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Geographic variations in marmots' alarm calls cause different responses

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Abstract

Population differences in acoustic signals, have 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 differences can have major impacts on the ability of conspecifics to communicate. Surprisingly, population differences in alarm calls and their consequences on the communication process have been so far neglected despite their crucial role on individuals' survival. Working with four wild populations of Alpine marmots (*Marmota marmota*), we found differences in the acoustic structure of their alarm calls. These differences can neither be explained by geographic, nor genetic distances but rather by other mechanisms including random processes. Moreover, playback experiments provided evidence that receivers discriminate among alarm calls from their own *versus* other populations, with responses being lower in intensity when the call bout played back originated from their own population. Research on the mechanistic causes of geographical markers and on the relationship between geographical variation, reliability of the signal and behavioural responses are now required to better understand how predation pressure and natural selection could drive the evolution of communication.

Significance statement

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

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

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Introduction

Considerable geographic differences among populations in acoustic signals, usually termed dialects, geographic markers or geographic variations (Conner 1982), have been documented across the whole animal kingdom (in mammals (Lameira et al. 2010); in birds (Krebs et al. 1980); in anurans (Velásquez 2014); in fish (Parmentier et al. 2005); in invertebrates (Zuk et al. 2001); for review, see (Wilczynski and Ryan 2001). This signal variation could arise from various processes, such as genetic and/or cultural drifts between isolated populations (Baker 1982; Davidson and Wilkinson 2001; Mundinger 1982; Janik and Slater 2003), and are particularly relevant to our understanding of the divergent evolution in communication systems (Campbell et al. 2010; Wilczynski and Ryan 2001). Signals have been shown to be influenced by many factors, including geographic barriers (Thomas et al. 1988; Cleator et al. 1989; Thomas et al. 1992; Perry and Terhune 1999), environment (, habitat structure, background noise (Hunter and Krebs 1979; Wiley and Richards 1982; van Parijs et al. 2003; Nichols and Goldizen 2006)) and sexual selection (*e.g.*, female preferences (Slater 1986)).

Signal geographic variation implies a structural variation from the emitter point of view but must also involve detection and discrimination processes by the receiver to have a major impact on the ability of conspecifics to communicate. For instance, an extensive literature on songbirds shows that dialects can strongly affect breeding behaviour, especially mate attraction and intra-sexual competition (Searcy et al. 2002). Many studies suggest that individuals discriminate among songs from conspecific populations, preferring the signal of their local population in a context of mate choice and intra-sexual competition (Baker 1982; Searcy et al 2002; Gray 2005; Boul et al 2007; Podos 2007; Nichols 2008; Uy et al. 2009; Bradely et al. 2013; Mortega et al.

2014; Lin et al. 2016); even in close populations (Leader et al. 2002; but see Colbeck et al. 2010; Danner et al. 2011). Dialects can then act as pre-zygotic 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 been suggested in mammals, although evidence is much more limited (Maeda and Masataka 2010).

Studies investigating geographic variation in acoustic signals and its perception in behavioural contexts other than sexual selection in avian species remain scarce (Charrier et al. 2013). Alarm calls, shaped by natural selection, could be as relevant to speciation as acoustic sexual signals because of their direct consequences on individuals' survival. Despite this crucial role, dialects in alarm calls have been so far poorly investigated because they are not as complex as bird nuptial and territorial songs. Although some of them are sophisticated (e.g., syntax in mobbing calls (Suzuki 2016); complex signal structure in monkey screams (Zuberbühler 2009)), alarm calls are often a repetition of one same call unit (Randler et al. 2011). Both the structure of the note and its repetition rate can be used to encode information (Manser 2001, but see Rendall et al. 2009). Nevertheless, it has been shown that alarm calls with highly stereotyped structures can convey information about the age or the sex of the emitter (Blumstein and Muñoz 2005). Geographic differences in alarm calls have been documented in several mammalian species (Slobodchikoff et al 1998; Matrosova et al. 2012; Schlenker et al. 2014, Francescoli 2002; Eiler and Banack 2004) but were found sometimes absent in yellow-bellied marmots, *Marmota flaviventris* (Blumstein and Armitage 1997). When present, their perceptual salience remains unknown (Zuberbühler 2009). Hence the importance of such variation for the communication process is currently unknown and further playback experiments are necessary to determine their biological relevance.

We investigated the existence of structural differences in alarm calls produced by Alpine marmots (*Marmota marmota*) from two native (Alps) and two reintroduced (Pyrenees) populations, and whether receivers discriminate among them. Alpine marmots are cooperatively breeding ground-dwelling territorial squirrels living in family groups. Territory surveillance is insured by all individuals from a family and, once a predator is detected, marmots can produce alarm calls warning 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). 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 divergence and genetic differentiation, as well as their consequences in terms of between-population recognition. We first investigated whether geographic variations are encoded in the acoustic structure of alarm calls produced by marmots from the four studied populations. Although dynamic interplay between song learning mechanisms and geographic isolation have been evidenced to be at the origin of dialects in birds songs (Podos and Warren 2007), the processes underlying the evolution of alarm call dialects remains to be described. Moreover, due to the fact that songs in oscines are usually learned (Kroodsma 2004), song dialects have been found to result from short-term, cultural, or ecological processes rather than to have a genetic basis (*e.g.*, Halfwerk and Slabbekoorn 2009; Ruegg et al. 2006). Conversely, alarm calls structure have a substantial genetic basis (Blumstein 2007; Blumstein et al. 2013) and consequently, cultural drift is unlikely to occur. Therefore, we expect to observe a strong relationship between alarm call divergence and genetic differentiation between the two reintroduced populations, which are geographically and genetically isolated from the two native populations (Bichet et al. 2016). We also conducted playback experiments to determine whether receivers perceived the differences in alarm calls produced by different populations and changed their anti-predatory response.

Methods

Ethical Note

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 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).

Study populations

We studied Alpine marmots from four populations (Supplementary Material S1): Sassière and Tignes are native populations of the Western Alps located 10 km apart, while Cerdanya (La Bastida) and Ripollès (Pardines) are reintroduced (from 1948 until 1988, Couturier 1955; Ramousse et al. 1992) populations situated 500 km away from the first ones in the South-eastern Pyrenees and which are 45 km apart. Ripollès is genetically closer to the two native populations than Cerdanya (Bichet et al. 2016). Within each population, intensive behavioural 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).

Does the acoustic structure of alarm calls differ between populations?

Each year (2011-2014), alarm calls were recorded in the four populations between mid-April and mid-July from 8:00 a.m. to 6:00 p.m. the main activity period of the marmots. 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 a main burrow entrance in order to maximize the chances to record alarm calls. Once a recording was performed, we moved to another territory not adjacent to the previous one to record another individual (Sassière: N = 38 alarm calls; Tignes: N =31; Cerdanya: N = 92 and Ripollès; N =35), Marmots typically retreated into their burrows during the setup of the recording material but they re-emerged within few minutes. Then, individuals were exposed to a threatening situation in order to trigger the emission of alarm calls by a focal individual: a human or a human with a domestic dog (*Canis lupus familiaris*) appeared promptly from a hide situated 60 to 80 m away from burrows and ran in the direction of the marmot. This protocol worked in almost all cases. Alarm callers were aged up to 3 years (when they reach adult size and stop growing), which enabled us to distinguish three age classes: pups, yearlings and adults. Since body size and age might impact frequencies of vocalizations neither pups' nor yearlings' calls were analysed (Tubaro and Mahler 1998; Blumstein and Munos 2005; but see Matrosova et al. 2007). As marmots are monomorphic, it was not possible to sex the recorded emitters. Hence, if sex variation is present in the alarm call of Alpine marmot, there will likely be "noise" in our analysis. A sex difference could bias our analysis at the population level in case of (i) an acoustic difference among male and female which is currently not known in Alpine marmot, (ii) a large difference in the proportion of male and female responding to alarm call playback in our studied populations, and, (iii) a reverse proportion of males and females recorded according to populations. Although an absence of literature does not mean that sex does not play a role in alarm calls. Currently, we do not have evidence for none of the above, but we are confident that sex information did not bias our analysis.

Alarm call recordings were analysed using Avisoft SASlab Pro (version 5.2.09). They were low-pass filtered to remove all noise corresponding to low frequency (*i.e.* less than 1 kHz, mainly wind noise, Hamming windows, FFT 1024pts). The number of calling bouts recorded varied between individuals and whenever it was possible we processed a maximum of 6 alarm calls per calling bout. From the 38 calling bouts from Sassière, the 31 from Tignes, the 92 from Cerdanya and the 35 from Ripollès, we selected 137, 143, 459 and 145 alarm calls, and described the modulation of their fundamental frequency. For each call, we performed a spectrogram (FFT 1024pts, overlap 93.75%, Hamming windows, frequency resolution, 47Hz) and we then researched the frequency modulation pattern through a zero crossing analysis. All analysis were performed with the same settings (i.e. same measurements accuracy). The same person (CS) measured on zero crossing the initial, maximum and final frequencies (in kHz) and the durations (in ms) of the ascendant (AD), stationary (SD) and descendant (DD) phases of the fundamental frequency (Fig. 1). By doing so, we avoided to measure parameters which are highly altered during the propagation.

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 as a random effect on the intercept. The maximum and final frequencies were discarded due to their strong correlation with the other variables (see Table 1).

We then tested for potential differences in the acoustical 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 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 a 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) between populations correlated with either the genetic distance (linearized $F_{ST}=F_{ST}/(1-F_{ST})$) or the geographic distance (log-transformed) using Mantel tests (all permutations, Mantel 1967) implemented in the package vegan (Oksanen et al. 2018). Acoustic distances between populations were quantified as the pairwise differences in the linear discriminant functions. The genetic distances were quantified using pairwise F_{ST} values (Weir and Cockerham 1984) and the geographic distances as the linear distance in kilometres (see the Supplementary Material S2).

Does marmots' response to alarm calls vary depending on the population of origin of the emitter?

Between mid-May and mid-July, 2015, a playback experiment was carried out on marmots from La Sassière and Tignes from 8:00 a.m. to 6:00 p.m. Each individual was tested once with a call from Cerdanya, Sassière or Tignes. Forty-three tests were performed in La Sassière (16, 17 and 10 tests with alarm calls from La Sassière, Tignes and La Cerdanya respectively) and 34 tests were done in Tignes (12, 8 and 13 tests with alarm calls from Tignes, La Sassière and La Cerdanya respectively). To limit pseudoreplication, we picked up and broadcasted 7 recordings of calls (See previous section "Does the acoustic structure of alarm calls differ between populations?") for each of the three populations used. We chose signals with a high signal to noise ratio so as the response observed cannot be due to eventual uniqueness of one soundtrack. Moreover to avoid a neighbour-stranger effect we chose carefully the sound track used for each test to ensure that it was recorded in another part of each population, away from the tested marmot (at least 500m). To avoid habituation of the tested marmots due to repetitions of trials over a short time, the same calling bout was never displayed more than once to a given territory and two neighbouring territories were never tested successively.

Each playback bout was composed of 5 alarm calls within 3 s, mimicking an average natural bout (measurement performed on 30 bouts recorded in our study). The amplitude of alarm calls of Alpine marmots has not been measured accurately before (*i.e.* the precise distance between the emitter and the sound level meter, taking the exact position of the head of the marmot into account). Hence, we decided to match by ear the amplitude of the calls used during our test to a natural call. Alarm calls were emitted at 100 dB(C) using a speaker (SMC8060 Beyma amplified loudspeaker) connected to a Fostex FR2LE, measurement done with a Lutron SL-4001, C weighting, slow settings). This amplitude is closed to the value measured on yellow-bellied marmot *Marmota flaviventris* (95-100 dB (Lea and Blumstein 2011)).

The speaker was placed on the ground in the upper part of the focal territory directed towards the main burrow entrance. Marmots typically retreated into their burrows during the installation but they re-emerged within minutes. Once a marmot was between 5 m and 10 m away from any burrow entrance and displayed a normal activity such as foraging, an observer placed outside the focal family group triggered the alarm call playback (beginning of the trial). At the same time, observers filmed the focal adult individual of the focal family group 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.

All video recordings were displayed in AVS Video Editor (version 7.1) in slow motion (x0.25) by a unique observer, blind to playback treatment, to ensure an accurate identification of behaviours as well as to record their duration with an accuracy of 0.01s. In one case over 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 kept this experiment in the analyses. We collected the occurrence (coded as a binary outcome) of vigilance, flight/running, entrance into a burrow, time spent vigilant and time until the focal individual resumed foraging. Vigilance behaviour 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 behaviour towards a burrow entrance and was considered to be a more extreme response than any vigilance posture, without necessarily entering in the burrow.

Statistical analyses

To test whether the response to alarm calls depends on the origin of the signal, the occurrence of flight and of the entry in 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 over 77), individuals became vigilant, the frequency of this behaviour 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 response of the focal individual in four categories: no response, vigilance only, flight and entry in a burrow; and we entered this ordinal 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 call originated from the population of the focal individual or from another population by entering the origin of the playback as a twomodalities factor (same or other population) in interaction with the population of the focal individual (Tignes or Sassière) as explanatory variables. Second, we tested whether the geographic distance between the focal individual and the signaller could further impact the responses by entering the origin of the playback as a three-modalities factor (same, close, far) in interaction with the population of the focal individual (Tignes or Sassière) as explanatory variables. All analyses were performed using the R software (Version 3.1.1) and packages 'nlme' (Pinheiro et al. 2018), 'multcomp' (Hothom et al. 2008), 'ade4' (Dray and Dufour 2007), 'MASS' (Venables and Ripley 2002).

Results

Does the acoustic structure of alarm calls differ among populations?

Marmot alarm calls from the four populations differed significantly in their initial frequencies ($F_{1, 190} = 4.75$, $P < 0.01$), ascendant phases' durations ($F_{3, 190} = 11.58$, $P < 0.001$), stationary phases' durations ($F_{3, 190} = 9.57$, $P < 0.001$) but not in their descendant phases' durations ($F_{3, 190} = 2.45$, $P = 0.07$).

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. Effectively, calls were correctly classified in 41.96 [35.71; 48.35] % 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 62.49 [54.17; 69.79] % to 71.64 [63.70; 79.41] % when comparing Tignes with Ripollès and Tignes with Cerdanya respectively (Table 2). All the acoustical variables (initial frequencies, ascendant, stationary and descendant phases' durations) contributed to the discrimination among the populations, but they seemed contribute differently to the differentiation between each pair of populations (Fig. 2).

The acoustic distances between two populations was neither explained by their genetic distance (Spearman = -0.77, N = 6, p = 0.92) nor by their geographic distance (Spearman = -0.71, N = 6, p = 1.00). Moreover, there is no evidence of genetic isolation by distance in our four populations (*i.e.* the genetic distances and the geographic distances were not correlated, spearman's Rho = 0.43, N = 6, p = 0.17).

Does marmots' response to alarm calls vary depending on the population of origin of the emitter?

Marmots exhibited lower intensity responses to an alarm call from their own population than to an alarm call from another population ($\beta = -1.23 \pm 0.52$, 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 call originated from its own population compared to another one. More specifically, marmots showed a significantly lower propensity to flee ($\beta = -1.45 \pm 0.58$, z = -2.50, N = 76, p = 0.01, fig. 3-a) when the alarm call originated from their own population than from nearby *versus* geographically remote population. Although the rest of our results were not significant, marmots tended to be less likely to enter their burrow (7.14 ± 1.86% *vs.* 16.67 + 6.67%, $\beta = -0.96 \pm 0.83$, z = -1.15, N = 76, p = 0.25, fig. 3-b), to remain vigilant for a shorter time (median = 23.37s *vs.* 34.58s, β = 0.002 ± 0.006, z = 0.40, N = 74, p = 0.68, fig. 3-c), and to resume a normal activity more rapidly (median = 24.88s *vs.* 39.35s, β = 0.003 ± 0.005, z = 0.49, N = 68, p = 0.62, fig. 3-d) when the alarm call originated from their own population. The intensity of the response was similar for alarm calls produced in a nearby or in a geographically distant 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 (β = -0.20 ± 0.83, z = -0.24, N = 76, p = 0.81, fig. 3-a), to enter in a burrow (near: 88.00 + 2.64% vs. far: 78.26 + 3.91%, β = -0.71 ± 0.80, z = -0.89, N = 76, p = 0.37, fig. 3-b), the amount of time spent vigilant (near = 34.58s *vs.* far = 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 (near = 38.16s *vs.* far =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.

Discussion

In the present study we described for the first time the acoustic structure of alarm calls produced by adult Alpine marmots in two native and two reintroduced wild populations. We found that the acoustic structure of Alpine marmots alarm calls differed among the four populations, which enabled us to assign calls described by four acoustic parameters to their population of origin greater than by chance. Neither the genetic distance nor the geographic distance explained the acoustic differences between populations. Finally, the playback experiments provided evidence that receivers discriminate among alarm calls from their own *versus* other populations. Surprisingly, intensity of marmots' responses was lower when the playback calling bout originated from their own population than when it came from another population. Again, these responses did not differ depending on the genetic nor on the geographic distances.

Although alarms calls are a stereotyped signal, we found that the acoustic structure of Alpine marmots alarm calls differ among the four studied populations. Indeed, using four acoustic parameters to describe each alarm call it was possible to assign the signal to the population their emitters originated from with accuracy exceeding that expected by chance. The presence of geographic markers in acoustic signals have been shown repeatedly in bird songs (Nottebohm 1969; Mundinger 1982; Zimmermann et al. 2016). The richness of avian vocal repertories offer a wide range of possibilities for differentiation of geographic markers: changes in note combinations, presence or absence of some notes (particularly during new population finding) and/or rhythm of emission of different notes (Baker and Jenkins 1987; Handford 1988; Shieh et al. 2013). Nevertheless, such markers have also been found within single stereotyped acoustic elements, e.g. those produced by bottlenose dolphins, *Tursiops spp.* (Campbell 2004). Similarly to Campbell's (2004) study, we found that the general shape of acoustic elements, as short as 0.2s, differed among marmot populations.

We further found a clear difference in the shape of alarm calls used by two populations originating from two mountain ranges but also between populations separated by only 5 km (i.e., Sassière and Tignes). Acoustic divergence is well documented at a large spatial scale (Lougheed and Handford 1992; Wilczynski and Ryan 1999). For instance, Shizuka et al. (2016) reported the existence of 13 discrete song types in golden-crowned sparrows (*Zonotrichia atricapilla*) over 3,100 km in Alaska. But, geographic variations in acoustic signals can also be observed at a microgeographic scale. Leader et al. (2008) documented dialects in orange-tufted sunbird (*Nectarinia osea*) within two sub-populations separated by a sharp boundary but only 100 m apart from each other. Studies conducted at these different geographic scales still remain scarce in mammals. At least two studies working on a phylogenetically close species to the Alpine marmot, the Gunnison's prairie dog (*Cynomys gunnisoni*), have documented large or microgeographic differences in acoustic signals (Slobodchikoff et al. 1998; Perla and Slobodchikoff 2002).

Genetic distance has been repeatedly hypothesized to explain acoustic differences among populations (Wilczynski and Ryan 1999). However, in the present study, we did not find any correlation between the acoustic and the geographic distances or between the genetic and the acoustic distances. The peculiar status of our studied populations is unlikely to explain such a lack of relationship: the two reintroduced Pyrenean populations originated from Alpine populations that were farther away both geographically and genetically than the two Alpine populations considered here (Bichet et al. 2016). While Balaban (1988) found a correlation between signal characteristics and genetic distance in two populations of swamp sparrow (*Melopsiza georgiana*), a lack of congruence in divergence of acoustic signal and genetic characteristics has been repeatedly observed in birds (Hafner and Petersen 1985; Payne and Westneat 1988; Wright and Wilkinson 2001).

Four main hypotheses (i.e., learning, morphology and body mass differences, antipredator strategies and local adaptation) have been proposed to explain the occurrence of dialects. The learning hypothesis attributes acoustic differences among populations to the colonization of a new area by young individuals before they have learned the song structure from their parents (Thielcke 2008). Baker and Jenkins (1987) invoked this cultural bottleneck to explain the presence of dialects in isolated populations of chaffinches. However, an interspecific cross-fostering experiment with ground squirrels failed to provide evidence for the learning hypothesis (Matocha 1975). Although the failure to learn allospecific calls does not preclude learning the nuances of conspecific calls, the learning process is probably not very important in marmots. Moreover, the founders caught for the reintroduction events in Pyrenees were all adults. For all these reasons, the learning hypothesis is unlikely to explain geographic variation in the acoustic structure of alarm calls found in our study.

Among other factors that could lead to geographic variation, indirect selective pressures on acoustic signals may alter vocalization characteristics (Podos 2001). In Alpine marmot, mechanistic processes may cause the alarm call divergence. For instance, significant differences in both morphology and body mass between two (Sassière and Cerdanya) of the four studied populations have been found (Ferrandiz-Rovira et al, in prep). Mechanical links between morphology and acoustic signals exist in many groups (e.g. in anurans (Hoskin et al 2009; Lengagne 2017), in birds (Nowicki 1987; Fletcher and Tarnopolski 1990)). For example, a study conducted on Darwin's finches showed that the adaptive evolution of beaks for feeding also influences the acoustic structure of their songs (Podos 2001). Although less investigated, such link has also been found for alarm calls in the speckled ground squirrel (*Spermophilus suslicus*, Matrosova et al. 2012). Nevertheless, further studies are needed to firmly establish a direct link between morphology and alarm call structure.

Antipredator strategies such as the use of alarm call is also known to strongly interact with predator dangerousness and predation risk (Dutour et al. 2016,2017). Indeed, signal characteristics will differ according to predator characteristics (Zuberbülher, 2009). For instance, mustached tamarins (*Saguinus mystax*) produce different alarm calls for aerial *versus* terrestrial predators (Kirchhof and Hammerschmidt 2006) or for the level of perceived threat (Coss et al. 2007). In the present study, this is unlikely to explain the observed marmots' geographic variation because alarm calls have been elicited by a human approach at the same distance from burrows (60-80m) and hunting was forbidden in the different marmot populations.

In agreement with the local adaptation hypothesis, dialects could also result from the optimization of sound transmission within local environment along various transmission channel (Morton 1975). Signal alteration depends on vegetation cover density, atmospheric turbulence, or height above the ground at which a signal is transmitted (Wiley and Richards 1978; Lengagne et al. 1999). Moreover, information transfer efficiency is linked to the intensity and the quality of the background noise (Ryan and Brenowitz 1985; Lengagne and Slater 2002). In birds, a study on dialect suggested that call structure have been shaped by local propagation conditions (Doutrelant et al. 1998) and another study with Gunnison's prairie dog emphasized a link between alarm call structure and habitat characteristics: geographic variant calls resulted in significant differences in transmission performance through different environments (Perla and Slobodchikoff 2002). Nevertheless, in our case, although we did not measure vegetation coverage, the predominant vegetation formation consisted in high altitude alpine meadows which should be quite similar from a population to another. Vegetation is thus unlikely to affect alarm call propagation here. Hence, a minimal effect of the propagation conditions on the evolution of alarm call structure in the four studied populations is expected in agreement with previous studies on marmots conducted by Daniel and Blumstein (1998). Background noise level, one major component of the transmission channel also influence signal evolution. One way to improve signal efficiency in such case is to avoid overlapping with low frequency corresponding to background noise and thus to modify signal spectrum towards high pitched frequencies. Anthropogenic effects on alarm calls had already been hypothesized to explain differences in alarm calls' dialects of speckled ground squirrels (Matrosova et al. 2016). We observed important noise differences among the studied populations. The "Tignes" population is located in a mountain resort and could be considered as disturbed by anthropogenic noises (traffic, helicopter…). However, we did not observe higher, minimum or maximum frequencies in the alarm calls recorded in Tignes compared to the other studied populations for which there was no evidence of environmental noise.

In addition to direct or indirect selection processes we cannot preclude the fact that acoustic differences observed among populations were due to a random process. Consistent with this explanation, we found that multiple sets of acoustical variables stoodd out to discriminate between each pair of populations. This lack of consistency suggests that evolution of alarm call characteristics may not be explained by a unique selective process.

As fleeing in response to alarm calls should provide a selective advantage by increasing survival, one predicts that 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 categorized alarm calls as local or non-local calls. Such behavioural consequences imply that these acoustic differences are meaningful for them (Soha et al. 2016). Most studies focused on male territorial defence or female attraction in birds and their results are contrasted with stronger response to local songs in some cases, absence of preferences or mixed responses in others (see Becker 1983; Catchpole and Slater 2008). In an alarm context, experimental approaches with playbacks emphasized that intensity of animal response varies according to the past reliability of the signaller (Cheney and

Seyfarth 1988)) or to the familiarity of the members of the colony (Hare and Warkentin 2012). Nevertheless, no study has been conducted so far to determine whether animals discriminate and react differently to conspecific alarm calls according to its population (*i.e.* geographical origin). Our results thus show for the first time that animals discriminate among calls recorded in different populations and react accordingly. We expected that a divergence in the alarm signal induce an alteration of alarm information and, according to Hanson and Coss (2001), we expected a reduced behavioural response to alarm calls of non-local congeners in tested animals. Surprisingly, behavioural responses obtained after playback of alarm calls from different populations showed the reverse. Indeed, animals reacted more strongly to playback of alarm calls originating from foreign populations. Moreover, the intensity of the response was similar for alarm calls produced in a nearby or in a geographically distant population suggesting that these signals represent an equally threatening situation for animals. Our results may be explained by a difference in the predator pressure or predator strategy (Dutour et al. 2016). Even though we did not measure predator pressure precisely, the two main predators of marmots, the golden eagle (*Aquila chrysaetos*) and the red fox (*Vulpes vulpes*), were present in the four studied population. Hence, predator pressure is unlikely to explain differences observed in marmot responses to playback. Previous experiments on rodents showed that they discriminate alarm calls from neighbours (*i.e.* familiar) *versus* unfamiliar individuals. In the present study, marmots displayed a stronger response to alarm calls originating from foreign populations compared to their native one. Nevertheless, this familiar *versus* unfamiliar discrimination process cannot explain our results because all animals were tested with unknown signals even when they were tested with calls of their own population. Our results may be explained by the fact that marmots estimate the information reliability received and reacted accordingly. Blumstein et al. (2004) observed a stronger response to unreliable than reliable alarm calls in yellow-bellied-marmot (*Marmota flaviventris)* and suggests that such difference was due to the fact that a marmot hearing an unreliable alarm call makes its own independent assessment of relative risk and thus invests more in antipredator behaviour (but see opposite results in Richardson's ground squirrels *Spermophilus richardsonii*: Hare and Atkins 2001).

The presence of geographic markers in acoustic signals involved in a context of mate choice and intra-sexual competition have been repeatedly found in avian species (Nottebohm 1969; Mundinger 1982; Zimmrmann et al. 2016). Both random and selective processes linked to sexual selection that may act via genetic or cultural transmission pathways could explain their origin. In the context of alarm, further research on the proximate causes of geographical markers as well as on the relationship between geographical markers, reliability of the signal and behavioural responses 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.

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FIGURE LEGENDS

Fig. 1 Description of the acoustic structure of an Alpine marmot alarm call. (a) Sonogram of a calling bout composed by 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), enter in burrow proportion (b), time spent vigilant (c) and time before resuming normal activity (d) in relation to the 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.

TABLES AND TABLE LEGENDS

Table 1 Pearson's correlation coefficients between acoustic features (N = 894 alarm calls). *: 0.10 < P > 0.05; **: 0.05 < P > 0.001; ***: P < 0.001.

	Maximum frequency	Final frequency	Ascendant phase	Stationary phase	Descendant phase
Initial frequency	0.55***	0.67***	-0.17***	-0.02 (P=0.58)	0.09**
Maximum frequency		0.74***	0.36***	0.08**	0.30***
Final frequency			0.09**	0.07*	0.03 (P=0.36)
Ascendant phase				0.05 (P=0.11)	0.30***
Stationary phase					0.01 (P=0.84)

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=137)	65.03 [56.04; 73.63]	0.35 [0.30; 0.40]	-0.51 [-0.56; -0.47]	0.77 [0.73; 0.80]	0.19 [0.13; 0.25]	44.45
Cerdanya *vs.* Ripollès (N=145)	65.13 [56.25; 73.96]	0.69 [0.58; 0.78]	0.61 [0.48; 0.72]	0.13 [-0.04; 0.31]	-0.24 [-0.41; -0.07]	42.21
Sassière *vs.* Cerdanya (N=137)	64.41 [56.04; 72.53]	0.63 [0.51; 0.75]	0.58 [0.47; 0.68]	-0.18 [-0.35; -0.01]	-0.65 [-0.75; -0.54]	41.10
Sassière *vs.* Ripollès (N=137)	62.95 [54.95; 71.43]	0.04 [-0.02; 0.09]	0.06 [0.01; 0.12]	-0.36 [-0.41; -0.30]	-0.52 [-0.56; -0.47]	43.08
Tignes *vs.* Cerdanya (N=153)	71.64 [63.70; 79.41]	0.90 [0.84; 0.95]	0.13 [-0.02; 0.28]	0.50 [0.38; 0.61]	-0.47 [-0.58; -0.35]	41.85
Tignes *vs.* Ripollès (N=145)	62.49 [54.17; 69.79]	0.47 [0.43; 0.51]	-0.57 [-0.61; -0.53]	0.55 [0.51; 0.59]	-0.38 [-0.43; -0.33]	44.56

FIGURES

Fig. 1

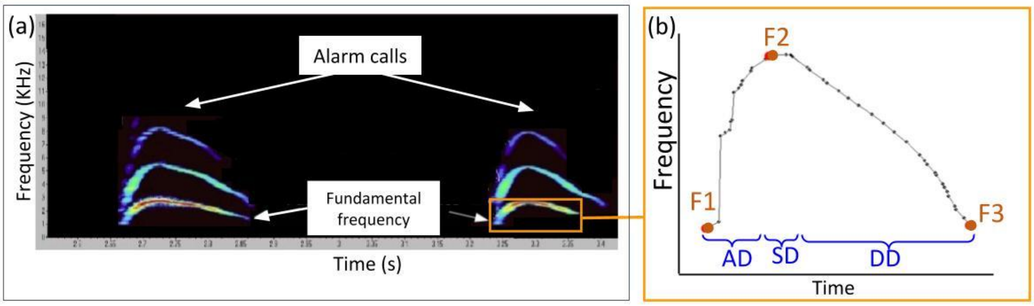


Fig. 2

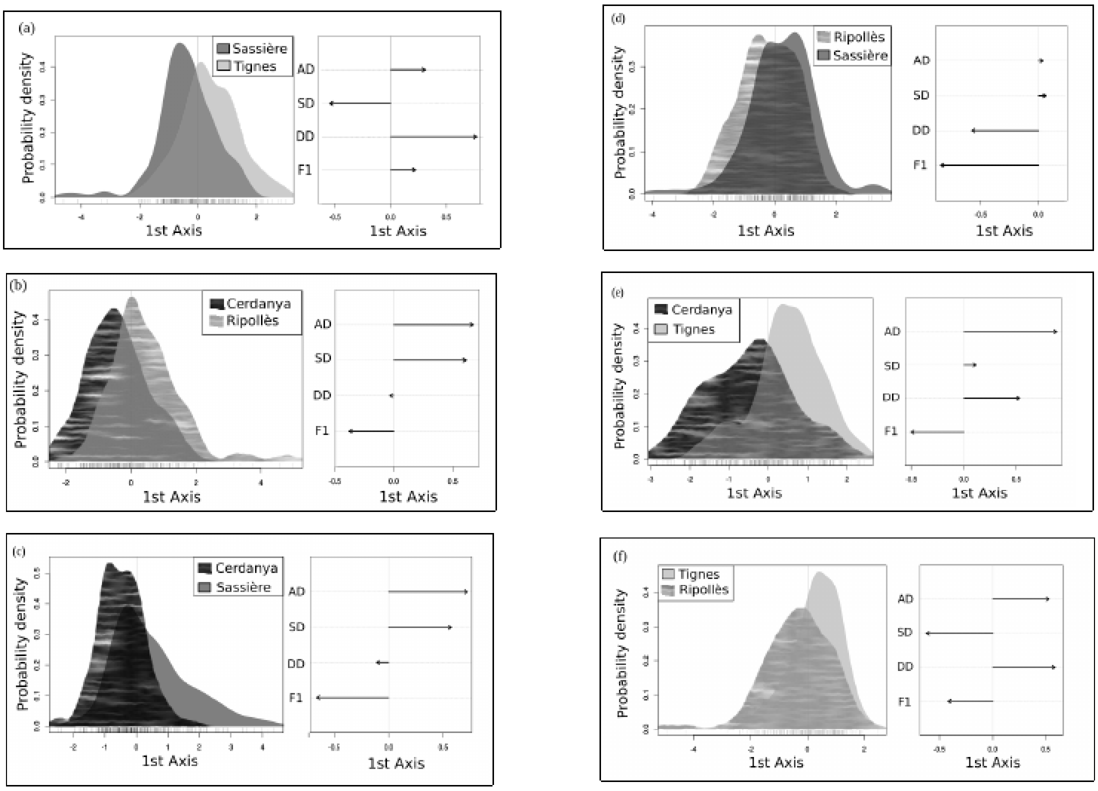

(a)
Sassière
Tignes
Probability density
1st Axis
AD
SD
DD
F1
1st Axis
(b)
Cerdanya
Ripollès
Probability density
1st Axis
AD
SD
DD
F1
1st Axis
(c)
Cerdanya
Sassière
Probability density
1st Axis
AD
SD
DD
F1
1st Axis
(d)
Ripollès
Sassière
Probability density
1st Axis
AD
SD
DD
F1
1st Axis
(e)
Cerdanya
Tignes
Probability density
1st Axis
AD
SD
DD
F1
1st Axis
(f)
Tignes
Ripollès
Probability density
1st Axis
AD
SD
DD
F1
1st Axis

Fig. 3

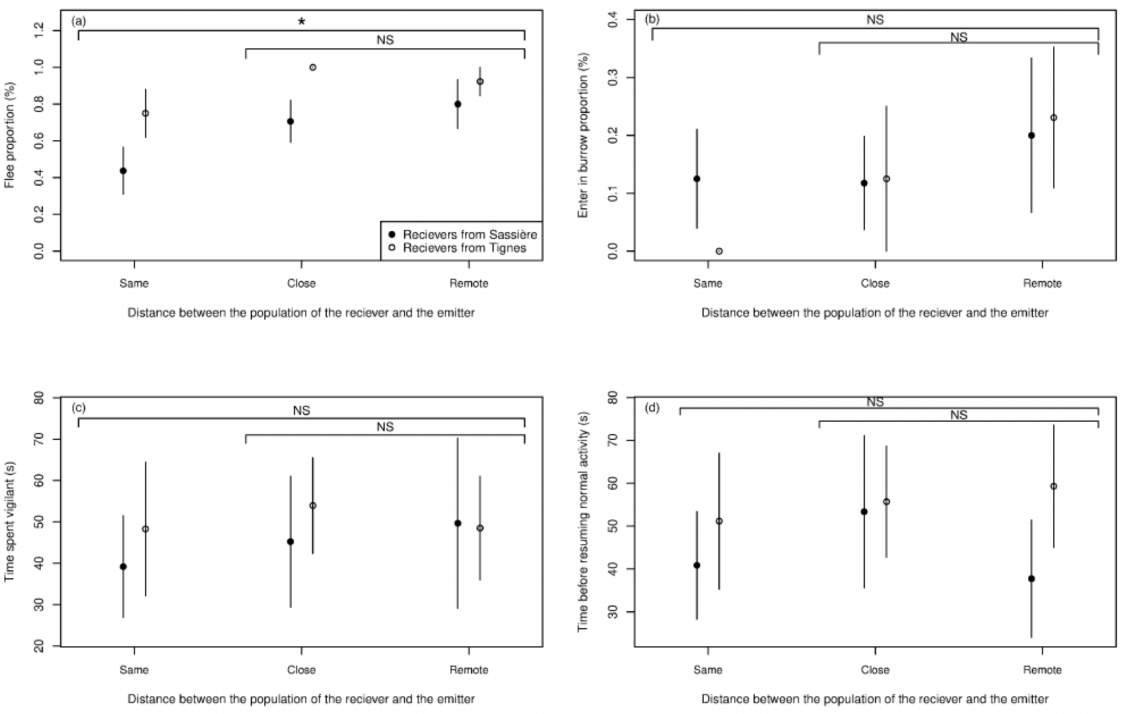

(a)
*
NS
Flee proportion (%)
Recievers from Sassière
Recievers from Tignes
Same
Close
Remote
Distance between the population of the reciever and the emitter
(b)
NS
NS
Enter in burrow proportion (%)
Same
Close
Remote
Distance between the population of the reciever and the emitter
(c)
NS
NS
Time spent vigilant (s)
Same
Close
Remote
Distance between the population of the reciever and the emitter
(d)
NS
NS
Time before resuming normal activity (s)
Same
Close
Remote
Distance between the population of the reciever and the emitter

Figure 1

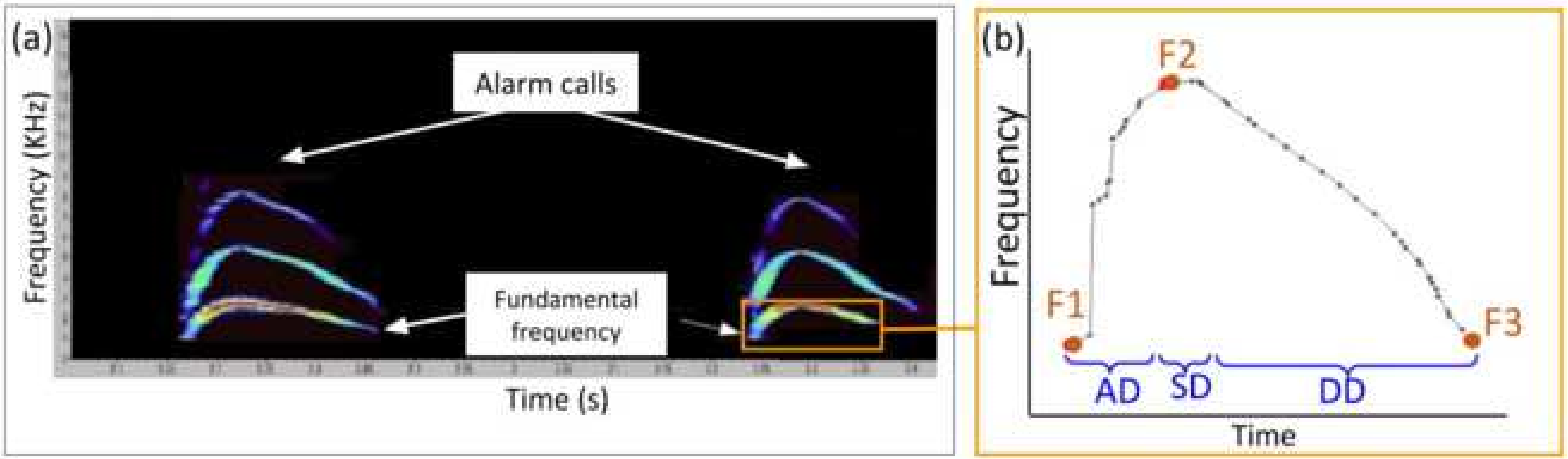

(a)
Alarm calls
Fundamental
frequency
Frequency (KHz)
Time (s)
(b)
F2
F1
F3
AD
SD
DD
Frequency
Time

Figure 2

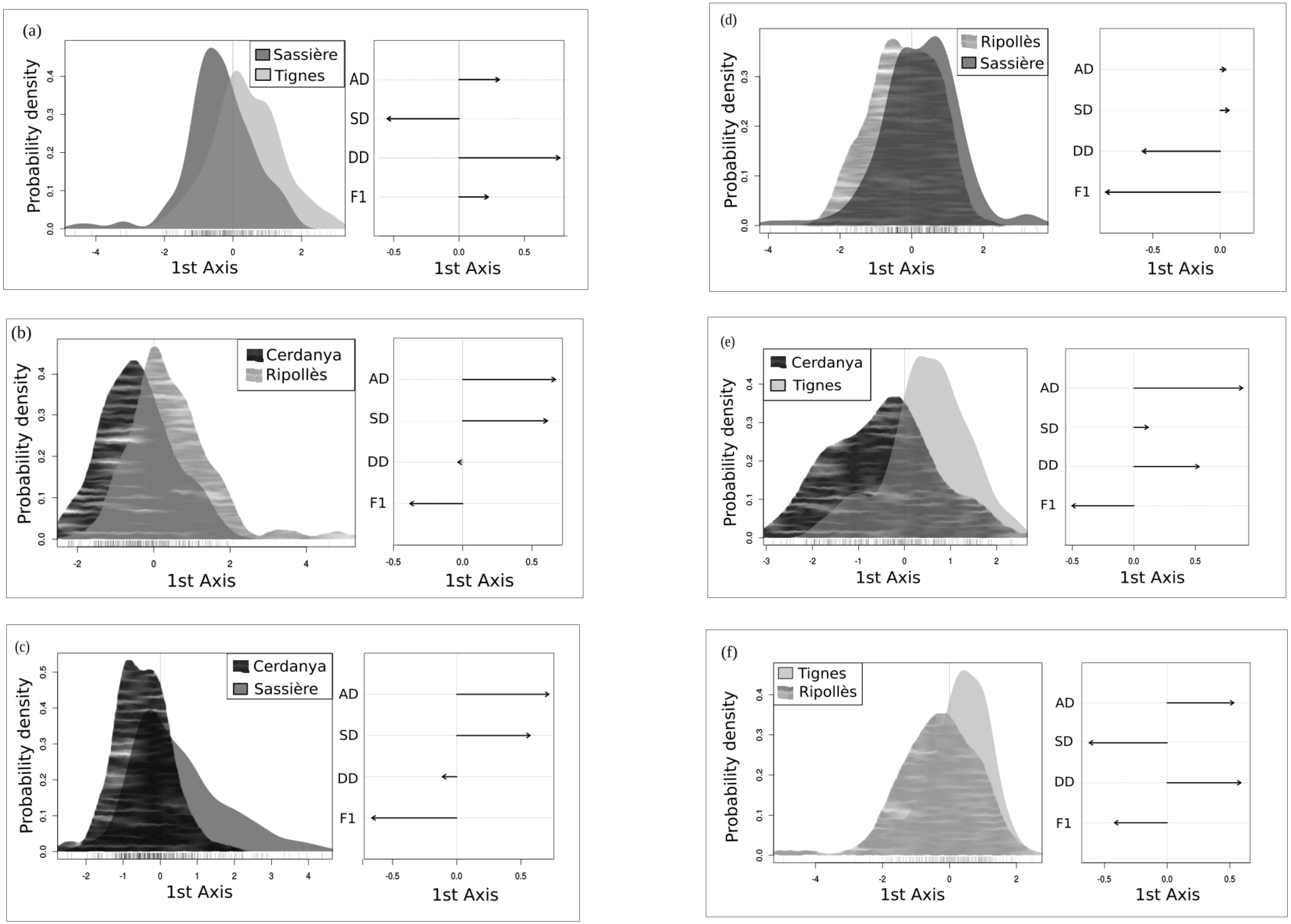
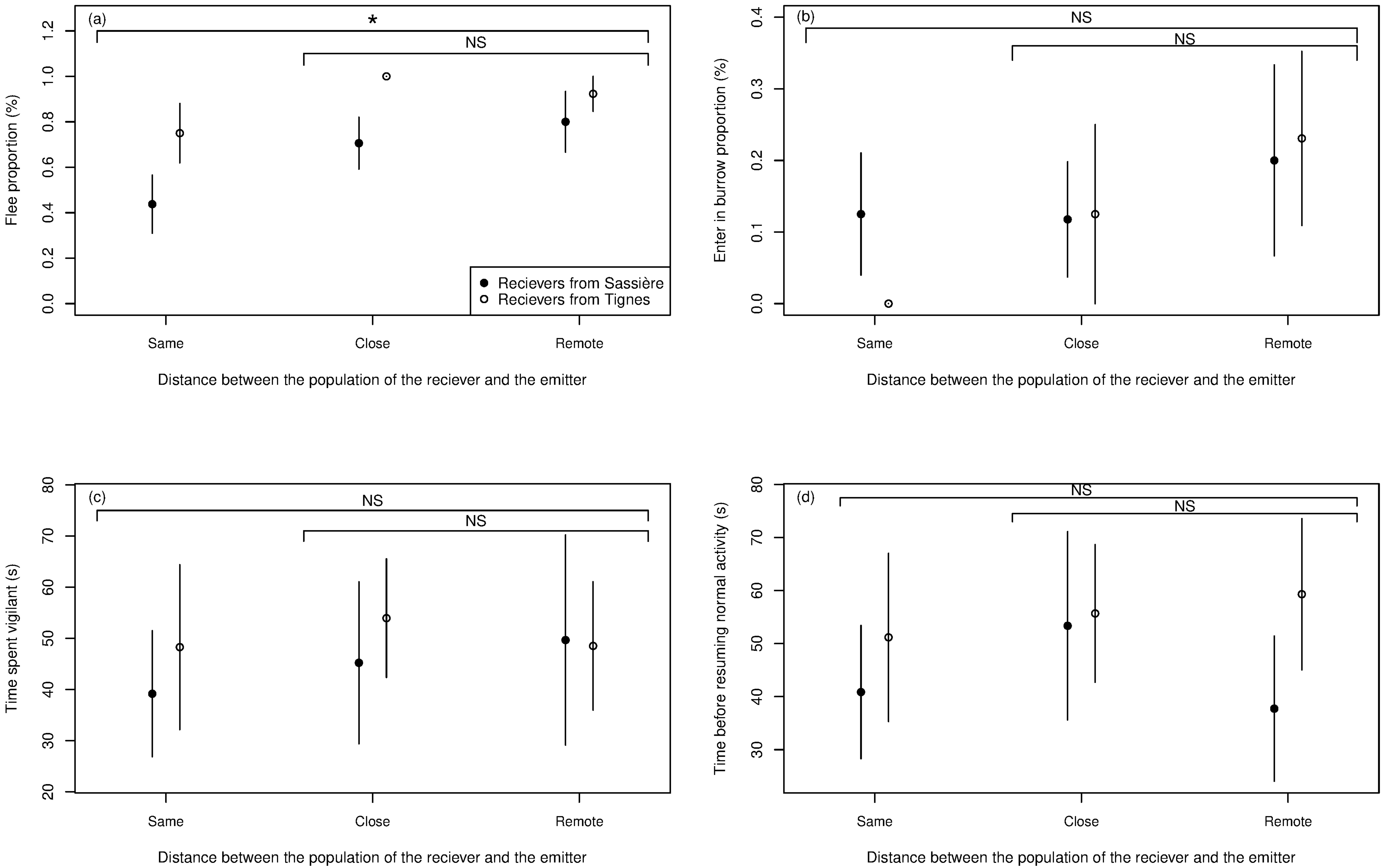

(a)
Sassière
Tignes
Probability density
1st Axis
AD
SD
DD
F1
1st Axis
(b)
Cerdanya
Ripollès
Probability density
1st Axis
AD
SD
DD
F1
1st Axis
(c)
Cerdanya
Sassière
Probability density
1st Axis
AD
SD
DD
F1
1st Axis
(d)
Ripollès
Sassière
Probability density
1st Axis
AD
SD
DD
F1
1st Axis
(e)
Cerdanya
Tignes
Probability density
1st Axis
AD
SD
DD
F1
1st Axis
(f)
Tignes
Ripollès
Probability density
1st Axis
AD
SD
DD
F1
1st Axis

Figure 3


(a)
*
NS
Flee proportion (%)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Recievers from Sassière
Recievers from Tignes
Same
Close
Remote
Distance between the population of the reciever and the emitter
(b)
NS
NS
Enter in burrow proportion (%)
0.0
0.1
0.2
0.3
0.4
Same
Close
Remote
Distance between the population of the reciever and the emitter
(c)
NS
NS
Time spent vigilant (s)
20
30
40
50
60
70
80
Same
Close
Remote
Distance between the population of the reciever and the emitter
(d)
NS
NS
Time before resuming normal activity (s)
30
40
50
60
70
80
Same
Close
Remote
Distance between the population of the reciever and the emitter

Supplementary Material 2

Click here to access/download
Supplementary Material
Appendix S2.docx