated from than to the other one (percentage of random classification: 50%). Percentages of correct classification ranged from 57.04 [54.07; 59.63] % to 76.18 [71.82; 80.91] % when comparing Sassière with Ripollès and Tignes with Cerdanya respectively (Table 2). All the acoustic variables (initial frequencies, ascendant, stationary and descendant phase durations) contributed to the discrimination among the populations, but they seemed to contribute differently to the variation between each pair of populations (Fig. 2).

The acoustic distances between two populations was neither explained by their genetic distance (Mantel Spearman = -0.37, N = 6, p = 0.76) nor by their geographic distance (Mantel Spearman = -0.26, N = 6, p = 0.80). Moreover, there is no evidence of genetic isolation by distance in our four populations (*i.e.* the genetic distances and geographic distances were not correlated, Mantel Spearman = 0.43, N = 6, p = 0.17).

Do marmot responses to alarm calls vary depending on the population of

origin of the emitter?

Marmots exhibited lower intensity responses to alarm calls from their own population than to alarm calls from another one (close or remote) (β = -1.23 ± 0.52, z = -2.40, N = 76, p = 0.02). The odds of a marmot showing a higher response decreased by a factor of 0.29 [0.10; 0.78] when the alarm calls originated from its own population compared to another one. More specifically, marmots showed a significantly lower propensity to flee (same = 57.14% *vs.* another = 83.33%, β = -1.45 ± 0.58, z = -2.50, N = 76, p = 0.01, Fig. 3-a) when the alarm calls originated from their own population than from another one (close or remote). The rest of our results were not significant, marmots were as likely to enter their burrow (same = 7.14% *vs.* another = 16.67%, β = -0.96 ± 0.83, z = -1.15, N = 76, p = 0.25, Fig. 3-b), to remain vigilant (same = 23.37s *vs.* another = 34.58s, β = 0.002 ± 0.006, z = 0.40, N = 74, p = 0.68, Fig. 3-c), and to resume a normal activity (same = 24.88s *vs.* another = 39.35s, β = 0.003 ± 0.005, z = 0.49, N = 68, p = 0.62, Fig. 3-d) when the alarm calls originated from their own population or from another one. The intensity of the response was similar for alarm calls produced in a close or in a remote population (odds ratio = 0.73 [0.19, 2.64], β = -0.31 ± 0.66, z = -0.47, N = 76, p = 0.64). The propensity to flee (close = 80.00% vs. remote = 86.96%, β = -0.20 ± 0.83, z = -0.24, N = 76, p = 0.81, Fig. 3-a), or to enter a burrow (close = 88.00% vs. remote = 78.26% , β = -0.71 ± 0.80, z = -0.89, N = 76, p = 0.37, Fig. 3-b), the amount of time spent vigilant (close = 34.58s *vs.* remote = 31.9s, β = -0.02 ± 0.33, z = -0.06, N =74, p = 0.95, Fig. 3-c) and elapsed time before resuming a normal activity (close = 38.16s *vs.* remote =39.35s, β = -0.001 ± 0.006, z = -0.29, N =68, p = 0.77, Fig. 3-d) did not vary with the geographic distance between the focal and the other population. Marmots of different populations behave similarly since no significant interaction between the population of the focal individual (Tignes or Sassière) and the origin of the playback was detected.

Discussion

In the present study we described for the first time the acoustic structure of alarm calls produced by adult Alpine marmots from two native and two reintroduced wild populations. We found that the acoustic structure of Alpine marmots' alarm calls differed among the four populations and that neither the genetic distance nor the geographic distance explained these differences. Moreover, the playback experiments provided evidence that receivers discriminate among alarm calls from their own *versus* other populations.

Although alarm calls are a stereotyped signal, we found that the acoustic structure of Alpine marmots' alarm calls differed among the four studied populations. The presence of geographic variation in acoustic signals has been shown repeatedly in rich vocal repertoires such as in bird songs (Nottebohm 1969; Mundinger 1982; Zimmermann et al. 2016). The richness of avian vocal repertoires offers a wide range of possibilities for geographic variation in note combinations, presence or absence of some notes and/or rhythm of emission of different notes (Baker and Jenkins 1987; Handford 1988; Shieh et al. 2013). However, such variation has been found also within single stereotyped acoustic elements (e.g. those produced by bottlenose dolphins, *Tursiops spp.*, Campbell 2004). In line with Campbell's (2004) study, we found that the structure of alarm calls, short of 0.2 s, differed among marmot populations.

Furthermore, we found a clear difference in the structure of alarm calls used by two populations originating from two mountain ranges but also between populations separated by only 10 km (*i.e.* Sassière and Tignes). Acoustic variation is well documented at a large spatial scale (Lougheed and Handford 1992; Wilczynski and Ryan 1999; Shizuka et al. 2016), but they can also be observed at a microgeographic scale. Leader et al. (2008) documented geographic variation in orange-tufted sunbird (*Nectarinia osea*) song within two sub-populations separated by 100 m and a sharp boundary. Studies conducted simultaneously at different geographic scales remain scarce, especially in mammals. To the best of our knowledge, only three studies on a phylogenetically close species to the Alpine marmot, the Gunnison's prairie dog (*Cynomys gunnisoni*), have documented both large and microgeographic differences in alarm calls (Slobodchikoff and Coast 1980; Slobodchikoff et al. 1998; Perla and Slobodchikoff 2002), although a recent study contradicts those results (Loughry et al. 2019).

We found that multiple sets of acoustic variables stood out as different between each pair of populations. This lack of consistency suggests that the evolution of alarm call characteristics could be explained by multiple selective forces or random processes rather than by a unique selective process. Four main hypotheses (*i.e.* genetic distance, learning local adaptation and random processes) have been proposed to explain the existence of geographic variation. In the present study, we did not find any correlation between the acoustic and the geographic distances or between the acoustic and the genetic distances. The peculiar status of our studied populations is unlikely to explain such a lack of relationship: the two reintroduced Pyrenean populations, although originating from Alpine populations, are farther away both geographically and genetically than the two native Alpine populations (Sassière and Tignes) are from each other (Bichet et al. 2016).

The learning hypothesis attributes acoustic variation among populations to the colonization of a new area by young individuals before they have learned the song structure from their parents (Thielcke 2008) or from social learning (Wich et al. 2012). The learning process is unlikely to generate the pattern we observed since the founders caught for the reintroduction events in Pyrenees were all adults (Ramousse and Le Berre 1995). Moreover, a genetic basis of vocalizations has been shown in several mammals including yellow-bellied marmots (e.g. Scherrer and

Wilkinson 1993; McCowan and Hooper 2002; Blumstein 2007).

Local adaptation resulting from differences in habitat characteristics or morphology could result in the signal of geographic variation we observed. Geographic variation could result from the optimization of sound transmission within the local environment (Morton 1975). Signal alteration depends on vegetation cover density, atmospheric turbulence, height above the ground at which a signal is transmitted (Wiley and Richards 1978; Lengagne et al. 1999) and the intensity and the quality of the background noise (Ryan and Brenowitz 1985; Lengagne and Slater 2002). In our study, although we did not measure vegetation coverage, the predominant vegetation formation (high altitude alpine meadows) is similar in all populations. Vegetation is thus unlikely to affect alarm call propagation here, as it was also concluded in a previous study on four other species of marmots (Daniel and Blumstein 1998). Background noise level has been hypothesized to influence signal evolution (e.g. in speckled ground squirrels, Matrosova et al. 2016). The Tignes population is located in a mountain resort disturbed by anthropogenic noises but we did not observe higher minimum or maximum frequencies in the alarm calls recorded in Tignes compared to the other less-disturbed populations. Alternatively, the geographic variation observed in alarm calls may be due to mechanistic processes involved in sound production. For instance, in a study between two (Sassière and Cerdanya) of the four studied populations significant differences in both morphology and body mass have been found (Ferrandiz-Rovira et al, in prep) and could explain the differences observed in alarm calls. Further studies are needed to firmly establish a direct link between morphology and alarm call structure.

Finally, rather than consider alarm call geographic variation due to selective forces, this variation could also be due to random processes. The intra-population variance found in this study may indeed be due to the effect of different stochastic processes that occurred in each population, which could have led to the existence of divergent alarm calls.

As fleeing in response to alarm calls should provide a selective advantage by increasing survival, marmots should react to all alarm calls despite discrimination abilities (but see trade-off between vigilance and foraging, Lima and Dill 1990). Surprisingly, our tests revealed that receivers perceived acoustic differences and discriminated between local and foreign alarm calls. Such behavioral consequences imply that these acoustic differences are meaningful for them (Soha et al. 2016). In our study, marmots reacted more strongly to playback of alarm calls originating from foreign populations. Moreover, the intensity of the response was similarly heightened for alarm calls produced in a close or in a remote population, suggesting that these signals are similarly perceived. Many studies focusing on male territorial defense or female attraction in birds provide contrasted results about geographic variation perception: stronger response to local songs in some cases, absence of preferences or mixed responses in others (see Becker 1983; Catchpole and Slater 2008). However, studies on whether animals distinguish and react differently to conspecific alarm calls originating from different populations were missing. Our study suggests that mammals may discriminate between calls recorded in their own population and calls recorded in other populations and react accordingly.

Experimental approaches with alarm call playbacks emphasized that intensity of animal response varies according to the familiarity (Hare and Warkentin 2012) or to the intelligibility of the signal. The classical familiar *versus* unfamiliar discrimination cannot explain our results because all animals were tested with unknown signals (*i.e.* signals never heard before either due to its novelty or unfamiliarity), even when originating from their own population. However, familiarity *sensu lato* could explain our results. Indeed, calls emitted by conspecific living within a same population could sound more familiar to the receivers than calls emitted by conspecific from another population, even if the receivers do not have prior knowledge about the identity of the signaler, as would be for humans the dialect of our natal region compared to another dialect.

Also, the fact that tested marmots reacted more strongly to alarm calls from other populations could be due to the fact that the existing alarm call differences among populations could make alarm calls hardly intelligible for individuals hearing alarm calls from another population. Thus, unintelligible signals could lead individuals to react more strongly to signals from other populations than their own as a precautionary reaction. If an individual is not sure to have understood the message of an alarm call (*i.e.* kind, localization and immediacy of the danger) but reacts accordingly it has higher chances to save its life in case of a real danger. On the contrary, if an individual hears an intelligible alarm call message (*i.e* from their own population) it can quickly assess the situation and, once proven that no danger is around because no predator was present during the playback trials, goes back to its normal behavior.

Finally, predator characteristics and associated perceived threats are known to alter alarm calls (Dutour et al. 2016, 2017). Indeed, signal characteristics could differ according to predator characteristics (Zuberbühler 2009). For instance, mammals can produce different alarm calls for aerial versus terrestrial predators (e.g. in mustached tamarins, *Saguinus mystax*, Kirchhof and Hammerschmidt 2006) or for the level of perceived threats (e.g. in bonnet macaques, *Macaca radiata*, Coss et al. 2007). Although this is unlikely to explain the observed geographic variation in marmots because alarm calls were elicited by a human at the same distance from burrows (60-80 m). However, we cannot definitely rule out that marmots had different perceptions of this same danger in the different populations, that they could modify the structure of their alarm call accordingly and that receivers' behavior was altered in response.

The existence of geographic markers in acoustic signals could arise from both random and selective processes that may act via genetic, cultural transmission or local adaptation pathways. In the context of alarm calls, further research on the proximate causes of geographic variation, the perception of dangers and the resulting emitted signals as well as the following behavioral responses of the receivers are now required to better understand how predation pressure and natural selection could drive the evolution of communication.

Electronic supplementary material

The online version of this article contains supplementary material, which is available to authorized users.

Ethical statement

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Fieldwork was conducted under permit number AP n82010/121 by the Préfecture de la Savoie. All handling and sampling were done by three co-authors (M.F.R, A.C and I.F) who are authorized for experimentation with animals by the French Ministry of Agriculture and Fisheries or Catalan Government (diplomas 0ETRY20090520, R45GRETAF110 and 53707-UAB-FELASA). The protocol was approved by the ethical committee of the University of Claude Bernard Lyon 1 (n8BH2012-92 V1).

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FIGURE LEGENDS

Fig. 1 Description of the acoustic structure of an Alpine marmot alarm call. (a) Spectrogram of a calling bout composed of two calls (FFT 1024pts, windows Hamming, overlap 100%). The colours represent the amplitude of the sound from blue (low amplitude) to red (high amplitude). (b) Tracking of the fundamental frequency of an alarm call by zero-crossing allowed us to measure three temporal parameters (in s): duration of the ascendant phase (AD), stationary phase (SD) and descendant phase (DD) and three frequency parameters (in Hz): initial (F1), maximum (F2) and final (F3) frequencies.

Fig. 2 Linear Discriminant Analysis (LDA) on the ascendant (AD), stationary (SD) and descendant (DD) phases' durations and the initial frequencies (F1) between each pair of populations: Sassière *versus* Tignes (a); Cerdanya *versus* Ripollès (b); Sassière *versus* Cerdanya (c); Sassière *versus* Ripollès (d); Tignes *versus* Cerdanya (e); and Tignes *versus* Ripollès (f). The plots on the left part of the figure represent the first axis of the LDA. The plots on the right part of the figure represent the contribution of the different acoustical variables to the discrimination between the considered pair of populations.

Fig 3 Flee proportion (a), entry in burrow proportion (b), time spent vigilant (in s) (c) and time before resuming normal activity (in s) (d) in relation to the geographic distance between the population of the receiver and the emitter (same, close - *i.e.* a geographically close population being Sassière and Tignes - and remote - *i.e.* a geographically remote population being Cerdanya). The black dots show the trials conducted in Sassière and the white dots show the trials conducted at Tignes. The error bars represent standard errors. Marmots showed a significantly lower propensity to flee ($\beta = -1.45 \pm 0.58$, $z = -2.50$, $N = 76$, $p = 0.01$, (a)) when the alarm calls originated from their own population than from a close or a geographically remote population. The other results were non significant.

TABLES AND TABLE LEGENDS

Table 1 Pearson's correlation coefficients between acoustic variables (N = 665 alarm calls). *: 0.05 < P > 0.01; **: 0.01 < P > 0.001; ***: P < 0.001.

	Maximum frequency	Final frequency	Ascendant phase	Stationary phase	Descendant phase
Initial frequency	0.56***	0.56***	-0.14***	-0.02 (P=0.58)	0.12**
Maximum frequency		0.99***	0.39***	0.10**	0.32***
Final frequency			0.39***	0.10*	0.32***
Ascendant phase				0.06 $^{(P=0.09)}$	0.28***
Stationary phase					-0.02 $^{(P=0.68)}$

Table 2 Percentage of correct classification and acoustic structure (AD, SD, DD and F1) correlations with the Linear Discriminant Analysis (LDA) canonical axis of the LDAs comparing each pair of studied populations. N: number of calls; AD: ascendant phases' durations; SD: stationary phases' durations; DD: descendant phases' durations; F1: initial frequency.

Compared populations	Mean correct classification [95% CI] (%)	Mean correlation [95% CI] of acoustical structures with the first canonical axis of each LDA				Pairwise differences in LD functions
		AD	SD	DD	F1	
Sassière *vs.* Tignes (N=110)	68.25 [65.45; 70.91]	0.33 [0.27; 0.46]	-0.41 [-0.49; -0.33]	0.87 [0.83; 0.92]	0.17 [0.07; 0.26]	46.51
Cerdanya *vs.* Ripollès (N=145)	69.89 [66.90; 73.10]	0.78 [0.71; 0.84]	0.60 [0.51; 0.69]	0.27 [0.16; 0.39]	-0.14 [-0.27; -0.02]	42.64
Sassière *vs.* Cerdanya (N=135)	67.60 [64.44; 70.74]	0.78 [0.69; 0.86]	0.63 [0.53; 0.73]	-0.03 [-0.17; 0.10]	-0.55 [-0.65; -0.44]	40.74
Sassière *vs.* Ripollès (N=135)	57.04 [54.07; 59.63]	0.03 [-0.07; 0.12]	0.06 [-0.02; 0.17]	-0.62 [-0.70; -0.54]	-0.81 [-0.87; -0.75]	42.15
Tignes *vs.* Cerdanya (N=110)	76.18 [71.82; 80.91]	0.89 [0.83; 0.94]	0.23 [0.10; 0.39]	0.63 [0.53; 0.73]	-0.32 [-0.44; -0.20]	46.90
Tignes *vs.* Ripollès (N=110)	66.00 [62.73; 69.55]	0.50 [0.36; 0.61]	-0.50 [-0.61; -0.38]	0.75 [0.67; 0.83]	-0.34 [-0.46; -0.21]	44.66

FIGURES

Fig. 1

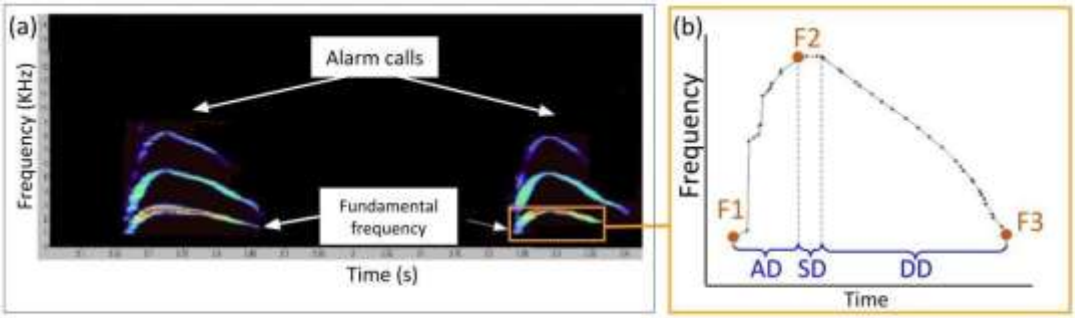


Fig. 2

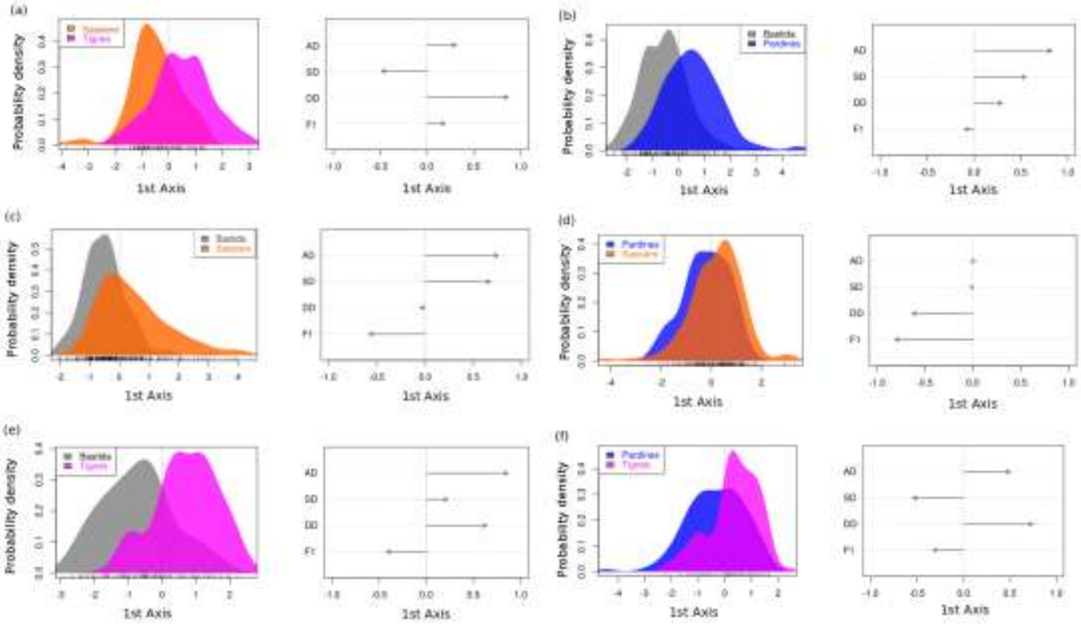
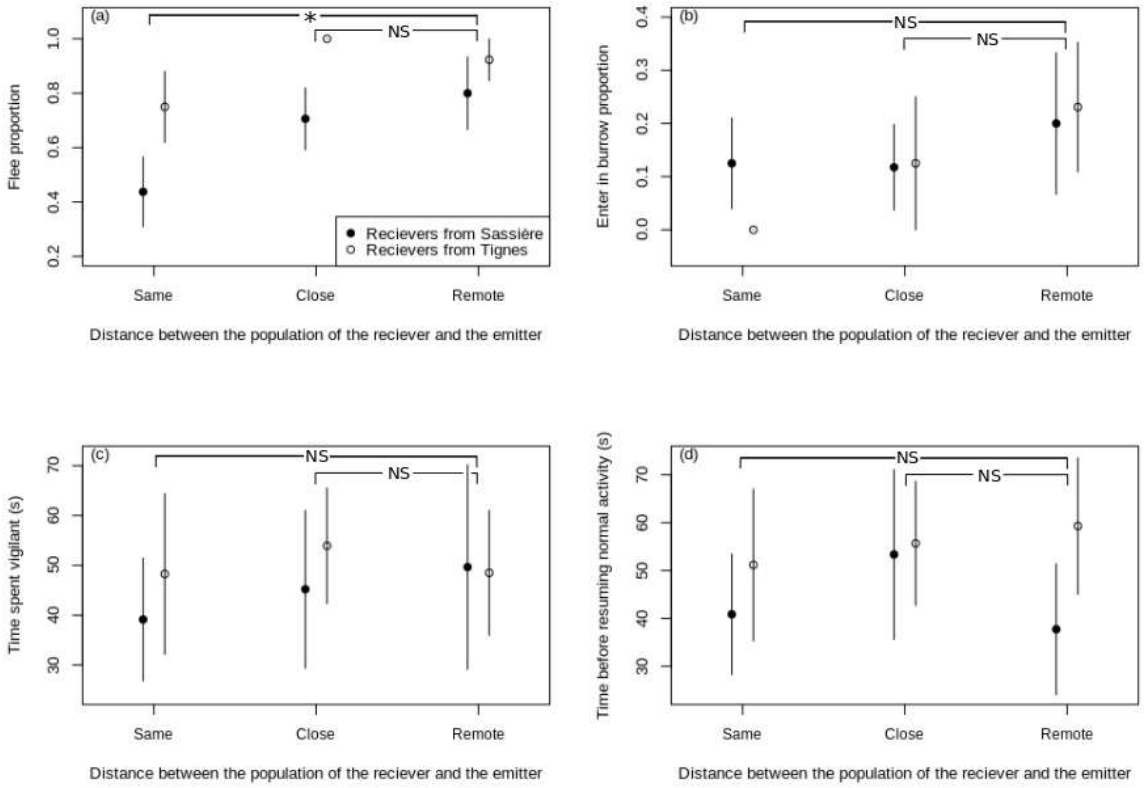


Fig. 3


(a)
Flee proportion
0.2
0.4
0.6
0.8
1.0
*
NS
● Recievers from Sassière
○ Recievers from Tignes
Same
Close
Remote
Distance between the population of the reciever and the emitter
(b)
Enter in burrow proportion
0.0
0.1
0.2
0.3
0.4
NS
NS
Same
Close
Remote
Distance between the population of the reciever and the emitter
(c)
Time spent vigilant (s)
30
40
50
60
70
NS
NS
Same
Close
Remote
Distance between the population of the reciever and the emitter
(d)
Time before resuming normal activity (s)
30
40
50
60
70
NS
NS
Same
Close
Remote
Distance between the population of the reciever and the emitter