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Geographic variations in marmots' alarm calls cause different responses

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Abstract:	<p>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 (<i>Marmota marmota</i>), 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.</p>	
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1 **Geographic variations in marmots' alarm calls cause different**
2 **responses**

3

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24 **Abstract**

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

41 **Significance statement**

42 Dialects can have major impacts on the ability of conspecifics to communicate.
43 Surprisingly, dialects in alarm calls have been neglected despite their crucial role on
44 individuals' survival. Alpine marmots have dialects in alarm calls and do discriminate
45 their own dialects from others, being more frightened by alarm calls from another
46 population than their own. Confronted with an unknown dialect, marmots may adopt a

47 self-preserving strategy and chose to run-away before assessing the danger.

48

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

51

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61 **Introduction**

62 Considerable geographic differences among populations in acoustic signals, usually
63 termed dialects, geographic markers or geographic variations (Conner 1982), have been
64 documented across the whole animal kingdom (in mammals (Lameira et al. 2010); in
65 birds (Krebs et al. 1980); in anurans (Velásquez 2014); in fish (Parmentier et al. 2005);
66 in invertebrates (Zuk et al. 2001); for review, see (Wilczynski and Ryan 2001). This
67 signal variation could arise from various processes, such as genetic and/or cultural drifts
68 between isolated populations (Baker 1982; Davidson and Wilkinson 2001; Mundinger
69 1982; Janik and Slater 2003), and are particularly relevant to our understanding of the
70 divergent evolution in communication systems (Campbell et al. 2010; Wilczynski and
71 Ryan 2001). Signals have been shown to be influenced by many factors, including
72 geographic barriers (Thomas et al. 1988; Cleator et al. 1989; Thomas et al. 1992; Perry
73 and Terhune 1999), environment (, habitat structure, background noise (Hunter and
74 Krebs 1979; Wiley and Richards 1982; van Parijs et al. 2003; Nichols and Goldizen
75 2006)) and sexual selection (*e.g.*, female preferences (Slater 1986)).

76 Signal geographic variation implies a structural variation from the emitter point
77 of view but must also involve detection and discrimination processes by the receiver to
78 have a major impact on the ability of conspecifics to communicate. For instance, an
79 extensive literature on songbirds shows that dialects can strongly affect breeding
80 behaviour, especially mate attraction and intra-sexual competition (Searcy et al. 2002).
81 Many studies suggest that individuals discriminate among songs from conspecific
82 populations, preferring the signal of their local population in a context of mate choice
83 and intra-sexual competition (Baker 1982; Searcy et al 2002; Gray 2005; Boul et al
84 2007; Podos 2007; Nichols 2008; Uy et al. 2009; Bradely et al. 2013; Mortega et al.

85 2014; Lin et al. 2016); even in close populations (Leader et al. 2002; but see Colbeck et
86 al. 2010; Danner et al. 2011). Dialects can then act as pre-zygotic barriers and play an
87 important role in speciation (Baker and Cunningham 1985; Slabbekoorn and Smith
88 2002; Price 2008; Wilkins et al. 2013). Similar patterns and processes have been
89 suggested in mammals, although evidence is much more limited (Maeda and Masataka
90 2010).

91 Studies investigating geographic variation in acoustic signals and its perception
92 in behavioural contexts other than sexual selection in avian species remain scarce
93 (Charrier et al. 2013). Alarm calls, shaped by natural selection, could be as relevant to
94 speciation as acoustic sexual signals because of their direct consequences on
95 individuals' survival. Despite this crucial role, dialects in alarm calls have been so far
96 poorly investigated because they are not as complex as bird nuptial and territorial songs.
97 Although some of them are sophisticated (e.g., syntax in mobbing calls (Suzuki 2016);
98 complex signal structure in monkey screams (Zuberbühler 2009)), alarm calls are often
99 a repetition of one same call unit (Randler et al. 2011). Both the structure of the note
100 and its repetition rate can be used to encode information (Manser 2001, but see Rendall
101 et al. 2009). Nevertheless, it has been shown that alarm calls with highly stereotyped
102 structures can convey information about the age or the sex of the emitter (Blumstein and
103 Muñoz 2005). Geographic differences in alarm calls have been documented in several
104 mammalian species (Slobodchikoff et al 1998; Matrosova et al. 2012; Schlenker et al.
105 2014, Francescoli 2002; Eiler and Banack 2004) but were found sometimes absent in
106 yellow-bellied marmots, *Marmota flaviventris* (Blumstein and Armitage 1997). When
107 present, their perceptual salience remains unknown (Zuberbühler 2009). Hence the
108 importance of such variation for the communication process is currently unknown and

109 further playback experiments are necessary to determine their biological relevance.

110 We investigated the existence of structural differences in alarm calls produced
111 by Alpine marmots (*Marmota marmota*) from two native (Alps) and two reintroduced
112 (Pyrenees) populations, and whether receivers discriminate among them. Alpine
113 marmots are cooperatively breeding ground-dwelling territorial squirrels living in
114 family groups. Territory surveillance is insured by all individuals from a family and,
115 once a predator is detected, marmots can produce alarm calls warning other individuals
116 before hiding in their burrows (Perrin et al. 1993). Marmot alarm calls are usually
117 composed of one stereotyped and frequency modulated single note (Perrin et al. 1993).
118 The comparison between the native populations in the Alps and the reintroduced
119 populations in the Pyrenees is of particular interest to investigate the relationship
120 between signal divergence and genetic differentiation, as well as their consequences in
121 terms of between-population recognition. We first investigated whether geographic
122 variations are encoded in the acoustic structure of alarm calls produced by marmots
123 from the four studied populations. Although dynamic interplay between song learning
124 mechanisms and geographic isolation have been evidenced to be at the origin of dialects
125 in birds songs (Podos and Warren 2007), the processes underlying the evolution of
126 alarm call dialects remains to be described. Moreover, due to the fact that songs in
127 oscines are usually learned (Kroodsma 2004), song dialects have been found to result
128 from short-term, cultural, or ecological processes rather than to have a genetic basis
129 (e.g., Halfwerk and Slabbekoorn 2009; Ruegg et al. 2006). Conversely, alarm calls
130 structure have a substantial genetic basis (Blumstein 2007; Blumstein et al. 2013) and
131 consequently, cultural drift is unlikely to occur. Therefore, we expect to observe a strong
132 relationship between alarm call divergence and genetic differentiation between the two

133 reintroduced populations, which are geographically and genetically isolated from the
134 two native populations (Bichet et al. 2016). We also conducted playback experiments to
135 determine whether receivers perceived the differences in alarm calls produced by
136 different populations and changed their anti-predatory response.

137

138 **Methods**

139 **Ethical Note**

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

146

147 **Study populations**

148 We studied Alpine marmots from four populations (Supplementary Material S1):
149 Sassièrè and Tignes are native populations of the Western Alps located 10 km apart,
150 while Cerdanya (La Bastida) and Ripollès (Pardines) are reintroduced (from 1948 until
151 1988, Couturier 1955; Ramousse et al. 1992) populations situated 500 km away from
152 the first ones in the South-eastern Pyrenees and which are 45 km apart. Ripollès is
153 genetically closer to the two native populations than Cerdanya (Bichet et al. 2016).
154 Within each population, intensive behavioural observations allowed us to precisely
155 identify family groups and to locate main burrows and territory borders. To conduct
156 genetic analyses, 151 unrelated individuals were captured in these four populations (all

157 the details about the sampling and the genetic analyses are given in the Supplementary
158 Material).

159

160 **Does the acoustic structure of alarm calls differ between populations?**

161 Each year (2011-2014), alarm calls were recorded in the four populations between mid-
162 April and mid-July from 8:00 a.m. to 6:00 p.m. the main activity period of the marmots.
163 An omnidirectional microphone (Sennheiser ME62-K6P) connected to a Fostex FR2LE
164 recorder (frequency sampling: 44.1 kHz, resolution: 16 bits) was placed approximately
165 2 m from a main burrow entrance in order to maximize the chances to record alarm
166 calls. Once a recording was performed, we moved to another territory not adjacent to
167 the previous one to record another individual (Sassi re: N = 38 alarm calls; Tignes: N
168 =31; Cerdanya: N = 92 and Ripoll s; N =35), Marmots typically retreated into their
169 burrows during the setup of the recording material but they re-emerged within few
170 minutes. Then, individuals were exposed to a threatening situation in order to trigger the
171 emission of alarm calls by a focal individual: a human or a human with a domestic dog
172 (*Canis lupus familiaris*) appeared promptly from a hide situated 60 to 80 m away from
173 burrows and ran in the direction of the marmot. This protocol worked in almost all
174 cases. Alarm callers were aged up to 3 years (when they reach adult size and stop
175 growing), which enabled us to distinguish three age classes: pups, yearlings and adults.
176 Since body size and age might impact frequencies of vocalizations neither pups' nor
177 yearlings' calls were analysed (Tubaro and Mahler 1998; Blumstein and Munos 2005;
178 but see Matrosova et al. 2007). As marmots are monomorphic, it was not possible to sex
179 the recorded emitters. Hence, if sex variation is present in the alarm call of Alpine
180 marmot, there will likely be "noise" in our analysis. A sex difference could bias our

181 analysis at the population level in case of (i) an acoustic difference among male and
182 female which is currently not known in Alpine marmot, (ii) a large difference in the
183 proportion of male and female responding to alarm call playback in our studied
184 populations, and, (iii) a reverse proportion of males and females recorded according to
185 populations. Although an absence of literature does not mean that sex does not play a
186 role in alarm calls. Currently, we do not have evidence for none of the above, but we are
187 confident that sex information did not bias our analysis.

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

203

204 *Statistical analyses*

205 To test for differences in the acoustic structure of alarm calls between the four
206 populations, we fitted four linear mixed-effect models with either the ascendant phase
207 (AD), the stationary phase (SD), the descendant phase (DD) duration or the initial
208 frequency (F1) as the response variable, the population as a fixed effect and the calling
209 bout as a random effect on the intercept. The maximum and final frequencies were
210 discarded due to their strong correlation with the other variables (see Table 1).

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

225 Then, we tested whether the acoustic distance (log-transformed) between
226 populations correlated with either the genetic distance (linearized $F_{ST} = F_{ST}/(1-F_{ST})$) or
227 the geographic distance (log-transformed) using Mantel tests (all permutations, Mantel
228 1967) implemented in the package *vegan* (Oksanen et al. 2018). Acoustic distances

229 between populations were quantified as the pairwise differences in the linear
230 discriminant functions. The genetic distances were quantified using pairwise F_{ST} values
231 (Weir and Cockerham 1984) and the geographic distances as the linear distance in
232 kilometres (see the Supplementary Material S2).

233

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

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

252 Each playback bout was composed of 5 alarm calls within 3 s, mimicking an

253 average natural bout (measurement performed on 30 bouts recorded in our study). The
254 amplitude of alarm calls of Alpine marmots has not been measured accurately before
255 (*i.e.* the precise distance between the emitter and the sound level meter, taking the exact
256 position of the head of the marmot into account). Hence, we decided to match by ear the
257 amplitude of the calls used during our test to a natural call. Alarm calls were emitted at
258 100 dB(C) using a speaker (SMC8060 Beyma amplified loudspeaker) connected to a
259 Fostex FR2LE, measurement done with a Lutron SL-4001, C weighting, slow settings).
260 This amplitude is closed to the value measured on yellow-bellied marmot *Marmota*
261 *flaviventris* (95-100 dB (Lea and Blumstein 2011)).

262 The speaker was placed on the ground in the upper part of the focal territory
263 directed towards the main burrow entrance. Marmots typically retreated into their
264 burrows during the installation but they re-emerged within minutes. Once a marmot was
265 between 5 m and 10 m away from any burrow entrance and displayed a normal activity
266 such as foraging, an observer placed outside the focal family group triggered the alarm
267 call playback (beginning of the trial). At the same time, observers filmed the focal adult
268 individual of the focal family group with a digital video camera (Sony® Handycam
269 model DCR-DVD650 or JVC® digital video model GZ-E 209). The trial was
270 considered completed when the focal individual entered into a burrow, resumed a
271 normal activity (*i.e.*, foraging) or 5 minutes after the beginning of the trial.

272 All video recordings were displayed in AVS Video Editor (version 7.1) in slow
273 motion (x0.25) by a unique observer, blind to playback treatment, to ensure an accurate
274 identification of behaviours as well as to record their duration with an accuracy of 0.01s.
275 In one case over 77, more than a single animal was present, we thus conducted the
276 statistical analyses with and without this trial but given that the results were

277 qualitatively identical and quantitatively similar, we chose to keep this experiment in the
278 analyses. We collected the occurrence (coded as a binary outcome) of vigilance,
279 flight/running, entrance into a burrow, time spent vigilant and time until the focal
280 individual resumed foraging. Vigilance behaviour was defined as any posture where
281 marmots were standing on their rear feet, or standing on their four feet but suddenly
282 putting their head up and maintaining it above the horizontal plane of their body.
283 Flight/running was defined as an escape-related behaviour towards a burrow entrance
284 and was considered to be a more extreme response than any vigilance posture, without
285 necessarily entering in the burrow.

286

287 *Statistical analyses*

288 To test whether the response to alarm calls depends on the origin of the signal, the
289 occurrence of flight and of the entry in a burrow were entered as response variables in
290 two generalized linear models (GLMs) with a logit link and the variance given by a
291 binomial distribution. Since in nearly all playback trials (72 over 77), individuals
292 became vigilant, the frequency of this behaviour could not be considered.

293 The time spent vigilant and the time before resuming a normal activity were further
294 entered as response variables in two other GLMs with a logarithmic link and the
295 variance given by a Gamma distribution. In the last model, we categorized the intensity
296 of the response of the focal individual in four categories: no response, vigilance only,
297 flight and entry in a burrow; and we entered this ordinal variable in an ordered logistic
298 regression model. In each of these five models, we first tested whether the responses
299 were different when the playback alarm call originated from the population of the focal
300 individual or from another population by entering the origin of the playback as a two-

301 modalities factor (same or other population) in interaction with the population of the
302 focal individual (Tignes or Sassièrè) as explanatory variables. Second, we tested
303 whether the geographic distance between the focal individual and the signaller could
304 further impact the responses by entering the origin of the playback as a three-modalities
305 factor (same, close, far) in interaction with the population of the focal individual (Tignes
306 or Sassièrè) as explanatory variables. All analyses were performed using the R software
307 (Version 3.1.1) and packages 'nlme' (Pinheiro et al. 2018), 'multcomp' (Hothorn et al.
308 2008), 'ade4' (Dray and Dufour 2007), 'MASS' (Venables and Ripley 2002).

309

310 **Results**

311 **Does the acoustic structure of alarm calls differ among populations?**

312 Marmot alarm calls from the four populations differed significantly in their initial
313 frequencies ($F_{1, 190} = 4.75$, $P < 0.01$), ascendant phases' durations ($F_{3, 190} = 11.58$, $P <$
314 0.001), stationary phases' durations ($F_{3, 190} = 9.57$, $P < 0.001$) but not in their descendant
315 phases' durations ($F_{3, 190} = 2.45$, $P = 0.07$).

316 Despite a strong overlap among the four populations when compared altogether
317 (Supplementary Material S3), alarm calls were always assigned more often to the
318 population their emitter originated from than to any other population. Effectively, calls
319 were correctly classified in 41.96 [35.71; 48.35] % of the cases (while the percentage of
320 random classification would have been 25%). When populations were compared two by
321 two, alarm calls were once again always better attributed to the population their emitters
322 originated from than to the other one (percentage of random classification: 50%).
323 Percentages of correct classification ranged from 62.49 [54.17; 69.79] % to 71.64
324 [63.70; 79.41] % when comparing Tignes with Ripollès and Tignes with Cerdanya

325 respectively (Table 2). All the acoustical variables (initial frequencies, ascendant,
326 stationary and descendant phases' durations) contributed to the discrimination among
327 the populations, but they seemed contribute differently to the differentiation between
328 each pair of populations (Fig. 2).

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

334

335 **Does marmots' response to alarm calls vary depending on the** 336 **population of origin of the emitter?**

337 Marmots exhibited lower intensity responses to an alarm call from their own population
338 than to an alarm call from another population ($\beta = -1.23 \pm 0.52$, $z = -2.40$, N = 76, p =
339 0.02). The odds of a marmot showing a higher response decreased by a factor of 0.29
340 [0.10; 0.78] when the alarm call originated from its own population compared to
341 another one. More specifically, marmots showed a significantly lower propensity to flee
342 ($\beta = -1.45 \pm 0.58$, $z = -2.50$, N = 76, p = 0.01, fig. 3-a) when the alarm call originated
343 from their own population than from nearby *versus* geographically remote population.
344 Although the rest of our results were not significant, marmots tended to be less likely to
345 enter their burrow ($7.14 \pm 1.86\%$ vs. $16.67 \pm 6.67\%$, $\beta = -0.96 \pm 0.83$, $z = -1.15$, N = 76,
346 p = 0.25, fig. 3-b), to remain vigilant for a shorter time (median = 23.37s vs. 34.58s, $\beta =$
347 0.002 ± 0.006 , $z = 0.40$, N = 74, p = 0.68, fig. 3-c), and to resume a normal activity
348 more rapidly (median = 24.88s vs. 39.35s, $\beta = 0.003 \pm 0.005$, $z = 0.49$, N = 68, p = 0.62,

349 fig. 3-d) when the alarm call originated from their own population. The intensity of the
350 response was similar for alarm calls produced in a nearby or in a geographically distant
351 population (odds ratio = 0.73 [0.19, 2.64], $\beta = -0.31 \pm 0.66$, $z = -0.47$, $N = 76$, $p = 0.64$).
352 The propensity to flee ($\beta = -0.20 \pm 0.83$, $z = -0.24$, $N = 76$, $p = 0.81$, fig. 3-a), to enter in
353 a burrow (near: 88.00 + 2.64% vs. far: 78.26 + 3.91%, $\beta = -0.71 \pm 0.80$, $z = -0.89$, $N =$
354 76 , $p = 0.37$, fig. 3-b), the amount of time spent vigilant (near = 34.58s vs. far = 31.9s, β
355 = -0.02 ± 0.33 , $z = -0.06$, $N = 74$, $p = 0.95$, fig. 3-c) and elapsed time before resuming a
356 normal activity (near = 38.16s vs. far = 39.35s, $\beta = -0.001 \pm 0.006$, $z = -0.29$, $N = 68$, $p =$
357 0.77 , fig. 3-d) did not vary with the geographic distance between the focal and the other
358 population.

359

360 **Discussion**

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

372 Although alarms calls are a stereotyped signal, we found that the acoustic

373 structure of Alpine marmots alarm calls differ among the four studied populations.
374 Indeed, using four acoustic parameters to describe each alarm call it was possible to
375 assign the signal to the population their emitters originated from with accuracy
376 exceeding that expected by chance. The presence of geographic markers in acoustic
377 signals have been shown repeatedly in bird songs (Nottebohm 1969; Mundinger 1982;
378 Zimmermann et al. 2016). The richness of avian vocal repertoires offer a wide range of
379 possibilities for differentiation of geographic markers: changes in note combinations,
380 presence or absence of some notes (particularly during new population finding) and/or
381 rhythm of emission of different notes (Baker and Jenkins 1987; Handford 1988; Shieh
382 et al. 2013). Nevertheless, such markers have also been found within single stereotyped
383 acoustic elements, e.g. those produced by bottlenose dolphins, *Tursiops spp.* (Campbell
384 2004). Similarly to Campbell's (2004) study, we found that the general shape of
385 acoustic elements, as short as 0.2s, differed among marmot populations.

386 We further found a clear difference in the shape of alarm calls used by two
387 populations originating from two mountain ranges but also between populations
388 separated by only 5 km (i.e., Sassière and Tignes). Acoustic divergence is well
389 documented at a large spatial scale (Lougheed and Handford 1992; Wilczynski and
390 Ryan 1999). For instance, Shizuka et al. (2016) reported the existence of 13 discrete
391 song types in golden-crowned sparrows (*Zonotrichia atricapilla*) over 3,100 km in
392 Alaska. But, geographic variations in acoustic signals can also be observed at a
393 microgeographic scale. Leader et al. (2008) documented dialects in orange-tufted
394 sunbird (*Nectarinia osea*) within two sub-populations separated by a sharp boundary but
395 only 100 m apart from each other. Studies conducted at these different geographic scales
396 still remain scarce in mammals. At least two studies working on a phylogenetically

397 close species to the Alpine marmot, the Gunnison's prairie dog (*Cynomys gunnisoni*),
398 have documented large or microgeographic differences in acoustic signals
399 (Slobodchikoff et al. 1998; Perla and Slobodchikoff 2002).

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

412 Four main hypotheses (i.e., learning, morphology and body mass differences,
413 antipredator strategies and local adaptation) have been proposed to explain the
414 occurrence of dialects. The learning hypothesis attributes acoustic differences among
415 populations to the colonization of a new area by young individuals before they have
416 learned the song structure from their parents (Thielcke 2008). Baker and Jenkins (1987)
417 invoked this cultural bottleneck to explain the presence of dialects in isolated
418 populations of chaffinches. However, an interspecific cross-fostering experiment with
419 ground squirrels failed to provide evidence for the learning hypothesis (Matocha 1975).
420 Although the failure to learn allospecific calls does not preclude learning the nuances of

421 conspecific calls, the learning process is probably not very important in marmots.
422 Moreover, the founders caught for the reintroduction events in Pyrenees were all adults.
423 For all these reasons, the learning hypothesis is unlikely to explain geographic variation
424 in the acoustic structure of alarm calls found in our study.

425 Among other factors that could lead to geographic variation, indirect selective
426 pressures on acoustic signals may alter vocalization characteristics (Podos 2001). In
427 Alpine marmot, mechanistic processes may cause the alarm call divergence. For
428 instance, significant differences in both morphology and body mass between two
429 (Sassi re and Cerdanya) of the four studied populations have been found (Ferrandiz-
430 Rovira et al, in prep). Mechanical links between morphology and acoustic signals exist
431 in many groups (e.g. in anurans (Hoskin et al 2009; Lengagne 2017), in birds (Nowicki
432 1987; Fletcher and Tarnopolski 1990)). For example, a study conducted on Darwin’s
433 finches showed that the adaptive evolution of beaks for feeding also influences the
434 acoustic structure of their songs (Podos 2001). Although less investigated, such link has
435 also been found for alarm calls in the speckled ground squirrel (*Spermophilus suslicus*,
436 Matrosova et al. 2012). Nevertheless, further studies are needed to firmly establish a
437 direct link between morphology and alarm call structure.

438 Antipredator strategies such as the use of alarm call is also known to strongly
439 interact with predator dangerousness and predation risk (Dutour et al. 2016,2017).
440 Indeed, signal characteristics will differ according to predator characteristics
441 (Zuberb lher, 2009). For instance, mustached tamarins (*Saguinus mystax*) produce
442 different alarm calls for aerial *versus* terrestrial predators (Kirchhof and
443 Hammerschmidt 2006) or for the level of perceived threat (Coss et al. 2007). In the
444 present study, this is unlikely to explain the observed marmots’ geographic variation

445 because alarm calls have been elicited by a human approach at the same distance from
446 burrows (60-80m) and hunting was forbidden in the different marmot populations.

447 In agreement with the local adaptation hypothesis, dialects could also result
448 from the optimization of sound transmission within local environment along various
449 transmission channel (Morton 1975). Signal alteration depends on vegetation cover
450 density, atmospheric turbulence, or height above the ground at which a signal is
451 transmitted (Wiley and Richards 1978; Lengagne et al. 1999). Moreover, information
452 transfer efficiency is linked to the intensity and the quality of the background noise
453 (Ryan and Brenowitz 1985; Lengagne and Slater 2002). In birds, a study on dialect
454 suggested that call structure have been shaped by local propagation conditions
455 (Doutrelant et al. 1998) and another study with Gunnison's prairie dog emphasized a
456 link between alarm call structure and habitat characteristics: geographic variant calls
457 resulted in significant differences in transmission performance through different
458 environments (Perla and Slobodchikoff 2002). Nevertheless, in our case, although we
459 did not measure vegetation coverage, the predominant vegetation formation consisted in
460 high altitude alpine meadows which should be quite similar from a population to
461 another. Vegetation is thus unlikely to affect alarm call propagation here. Hence, a
462 minimal effect of the propagation conditions on the evolution of alarm call structure in
463 the four studied populations is expected in agreement with previous studies on marmots
464 conducted by Daniel and Blumstein (1998). Background noise level, one major
465 component of the transmission channel also influence signal evolution. One way to
466 improve signal efficiency in such case is to avoid overlapping with low frequency
467 corresponding to background noise and thus to modify signal spectrum towards high
468 pitched frequencies. Anthropogenic effects on alarm calls had already been

469 hypothesized to explain differences in alarm calls' dialects of speckled ground squirrels
470 (Matrosova et al. 2016). We observed important noise differences among the studied
471 populations. The "Tignes" population is located in a mountain resort and could be
472 considered as disturbed by anthropogenic noises (traffic, helicopter...). However, we
473 did not observe higher, minimum or maximum frequencies in the alarm calls recorded
474 in Tignes compared to the other studied populations for which there was no evidence of
475 environmental noise.

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

482 As fleeing in response to alarm calls should provide a selective advantage by
483 increasing survival, one predicts that marmots should react to all alarm calls despite
484 discrimination abilities (but see trade-off between vigilance and foraging, Lima and Dill
485 1990). Surprisingly, our tests revealed that receivers perceived acoustic differences and
486 categorized alarm calls as local or non-local calls. Such behavioural consequences
487 imply that these acoustic differences are meaningful for them (Soha et al. 2016). Most
488 studies focused on male territorial defence or female attraction in birds and their results
489 are contrasted with stronger response to local songs in some cases, absence of
490 preferences or mixed responses in others (see Becker 1983; Catchpole and Slater 2008).
491 In an alarm context, experimental approaches with playbacks emphasized that intensity
492 of animal response varies according to the past reliability of the signaller (Cheney and

493 Seyfarth 1988)) or to the familiarity of the members of the colony (Hare and Warkentin
494 2012). Nevertheless, no study has been conducted so far to determine whether animals
495 discriminate and react differently to conspecific alarm calls according to its population
496 (*i.e.* geographical origin). Our results thus show for the first time that animals
497 discriminate among calls recorded in different populations and react accordingly. We
498 expected that a divergence in the alarm signal induce an alteration of alarm information
499 and, according to Hanson and Coss (2001), we expected a reduced behavioural response
500 to alarm calls of non-local congeners in tested animals. Surprisingly, behavioural
501 responses obtained after playback of alarm calls from different populations showed the
502 reverse. Indeed, animals reacted more strongly to playback of alarm calls originating
503 from foreign populations. Moreover, the intensity of the response was similar for alarm
504 calls produced in a nearby or in a geographically distant population suggesting that
505 these signals represent an equally threatening situation for animals. Our results may be
506 explained by a difference in the predator pressure or predator strategy (Dutour et al.
507 2016). Even though we did not measure predator pressure precisely, the two main
508 predators of marmots, the golden eagle (*Aquila chrysaetos*) and the red fox (*Vulpes*
509 *vulpes*), were present in the four studied population. Hence, predator pressure is
510 unlikely to explain differences observed in marmot responses to playback. Previous
511 experiments on rodents showed that they discriminate alarm calls from neighbours (*i.e.*
512 familiar) *versus* unfamiliar individuals. In the present study, marmots displayed a
513 stronger response to alarm calls originating from foreign populations compared to their
514 native one. Nevertheless, this familiar *versus* unfamiliar discrimination process cannot
515 explain our results because all animals were tested with unknown signals even when
516 they were tested with calls of their own population. Our results may be explained by the

517 fact that marmots estimate the information reliability received and reacted accordingly.
518 Blumstein et al. (2004) observed a stronger response to unreliable than reliable alarm
519 calls in yellow-bellied-marmot (*Marmota flaviventris*) and suggests that such difference
520 was due to the fact that a marmot hearing an unreliable alarm call makes its own
521 independent assessment of relative risk and thus invests more in antipredator behaviour
522 (but see opposite results in Richardson's ground squirrels *Spermophilus richardsonii*:
523 Hare and Atkins 2001).

524 The presence of geographic markers in acoustic signals involved in a context of
525 mate choice and intra-sexual competition have been repeatedly found in avian species
526 (Nottebohm 1969; Mundinger 1982; Zimmmann et al. 2016). Both random and
527 selective processes linked to sexual selection that may act via genetic or cultural
528 transmission pathways could explain their origin. In the context of alarm, further
529 research on the proximate causes of geographical markers as well as on the relationship
530 between geographical markers, reliability of the signal and behavioural responses are
531 now required to better understand how predation pressure and natural selection could
532 drive the evolution of communication.

533

534 **Electronic supplementary material**

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

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807 **FIGURE LEGENDS**

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

816

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

824

825 **Fig 3** Flee proportion (a), enter in burrow proportion (b), time spent vigilant (c) and
826 time before resuming normal activity (d) in relation to the distance between the
827 population of the receiver and the emitter (same, close - *i.e.* a geographically close
828 population being Sassièrè and Tignes - and remote - *i.e.* a geographically remote
829 population being Cerdanya). The black dots show the trials conducted in Sassièrè and
830 the white dots show the trials conducted at Tignes. The error bars represent standard

831 errors.

832 **TABLES AND TABLE LEGENDS**

833

834 **Table 1** Pearson's correlation coefficients between acoustic features (N = 894 alarm

835 calls). *: 0.10 < P > 0.05; **: 0.05 < P > 0.001; ***: P < 0.001.

836

	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)

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

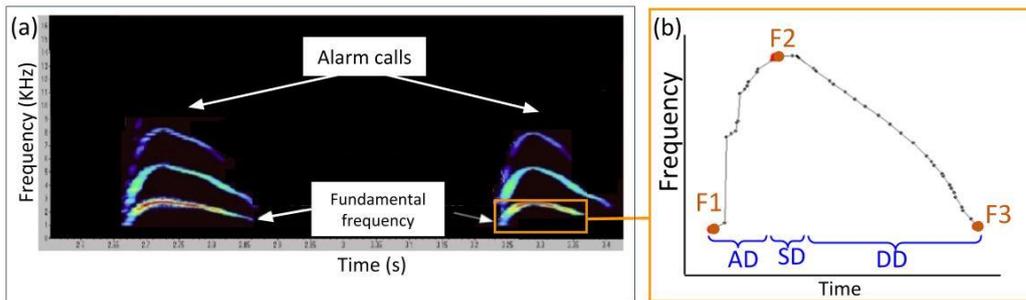
840

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

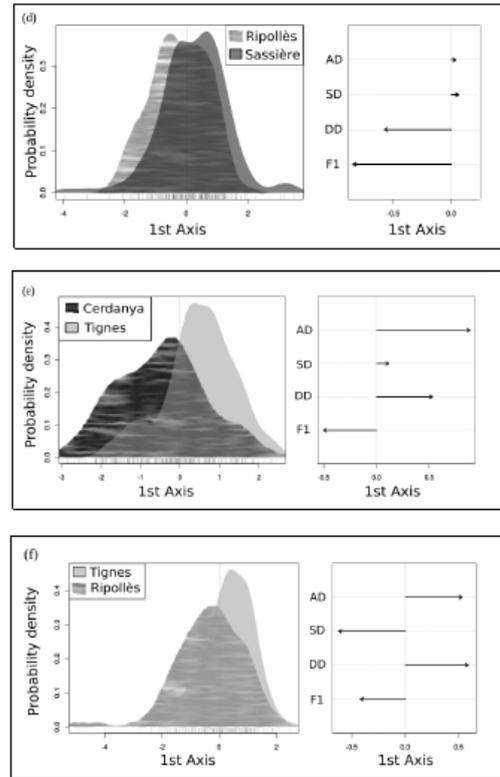
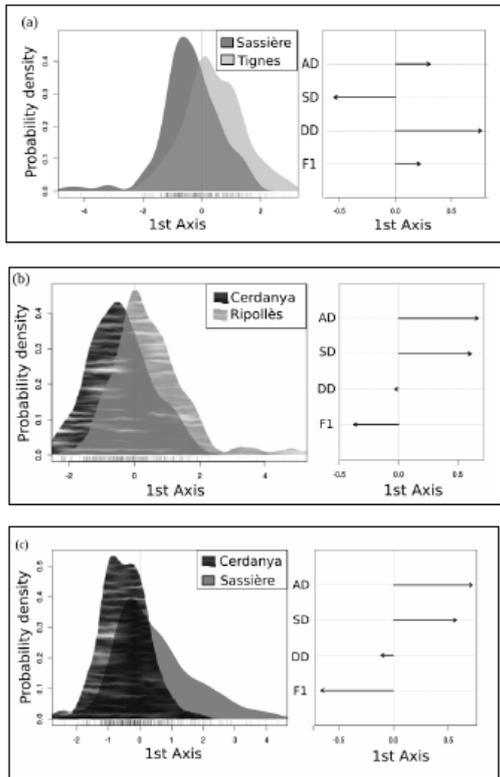
841 **FIGURES**

842

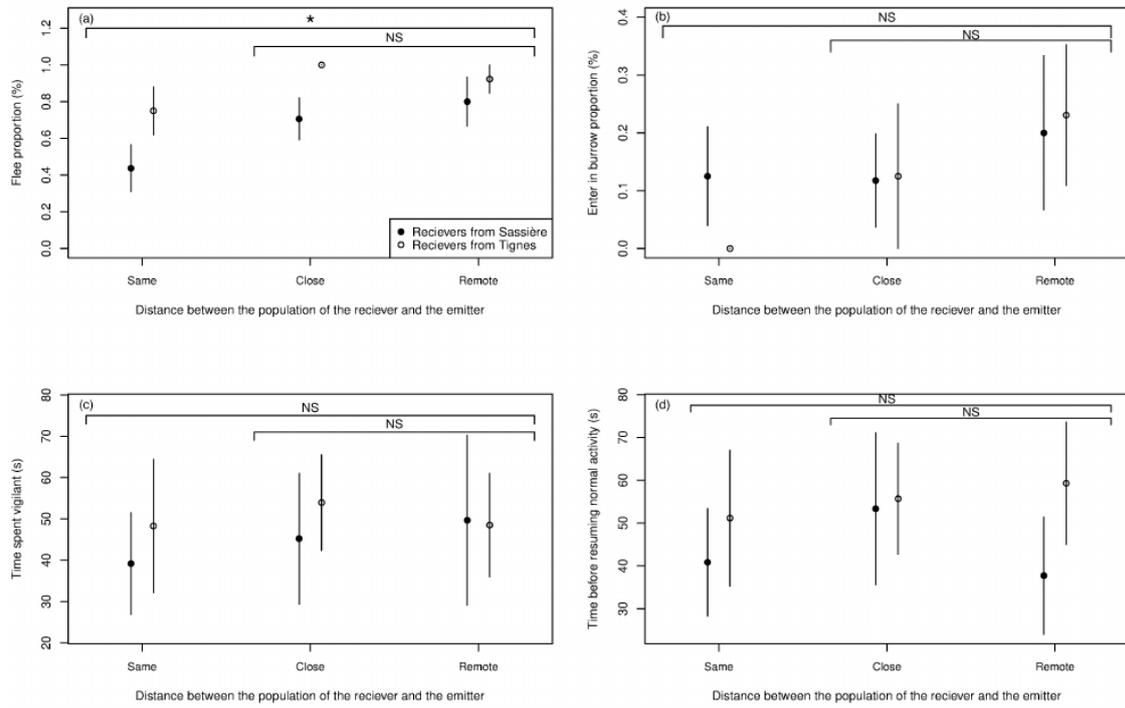
843 **Fig. 1**



844



847 **Fig. 3**
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850

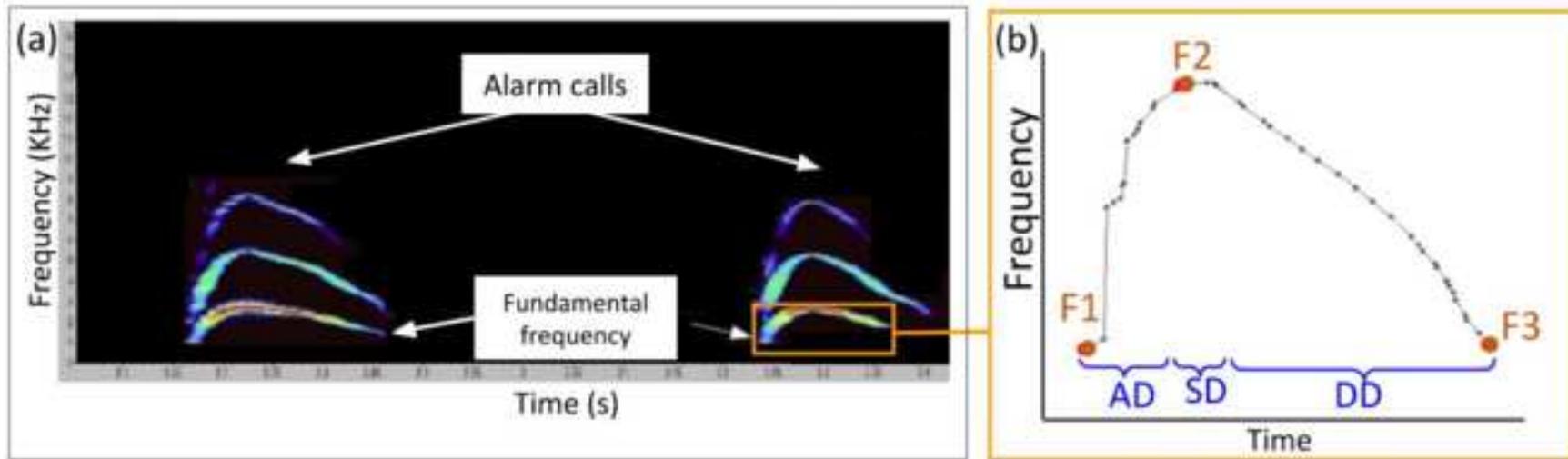


Figure 2

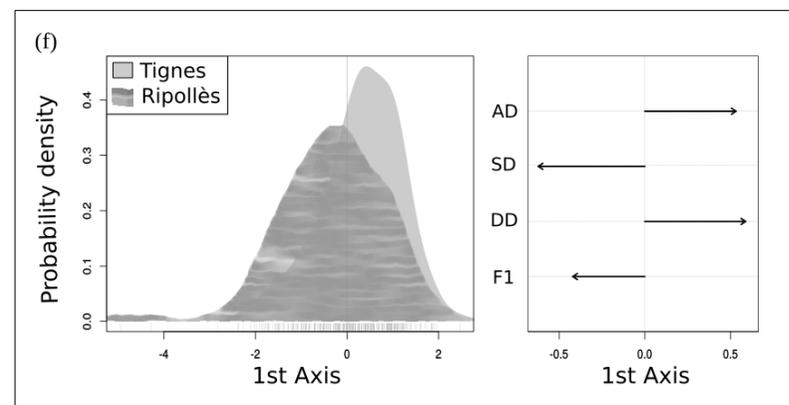
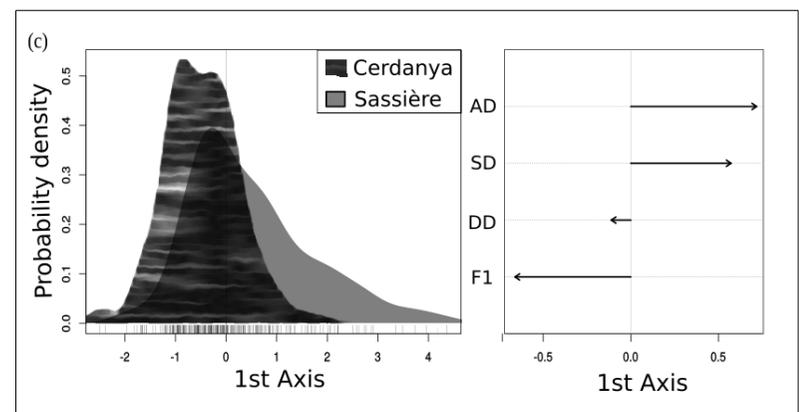
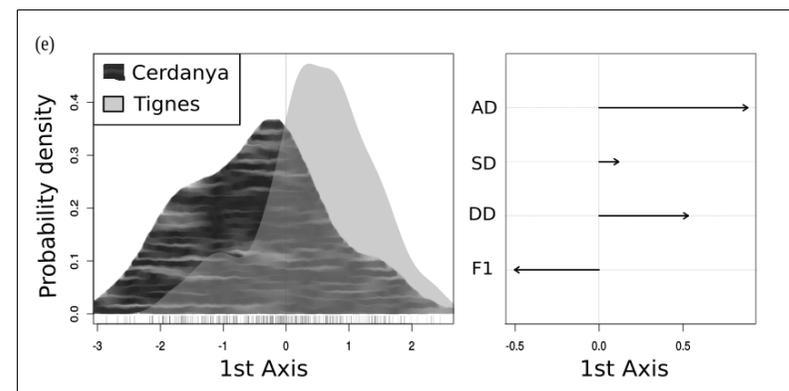
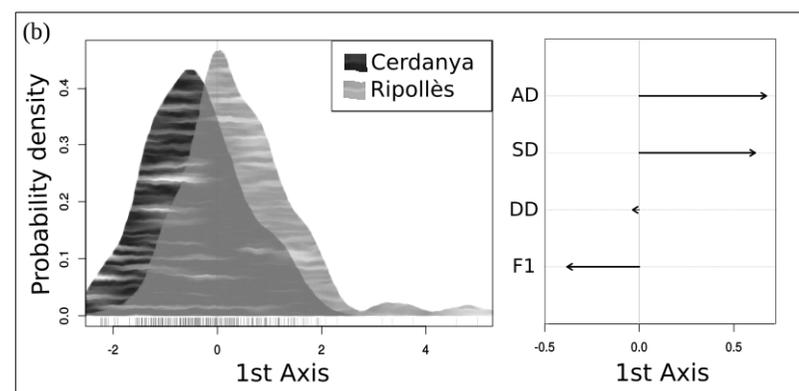
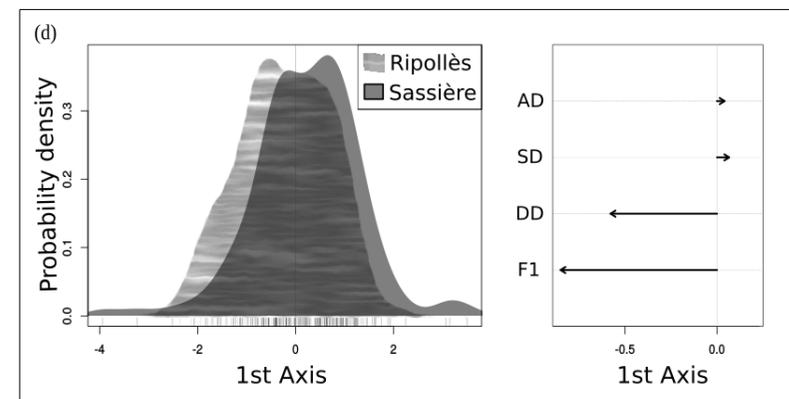
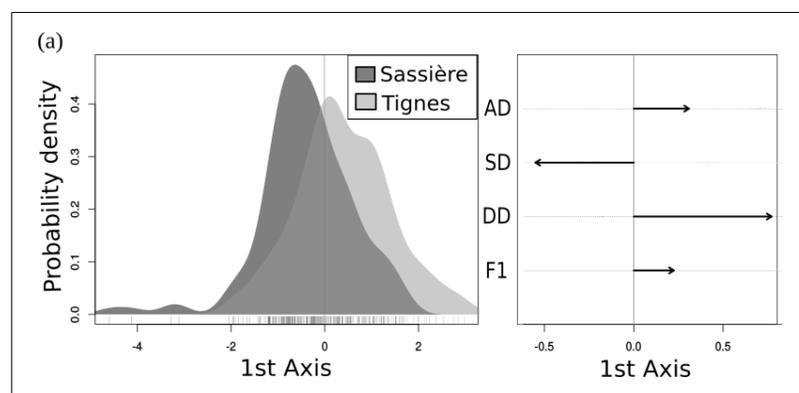
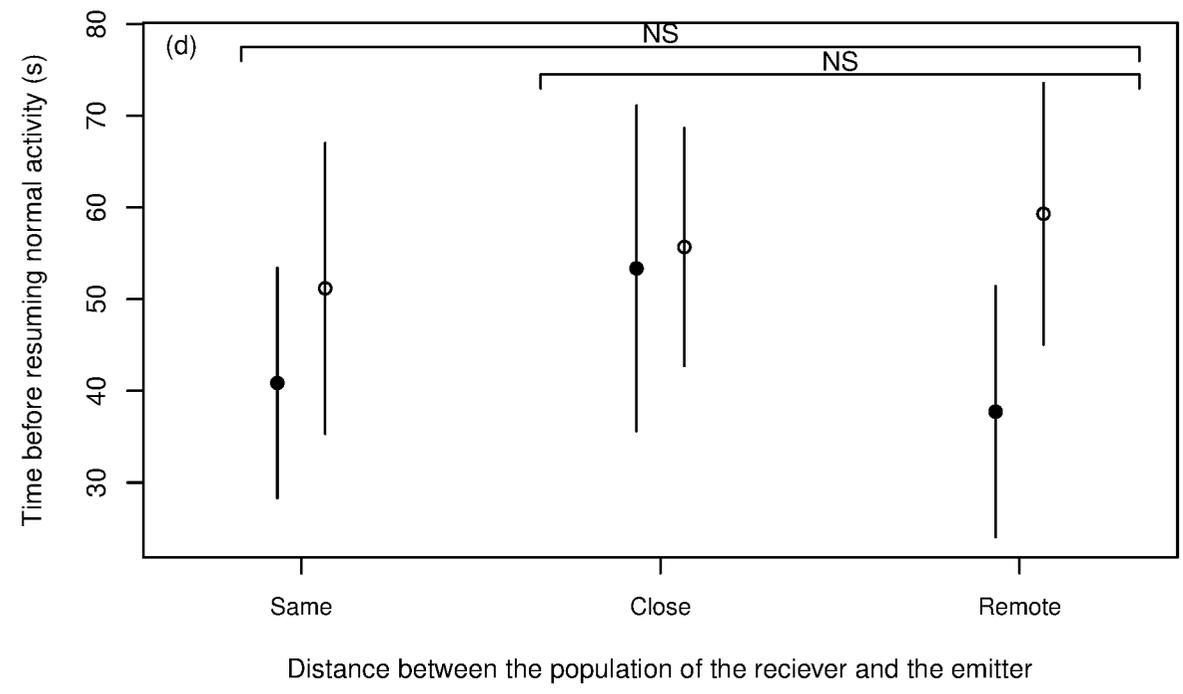
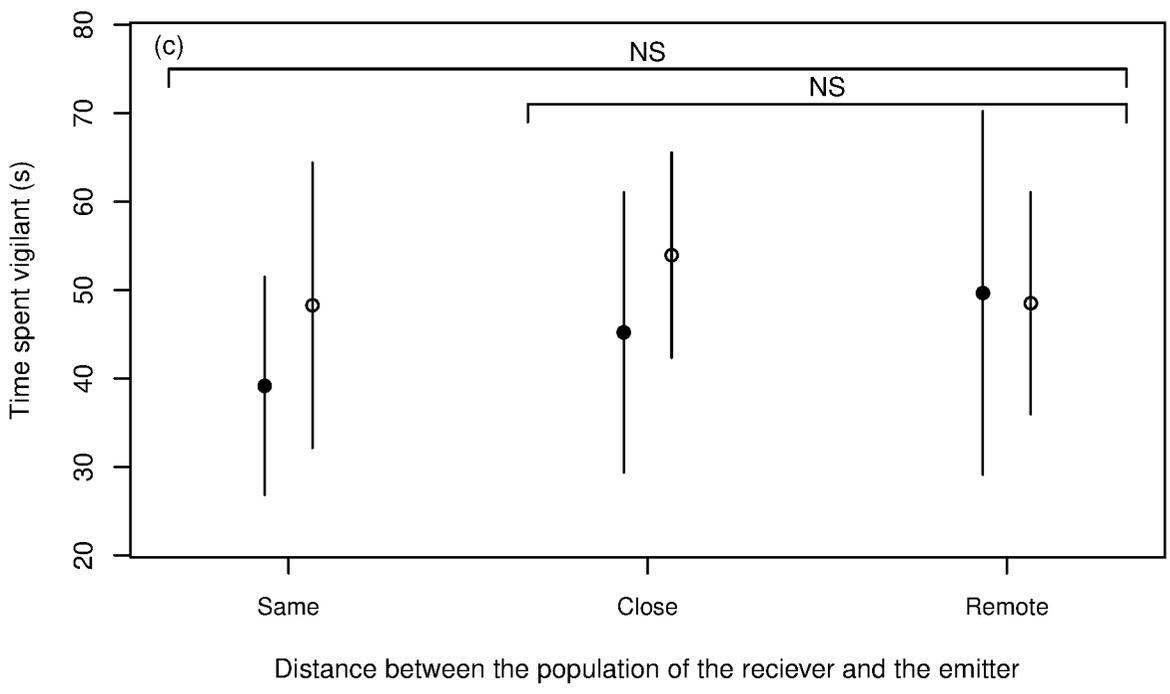
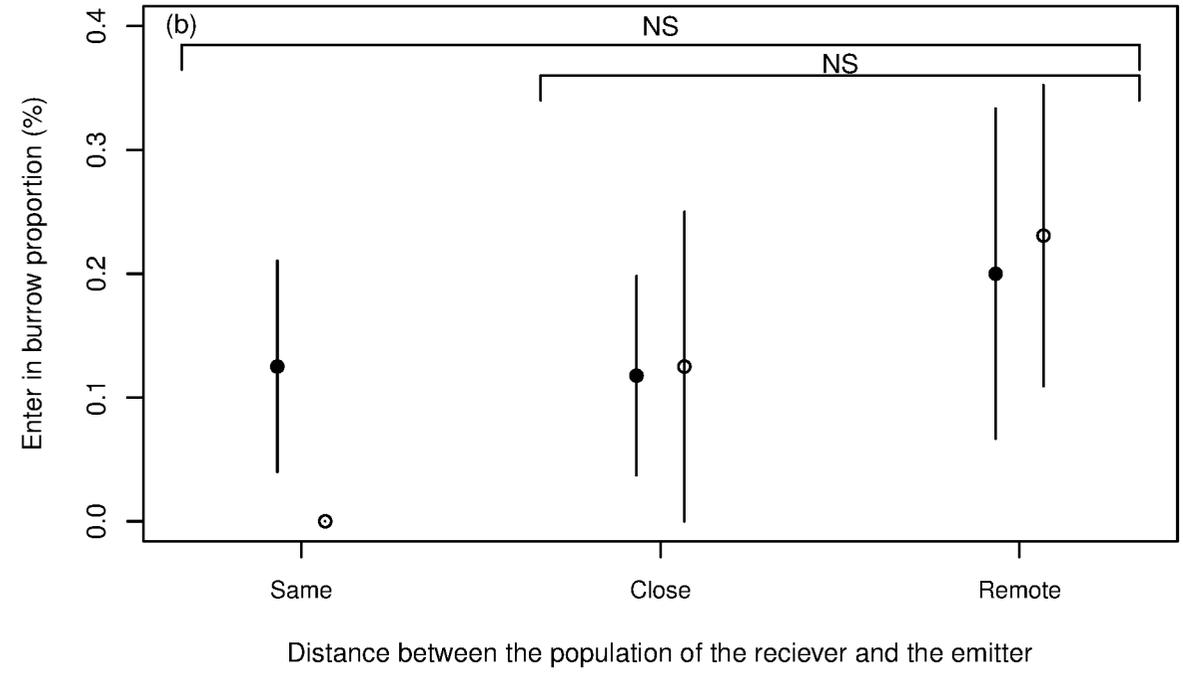
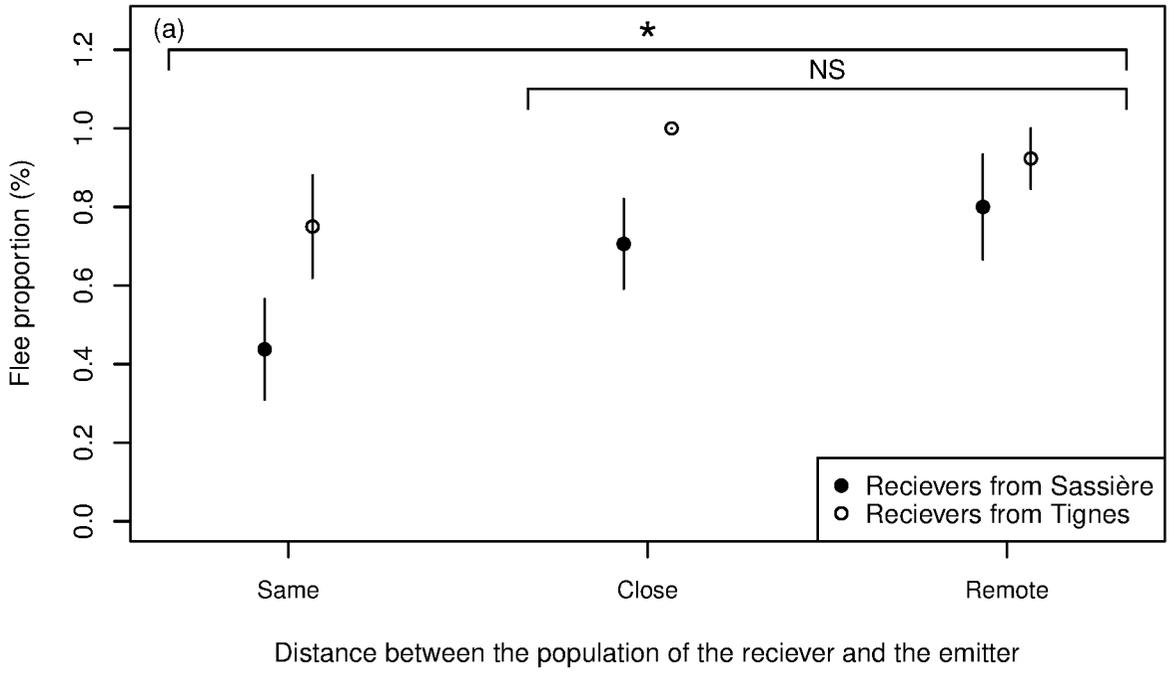
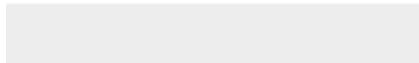
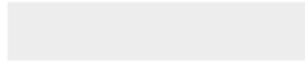


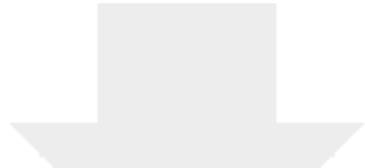
Figure 3





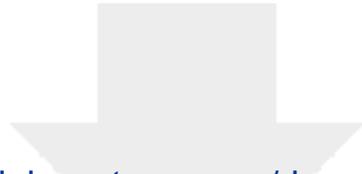
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