Title

Socio-acoustic co-selection? Vocal encoding of sociability prevails over emotions in sheep bleats

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Abstract

Vocalisations of animals are good indicators of their emotions. Temperament is known to influence the regulation and expression of emotions. However, how animal temperament affects their vocalisations and particularly their vocal expressions of emotions remains largely unexplored. Sociability is often measured as the behavioural reactivity to social separation and is a temperament trait intrinsically

linked to emotional reactivity. Most social species respond to this challenging situation using contact calls. Here, we investigated whether the acoustic structure of these calls reflect sociability, emotions or both. We used 42 female lambs from two diverging sheep lines selected for high or low sociability. High bleats were recorded both in isolation (social challenge) and before receiving a food treat (non social context) to investigate the link between vocalisations, emotions and heritable sociability. The acoustic features of isolation bleats differed between the lines, but it was not the case for pre-feeding bleats. Surprisingly, the genetic selection index and social behaviour were better predictors of the structure of isolation bleats than the arousal. Last, encoding of individuality in isolation bleats was impaired by the genetic selection. Our findings suggest a socio-acoustic co-selection: selecting for sociable animals affects vocal signatures in calls produced during a social separation.

1. Introduction

Vocalisations encode both static (i.e. relatively stable) information about the sender, such as its sex, identity and social rank [1,2], as well as more dynamic information about its internal motivation and emotional states [3]. Indeed, variation in acoustic signaling in term of call types, rate of production of these types, or spectro-temporal features of vocalisations arising as a function of the sender's emotions have been discovered in many non-human animals and show construct validity as indicators of emotional states [3–5]. Regulation and expression of dynamic features such as emotions depend on more stable traits, namely personality, in humans [6,7], with some evidence suggesting that this is also the case in non-human animals [8]. As a result, a link between temperament or personality and vocal expressions of emotions is expected, but has rarely been explored in non-human animals.

Temperament traits and personalities are defined as consistent individual differences in response to species-specific environmental or social stimuli [9]. Temperament traits are heritable, and selective breeding of divergent lines for opposite temperament traits provide experimental models to study phenotypic consequences of heritable components in ecology [9,10], medical [11] or agricultural research [12]. Boldness-shyness, exploration-avoidance, activity, sociability, aggressiveness or emotional reactivity, are temperament traits commonly assessed in both humans and non-human animals [9]. Most of these traits are inherently linked to, and measured as, individual differences in physiological and behavioural responses to challenging situations [9–11], and are thus intrinsically linked to emotions [13,14]. In particular, sociability may be defined as "an individual's tendency or propensity to associate with other individuals, where the association is not driven by reproduction or aggression" [15] and may

be partly measured as “an individual’s reaction to the absence of conspecifics” [9], i.e. quantifying the stress response and/or exacerbated behaviours to isolation.

In response to challenging situations, many animal species respond using vocalisations (alarm calls, contact calls) [12]. Yet, studies on the link between the acoustic structure of vocalisations (i.e. variation in duration, frequencies and amplitude) and temperament traits are rare, despite their potential to highlight non-invasive and rapid indicators of temperament. A few studies have incorporated call rate in their behavioural assessment of temperament [13,16–19]. In some cases, call types were produced in a context-specific way [16,20], which may emphasize the functional link between a call type and a situation. Going further into the acoustic structure of vocalisations produced during temperament assessment, Leliveld et al (2007) focused on two common call types emitted by pigs, grunts and squeals, to investigate vocal correlate of emotional reactivity [21]. They showed that grunts could be consistently divided into high and low frequency grunts and that high frequency grunts were indicative of more active, more explorative and less stressful individuals. As vocalisations are strongly affected by emotions [3–5], and the range of emotions experienced by an individual is driven by its temperament, we can predict that the acoustic structure of vocalisations may reflect the temperament of non-human animals in a broader range of species as currently demonstrated [21], similarly to vocal correlates of personality in human voice [22]. Within a social group, acoustic correlates of temperament could be very useful for receivers, as it would allow them to predict the behaviour of others.

Sheep (*Ovis aries)* are highly gregarious and vocal species [23]. Their vocal repertoire contains two main structural call types: high and low bleats, differentiated by the opening of the month during the call (open and closed, respectively). High and low bleats are context specify; namely, high bleats are usually produced in higher arousal states, especially in negative situations such as isolation from conspecifics [23], and low bleats in lower arousal states, such as during short distance mother-lamb communication [24,25]. The acoustic structure of sheep bleats encodes individuality [24], hence enabling vocal recognition, especially between mother and lamb [25,26] as well as between kin and non-kin lambs [27], which is at the basis of the strong social bonds on which the lamb’s survival depends at early stages of its live. Several temperament traits, such as ‘exploration-avoidance’, ‘boldness-shyness’ or ‘sociability’, were identified in sheep [28]. Vocal production rate (i.e. the number of vocalisation produced per time unit) was found as indicative of sociability [28]. In addition, selective breeding based on sociability revealed that the rate of calls emitted during isolation from conspecifics was heritable and that highly sociable sheep genetic line produced bleats at a higher rate than less sociable ones

[29]. However, whether the acoustic structure of sheep bleats also encodes sociability remains unknown.

In the present study, we studied a population of sheep consisting of two divergent lines of female sheep selected for high or low sociability for over 11 years (at the time of data collection). We evaluated the consequences of three generations of selection for sociability on the structure of sheep bleats recorded in two contexts that trigger emotions of different valence (negative/unpleasant versus positive/pleasant) of moderate to high arousal and vary in whether they are social-related (isolation from conspecifics) or not social-related (prior to a repeated and announced food treat delivery), to investigate the drivers of bleat structure and study potential vocal correlates of temperament in sheep. We designed two non-exclusive hypotheses to explain bleat structure: the emotional hypothesis and the socio-acoustic co-selection hypothesis, while (partially) controlling for heritability of the structure of the vocal apparatus. According to the emotional hypothesis, the isolation test should trigger a higher level of arousal for high-sociability sheep than less sociable ones, which should explain variation in acoustic structure, while the 'anticipation' of a food treat should not provoke differential arousal states between the two lines. Considering vocal indicators of negative arousal, which are consistent across species [3], this should result in bleats of longer duration and with higher frequencies in the isolation bleats of high-sociability sheep. Based on the socio-acoustic co-selection hypothesis, selection for sociability should be the main driver of the acoustic structure of bleats, meaning that genetic selection for sociability and social behaviour should better explain variation in acoustic structure than emotional arousal. We first tested for an acoustic signature of the two divergent lines of sheep, in the two recorded contexts. We predicted that if selection for sociability drives the acoustic structure of bleats, a 'line acoustic signature' should be stronger in calls produced in a social context (isolation) than in a non social context (food treat 'anticipation'). We then tested the importance of several predictors (biometry, emotional arousal, selection index and social behaviour) on the acoustic structure of isolation bleats while partially controlling for pedigree effects to disentangle between the emotional and socio-acoustic co-selection hypotheses. Last, as the salience of vocal individuality (i.e. how much acoustic structure differ between individuals) has been shown to be affected by both emotional arousal [30] and valence [31], we additionally evaluated the potential consequences of the divergent selection for sociability on individual signature in bleats, a key stone for social communication.

2. Materials and Methods

2.1 Ethical note

This study was approved by the Animal Ethics Committee SCIENCE ET SANTE ANIMALES N°115 of the Veterinary School of Toulouse under the number: SSA_2022_003.

2.2 Animals and management

The experimental animals were Romane meat sheep, reared at the INRAE experimental farm of La Fage (Saint-Jean Saint-Paul, Causse du Larzac, France) exclusively outdoors. The flock comprised about 250 reproductive females reared on 280 ha of rangelands (see [32] for details about the farming system and management characteristics). This population has been established in 2000 and selections started in 2011. Lambs used in this study were born outdoors in the spring (from April 2nd to May 2nd 2022), and remained with their dams until weaning (late June) with limited contact with humans. Lambs had access to additional food concentrate from four weeks of age. At 1 day of age, lambs and mothers were subjected to an isolation test in the same environment as the arena test (presented below) and a human approach test. Lambs were weighed on June 14th 2022. From July 4th to 14th 2022, 152 female lambs were subjected to individual tests, upon which 42 female lambs were selected according to their high (S+) or low (S-) reactivity to social isolation to constitute the two divergent lines (see next section for procedure). Four groups of five female lambs within each line were composed (two lambs, selected as replacement were not introduced into the groups). Three lambs (3S+) died from respiratory disease early after the constitution of the groups, one of which was replaced, leading to groups of three to five lambs. Sheep were individually marked with color spray and brought to pasture fields (approx. 50*50m). Lambs had unlimited access to water and grass and were supplemented in hay due to a very dry season in 2022. Every morning, between 7:00 and 10:00, at random times and for 5 weeks, each group received a 800g portion of barley.

2.3 Divergent selection based on behavioural reactivity to isolation and human presence

2.3.1 Tests procedure

The tests were performed between July 4th and 14th 2022 on 152 females. The day before being tested, 20 to 30 lambs were removed from the rest of the flock, moved to a barn with both indoor (approxi-

mately 15m×5 m) and outdoor space (approximately 20m×7 m), and with ad libitum access to water, hay and concentrate food. The following day, lambs were individually tested in an arena test (morning) and a corridor test (afternoon). At the end of each testing day, lambs returned to pasture with the with the rest of the flock (see below for a summary of the tests [33,34] for a detailed description).

Arena test:

The test arena was a wooden pen (7m long and 2m wide), with the entrance for the tested lamb on one side and an additional pen with three flock-mates separated by a grid and an optional wooden panel on the other side. In a nutshell, each lamb was placed by an operator in the corridor and was exposed to three consecutive phases: visual contact with the flock-mates (30s), no visual contact with the flock-mates (isolation phase, 60s), visual contact with the flock-mates while a human stood between the tested lamb and the flock-mates (reunion phase, 60s). Several behaviours (live data) were collected during the test and used for the selection of the animals (number of low and high bleats, displacement). The test was also video recorded to score the behaviour of each lamb in further details later on during each phase.

Corridor test:

The test corridor was a non-opaque squared corridor (1m large and 25m long). Each lamb was placed there by an operator and exposed to two phases: isolation (30s) and exposure to a moving human (60s). The vocalisations were not used in the present study due too their lower quality, but the first phase of the test was used for selection (see below).

2.3.2 Selection indexes and divergent lines

To summarize, the selection for social traits is based on the reactivity to social separation from conspecifics, and the main variable used the selection is the number of bleats produced by the lamb during the isolation and reunion phases of the arena test and during the isolation of the corridor test (see [29] for a detailed description of the statistical procedure). A PCA was applied on the 3 variables corresponding to the number of vocalisations emitted during the 3 phases of tests to construct a synthetic variable (i.e. first principal component). For genetic selection of the divergent lines, the Social Selection Index (hereafter ‘SSI’), corresponding to individual estimated breeding values, was calculated using genetic animal mixed model and considering this synthetic variable and pedigree information. Extremes individuals were chosen based on the SSI to constitute high and low divergent lines. The 42 most extremes individuals for the SSI were selected: the 21 individuals with the highest SSIs, composing the S+ line, *i.e* individuals highly reactive to separation from conspecifics, and the 21 individuals

with the lowest SSIs, composing the S- line, *i.e* individuals with low behavioural expression during separation from conspecifics. Intermediate individuals were not selected.

Similarly, two additional divergent lines were selected for human reactivity (see detailed procedure in [29]). A symmetrical pattern was used to constitute the H+ and H- lines by selecting individuals with the highest and lowest Human Selection Indexes (hereafter 'HSI'), respectively expressing the highest and lowest proximity to the human. The S+ and S- lines were selected for having simultaneously intermediate values for the HSI.

The divergent selections had been carried out for 11 years at the time of the study. Considering the breeding cycle of the sheep and overlaps between generations, this study is composed of animals after 2 (19%), 3 (53%) or 4 (22%) and 5 (6%) generations. Heritability of sociability and human reactivity traits have already been quantified and are available in [29], including the behavioural profiles of S+ and S- sheep. Overall, S+ lambs produce significantly more calls, have a higher locomotion and maintain closer proximity towards conspecifics than S- lamb [29].

2.4 Recording contexts and analysis of vocalisations structure

2.4.1 Isolation bleats

An omnidirectional microphone (Sennheiser MK20), connected to a recorder (Marantz PMD670, frequency sampling 44100 Hz) was placed on the top of the arena at 2.5m from the ground (between 2.3 and 5m from the lamb). Calls, produced during isolation phase of the arena test, when the lamb was fully separated from the flock mates, were recorded. The context of production of these calls is thus a negative situation of relatively high arousal, with regards to the gregarious behaviour of sheep. In total, 152 females were tested and their bleats recorded.

2.4.2 Pre-feeding bleats

Eight groups composed of lambs of each line (17S+ and 20S- lambs) were given a barley reward right after shaking a metal barley box every morning for a week, resulting in an association between the sound and reward. Every day of the four following weeks, vocalisations produced before the delivery of the barley treat were recorded by the experimenter at group level using an omnidirectional microphone (Sennheiser MK20), connected to a recorder (Marantz PMD670), while located at 3 to 15 meters from the lamb. The identity of the caller was assigned when possible by the experimenter. The context of production of these calls could be a food anticipation context, which we assume as positive and

lower in arousal than the social isolation [35,36]. However, the valence of this context was not validated using emotion indicators.

2.4.3 Acoustic analysis of high bleats

Only high bleats, which were consistently produced in both contexts and by the two divergent lines, were analysed in the present study (hereafter 'bleats'). Isolation and pre-feeding bleats were analysed separately using the same methodology. All calls of good quality (no overlapping sound) were kept for acoustic analysis. For the isolation context, 1287 bleats were analysed; 322 bleats from 21 S+ females, 67 bleats from 14 S- females, and 898 bleats from 117 females that were recorded but not selected as S+ or S- due to their intermediate SSI (hereafter 'non-selected'). Regarding the pre-feeding context, 519 bleats were analysed; 262 bleats from 14 S+ females (including 64 from unknown callers), and 257 bleats from S- females (including 52 from unknown caller). The process for extracting acoustic parameters was automatically computed using the *extract_chunk_features_dir()* function from SoundChunk R-package [37], which compiles several low level functions of the Seewave and tuneR R package [38–40] (details on computation are available as supplementary material (section 'Additional content > Acoustics')). Sixteen acoustic parameters were extracted to describe the spectro-temporal features of the bleats: duration, energy quartiles (Q25, Q50, Q75 and IQR), spectral flatness, dominant frequency over the duration of the call (mean and standard deviation), fundamental frequency over the duration of the call (mean and standard deviation), mean and relative amplitude of the three first formants (table S1).

It has to be noted that the environment of the two recording contexts (indoor vs. outdoor) prevented us from running direct comparison of call structure between contexts.

2.5 Statistics

All statistics were performed in R version 4.4.0. [34]

2.5.1 Investigation of acoustic difference between socially selected lines

Two complementary approaches were used: on the one hand, a Linear Discriminant Analysis (LDA) on bleats from selected S+ and S- sheep was used built an acoustic score describing bleat structure and extract key potentially discriminant acoustic parameters. Predictions of linear discriminant functions on non selected animals allowed to test for their intermediate score using a linear mixed effect model (LMM). On the other hand, a permuted Discriminant Function Analysis (pDFA) was used to test for correct classification of the genetic line of the sheep based on the acoustic structure of their bleats.

Linear discriminant function analysis and test for intermediate acoustic structure in bleats from non selected females.

A LDA including the 16 acoustic parameters extracted from the bleats and with line as a factor was performed to search for the acoustic parameters more likely to discriminate the calls from S+ and S- lambs (*lda()* function from 'MASS' R package [41], no warning for collinearity between parameters was raised). One Acoustic Score (LD) was computed for the isolation calls (isoLD), and one for the pre-feeding calls (feedLD), by entering the isolation calls and pre-feeding calls in separate LDAs. The LDA ran on the isolation calls from S+ and S- lambs was then used to predict the Acoustic Score of the calls produced by non-selected females (*predict()* function from 'MASS' R package).

Following the LDA, a LMM (*lmer()* function, 'lme4' R package [42]) was built using the isoLD as response variable, in order to test for intermediate acoustic structure of non selected females. Two interacting fixed effects were used: 'group_line', a categorical factor with three levels (the two sheep lines (S+ or S-) and the group predicted by the LDA using the non-selected lambs) in interaction with the weight of the lamb ('Zweight', centered and reduced covariate, i.e. 'scaled'). The identity of the genetic father and identity of the animal were used as a nested random factor (animal ID within father ID):

```
isoLD ~ group_line*Zweight + (1|father_ID/animal_ID), data = all_females
```

Permuted Discriminant Function Analysis

Focusing on selected lambs only, pDFAs were computed within each recording context to test for discrimination of the line respectively in isolation calls and in pre-feeding calls. The statistical approach is described in [43] and the code used here was developed in R by Roger Mundry using the R 'MASS' package (built mainly using functions *lda()* and *predict()*).

To test for acoustic discrimination according to the factor line, nested pDFAs were computed within each recording context, with N=100 random selection and cross classification and N=1000 permutations, to generate p-values for correct classifications in general and within each group (confusion matrix of p-values). Two pDFA per context were ran: the first pDFA tested for correct classification of all calls without taking into account the individual identity of the emitter (so that all calls could be kept for analysis, even from unknown callers in pre-feeding calls and from individuals that had produced less than four calls in isolation). The second pDFA controlled for the individual identity of the lambs (removing calls produced by unknown callers and individuals having produced less than 4 calls). For the pDFA controlling for individual identity, only females that had produced at least four calls were kept to allow selection of three calls (training dataset) and cross classification on the remaining one(s). For

isolation calls, the first pDFA included calls from 21 S+ and 14 S- females, with median number of calls per female [first;last decile]=9[2;16], while the second pFDA included 21 S+ and eight S- females with median number of calls per female [first;last decile]=9[2;16]. For pre-feeding calls, the first pDFA inclluded calls from 12 S+, 10 S- females, among which, 116 calls out of 519 were produced by unknown callers but known line. The second pDFA included calls from seven S+ and nine S- females that had produced at least four calls, with median number of calls per female [first;last decile] = 12[4;34].

2.5.2 Study of predictors of acoustic structure of isolation bleats

To study predictors of the acoustic structure of the isolation bleats, we designed a LMM including the Acoustic Score (isoLD) as a response variable and several predictors as fixed effects. Since the Acoustic Score was built to discriminate calls from S+ and S- females, data from these selected sheep were excluded and the LMM was run on predicted isoLD corresponding to the calls of non-selected females only. According to our two hypotheses, isolation bleats structure may be driven by 1) the arousal level expressed during the recording (emotional hypothesis) and/or 2) the selection for sociability (socio-acoustic co-selection hypothesis). To disentangle between hypotheses, proxies of arousal and social behaviour were extracted from scoring the videos of the isolation and reunion phases to be tested along with the SSI. The detailed description of the procedure is available as supplementary material (section 'Behavioural profiles', table S2 and figure S1). In a nutshell, Principal Component Analyses allowed us to compute behavioural scores (PCs) that described the behaviour of S+ and S- sheep, using behaviour that could be symmetrically scored in both phases: mobility, interest toward either conspecifics or the rest of the environment and attraction toward conspecifics. Three behavioural scores showed significant difference or a trend between S+ and S- sheep and were kept as potential predictors of bleats structure: IsoPC1 was a proxy of vigilance and mobility (i.e. bodily activation, arousal), ReuPC1 was a proxy of interest toward conspecifics and ReuPC3 was a proxy of attraction toward conspecifics (see loadings of PCs in table S2). As controls, we were first able to partially control for potential heritability of vocal apparatus using the identity of the father (10 fathers) as random factor in the LMM. Second, based on the source-filter theory of sound production, bigger lambs are expected to produce calls containing lower frequencies [44], which could be a strong predictor of bleat acoustic structure. Hence, the weight of the lamb was used as interacting covariate in our LMM. Third, to control for effect of the diverging selections, the HSI was also included in our LMM, as a negative control for selections.

Therefore, the full model was composed of the following fixed effects: isoPC1 (proxy of the arousal of the lamb in isolation, emotional hypothesis), reuPC1 and reuPC3 (proxy of social interest and attraction

toward conspecifics, socio-acoustic co-selection hypothesis), the SSI and HSI (proxy of genetic selection for sociability and selection on human reactivity, socio-acoustic co-selection hypothesis), and the scaled weight of the lamb 'Zweight' (proxy of the size of the lamb, biometric effects) in interaction with all other factors. The random factor included the identity of the animal nested within the identity of the father (partially controlling for heritability of vocal apparatus structure and repetitions of several calls from the same individual):

```
isoLD ~ lmer (ZWeight *(isoPC1 + reuPC1 + reuPC3 + SSI  + HSI) + (1|father_ID/ani-
mal_ID), data = (intermediate) non selected females)
```

2.5.3 Impact of genetic selection for sociability on encoding of individuality in bleats

To test the impact of the genetic line on identity coding in isolation and pre-feeding bleats, nested pDFAs were computed within each context of recording to extract scores of correct classification according to the identity of the caller.

For the isolation bleats, the calls of 124 females (selected or not) were used in the analysis, and permutations for testing call classification according to individuals were restricted to within father identity ([min;max] = [1;18] females per father for permutations). For the pre-feeding bleats, the calls of 16 females were used in the analysis, and due to the lower sample size ([min;max] = [1;3] females per father), no restriction for permutations within father identity was applied. The analysis confirmed that the set of acoustic parameters used in this study allowed correct classification according to the caller, in both contexts (supplementary table S2), as previously shown in sheep on a different set of parameters [24]. The ratio of correctly cross-classified bleats, in each context, was then extracted from the diagonal of the prediction/reference confusion matrix as a proxy of success of cross classification of calls according to the identity of the caller (the higher the ratio, the higher the individuality) and used as response variable after square-root transformation in a LMM to test the effect of the genetic line (and predictors cited above when possible).

The LMM built for the isolation bleats included the same predictors as above (2.5.2) to explain the correct classification score: the SSI, isoPC1, reuPC1, reuPC3 and the weight of the lamb as interacting scaled covariate ('Zweight'). Arousal level may decrease identity coding in vocalisations [30]: if identity coding was affected by the level of arousal expressed during the test, then we expected a negative relationship between the ratio of correctly classified calls and isoPC1 (emotional hypothesis). If identity coding was affected by the selection for sociability and social behaviour, then we expected a rela-

tionship between the ratio of correctly classified calls and reuPC1, reuPC3 and/or the Social Selection Index (socio-acoustic co-selection hypothesis). The father identity was included as a random effect:

```
sqrt(ratio_of_correctly_cross_classified_bleats) ~ lmer(ZWeight *(isoPC1 + reuPC1
+ reuPC3 + SocialSelectionIndex) + (1|father_ID))
```

For the LMM built for the pre-feeding bleats, only selected females were recorded and no behavioural data was available, hence we included only the line (S+ vs. S-) in interaction with the weight of the lamb as an interacting scaled covariate ('Zweight'), along with father identity as a random effect:

```
sqrt(ratio_of_correctly_cross_classified_bleats) ~ lmer(ZWeight *line + (1|
father_ID))
```

For both models, the response variables were transformed using a square root function to reach symetrical distribution prior to modeling.

2.5.4 Model inspection and statistical tests on LMM

After visual inspection of the symmetrical distribution of the residuals, a type II Wald Chi-Square test on the full model was computed to test the effect of the predictors (*Anova()* function, 'car' R package). Slope estimates were extracted with *emtrends()* function ('emmeans' R package, [45]). When interactions between covariates were significant, pairwise comparisons of slopes were computed, fixing one covariate at mean, mean-se, mean+se to test the variation of the slope of the other covariate.

3 Results

3.1 Differences in acoustic signature of lambs selected for sociability

According to the loadings of acoustic parameters on the first linear discriminant function (LD) extracted from a LDA maximizing the difference between the two lines, the median frequency (Q50), mean fundamental frequency (fo_mean), the mean frequency of the second formant and its relative amplitude (F2_mean and F2_amp) consistently loaded highly on LD in bleats recorded in both contexts (table 1 and figure 1A, 1B). Other parameters loaded highly on the LD in one of the contexts but not the other: duration and mean dominant frequency (dfreq_mean) loaded more strongly on isoLD, carried out on isolation bleats, whereas the spectral flatness (sfm), the energy distribution (Q25, Q75 and IQR) and the variability of fundamental frequency (fo_sd) loaded more on the feedLD, carried out on pre-feeding bleats (figure 1B). For isolation bleats, the projection of the acoustic parameters of bleats produced by non-selected females on the linear discriminant function describing differences between S+

and S- bleats (isoLD) showed that non selected females had intermediate values (LMM, Chisq = 23.94, $p < 0.001$, figure 1C) between the bleats from S+ and S- females.

Table 1: Loadings of acoustic parameters on the first linear discriminant function (LD) extracted from the linear discriminant function analysis (LDA) performed on isolation calls, leading to the acoustic score 'isoLD', and on LD extracted from the LDA performed on pre-feeding calls, leading to the acoustic score 'feedLD'. In bold, loading values having absolute relative loading above 0.4, considered as descriptors of the linear discriminant function. Underligned acoustic parameter names illustrate consistently high loadings across both contexts, bold loading value illustrate parameters loadings significantly on LD in one of the context.

Acoustic parameter	**Loading on isoLD**	**Loading on feedLD**
duration	**0.515**	-0.174
<u>Q50</u>	**-0.745**	**-0.640**
Q25	0.131	**-0.526**
Q75	0.161	**0.895**
IQR	0.112	**-1.000**
sfm	-0.172	**0.932**
dfreq_mean	**1.000**	-0.036
dfreq_sd	**-0.866**	-0.051
<u>fo_mean</u>	**-0.895**	**0.488**
fo_sd	-0.260	**-0.545**
F1_mean	-0.087	-0.316
<u>F2_mean</u>	**-0.952**	**0.656**
F3_mean	0.129	-0.132
<u>F2_amp</u>	**0.926**	**0.639**
F3_amp	**-0.430**	-0.175

NB: dfreq_mean and dfreq_sd for pre-feeding LDA were square-root transformed

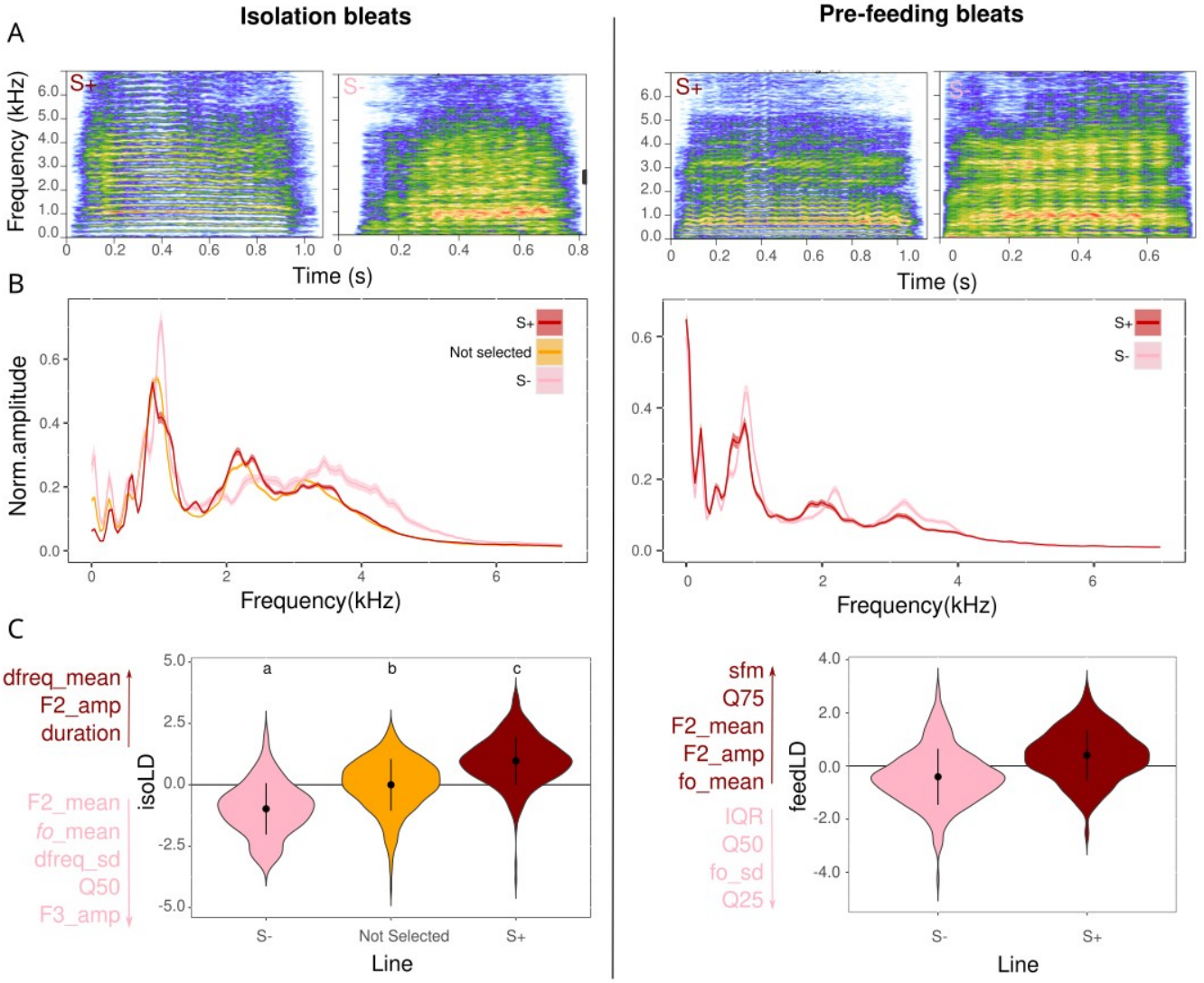

Figure 1: Acoustic differences in isolation bleats (left side of panel) and in pre-feeding bleats (right side of panel) according to the 'line' (S+, S- and intermediate not selected when considering the isolation calls). A: Spectrogram of calls of one representative S+ female and one representative S- female according to the context of recording (FFT wl = 2048 pts, ov= 90%, frequency sampling=44.1 kHz, amplitude range from blue to red color scale: -70 to 0dB). B: Averaged mean-spectrum (and standard deviation in shade) over the total amount of calls analyzed per line and contexts (Number of isolation calls: N=322 from S+, N=67 from S-, N=899 from not selected females; Number of pre-feeding calls: N=262 from S+, N=257 from S- females). C: Acoustic parameters explaining the Acoustic Score computed using a LDA on 'line' within each context of recording (isoLD for isolation and pre-feedLD for pre-feeding). For isolation calls, the predictions of isoLD for calls recorded in non-selected females and comparisons (groups with different letters illustrate statistical differences, see supplementary statistical tables S3 for model estimates and pairwise comparisons).

The pDFA on isolation and pre-feeding bleats showed that, without controlling for individual identity, the correct classification according to the line was significant on the training data set, i.e. selected calls (24% and 12% correct classification above percentage expected at random for isolation and pre-feeding calls, respectively; p = 0.001 for both; table 2A). However, correct cross classification (on the validat-

ing dataset) was only significant for isolation bleats and not pre-feeding bleats (31%, p = 0.001 vs. 15%, p = 0.223 correct classification above percentage expected at random for isolation and pre-feeding calls, respectively; table 2A). Since the results could be driven both by the line and individual vocal signatures of the lamb belonging to each line, a second analysis was performed, controlling for repeated measures of the same individuals. The analysis showed a trend for correct classification for isolation bleats according to the line (p = 0.105 and p = 0.084 for correct classification of calls using the training and validating (cross classification) dataset respectively, table 2A). Although the significance 0.05 threshold was not reached for cross classification in general, the confusion matrix of p-values showed significant discrimination of S+ bleats for isolation calls (table 2B). For pre-feeding bleats, no evidence of correct classification of the line was found neither considering classification scores (p = 0.501 and p = 0.435 for correct classification of calls according to the line using the training and validating (cross classification) dataset respectively, table 2A), nor inspecting the confusion matrix of p-values for each line (table 2B).

Table 2: Call classification according to the line (S+/S-) using pDFA in isolation bleats and in pre-feeding bleats. A: result of pDFA, percentage of classified and cross classified calls and p-values computed according to permuted (random) classification. B: confusion matrix of classification p-values for S+ and S- calls in each context.

	Isolation bleats		**Pre-feeding bleats**	
A. Results of pDFA on discrimination of calls from line S+ and S-				
Control factor (for selection)	X	Individual	X	Individual
Number of levels of control factor selected out of total	X	14/29	X	12/16
Correct classification of selected calls (%)	88.02	93.52	68.41	85.04
Expected correct classification of selected calls (%)	64.23	85.47	56.60	84.36
P value for classification of selected calls	**0.001**	**0.105**	**0.001**	**0.501**
Correct cross classification (%)	80.01	68.91	64.43	56.75
Expected correct cross classification of calls (%)	49.84	58.21	49.69	55.74
P value for cross classification of calls	**0.001**	**0.084**	**0.223**	**0.435**

B. Confusion matrices of P values of cross classification

Reference \ Prediction	S-	S+	S-	S+	S-	S+	S-	S+
S-	0.476	0.505	0.443	0.913	0.515	0.486	0.370	0.598
S+	1	**0.001**	0.947	**0.039**	0.875	0.126	0.586	0.459

3.2 Drivers of acoustic signature of isolation bleats

A significant positive relationship between the SSI and the acoustic score isoLD was found (LMM: Chisq = 8.15 p = 0.004, slope estimate [95%CI] = 0.36[0.10;0.63], figure 2), without any interaction with the weight of the lamb (LMM: Chisq = 0.31 p = 0. 576). Based on the acoustic loadings (table 1), this suggests that non-selected lambs having SSI closer to S+ sheep produced calls with higher mean dominant frequency, longer duration, and a more pronounced second formant. A significant interaction was found between the weight of the lamb and reuPC3 (LMM: Chisq = 4.29 p= 0.04). Further posthoc tests revealed that there was a positive relationship between reuPC3 and the acoustic score isoLD, especially for heavier lambs (lstrends posthoc tests, slope estimates [95%CI]: 0.27[0.06;0.48] and 0.13[-0.01;0.26] respectively for high and medium), but not for lighter lamb (-0.02[-0.19;0.16], figure 2). According to the loadings of the behavioural PCA (table S2), this suggests that lambs - at least heavy and medium weight ones - exhibiting closer proximity with their conspecifics in the reunion that followed the isolation phase produced bleats with a higher mean dominant frequency, longer duration, and a more pronounced second formant during isolation. There was no evidence for any effect of the behavioural scores isoPC1 - correlated with bodily activation during the isolation - or reuPC1 - correlated with exploration and bodily activation during the reunion - on isoLD (LMM: Chisq < 2.3 p > 0.13, supplementary table S6). There was also no evidence of any effect of the HSI on isoLD (LMM: Chisq = 0.006 p = 0.94, supplementary table S6).

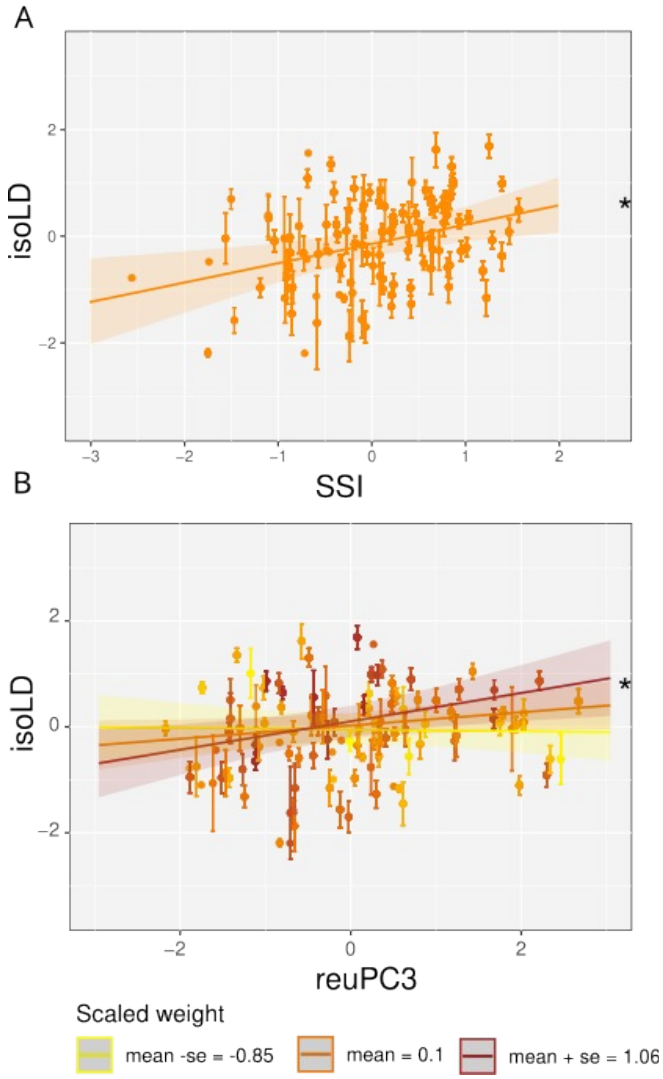

Figure 2: Model estimates and 95% confidence interval depicting the relationship between the Acoustic Score describing isolation bleat structure (isoLD) of non-selected females (hence having intermediate social selection score compared to selected animals) and (A) the Social Selection Index or (B)the behavioural score reuPC3, which positively correlated with proximity. For reuPC3, a significant interaction was found with the weight of the lamb, so slope estimated are showed based on the mean, mean + se and mean – se of the scaled weight used as covariate. Stars (*) show significant slopes. Statistical results of Anova() and model estimates are available as supplementary material tables S6 and S7 repsectively.

3.3 Consequence of genetic divergence for sociability on individuality coding in bleats

For isolation bleats, using 122 females that produced more than four calls during the arena test, the SSI was negatively correlated with the individuality in calls (measured as the ratio of correctly cross-classifed calls in the pDFA) (LMM: Chisq = 4.6 p = 0.03); higher SSI had slightly lower ratios of correctly cross-classified calls (slope estimates [95%CI]: -0.04[-0.09;0.002], figure 3). No evidence of any effect of other potential predictors (weight as interacting covariate of isoPC1, reuPC1, reuPC3) were found (LMM: Chisq < 1.59 p > 0.21, table S8). For pre-feeding bleats, using the 16 females that produced more than four calls during the pre-feeding sessions, no evidence of any effect of the line nor the

weight was found on the ratio of correctly cross-classified calls (LMM: Chisq < 0.95 p > 0.33, table S8).

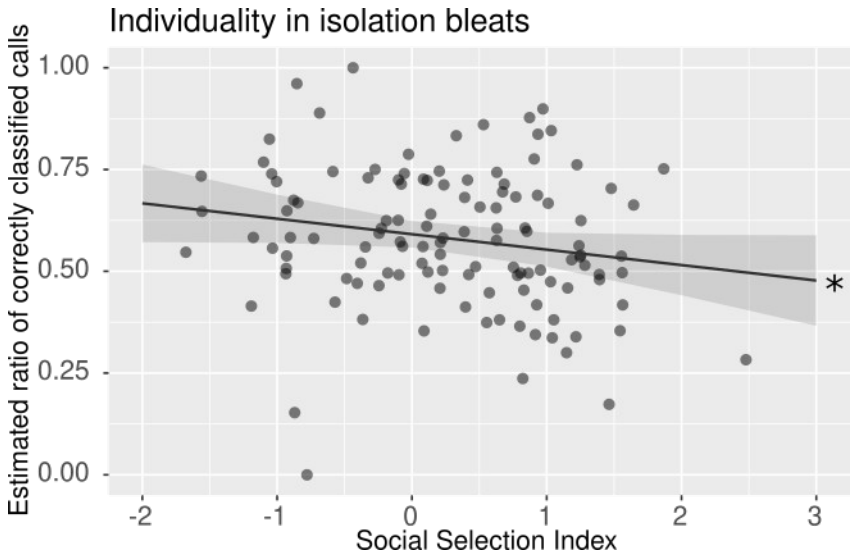

*Figure 3: Relationship between the ratio of correctly cross classified calls (proxy of individuality in spectro-temporal features of bleats) and the Social Selection Index. Model estimate (line) and 95% confidence interval represented (shade). Each point represents individual estimated value. *: significant slope. Statistical results of Anova() are available as supplementary material tables S8.*

Discussion

High bleats of lambs selected for high (S+) or low (S-) sociability (i.e. reactivity to social isolation) were recorded in emotional contexts, namely isolation and pre-feeding contexts. Our acoustic analysis showed that several acoustic parameters, such as median frequency, fundamental frequeny, frequency and relative amplitude of second formant , consistently contributed to the difference between the S+ and S- lines across contexts, while others were more context specific. Correct classification of calls according to the line was significant only for isolation bleats, particularly S+ bleats, and not pre-feeding bleats. This suggests that a sociability related ‘line acoustic signature’ may arise in isolation calls only. The Social Selection Index (SSI) and proximity to conspecifics expressed during the reunion phase were predictors of the acoustic structure of isolation bleats, which was not the case of emotional arousal. These results suggest that the temperament trait sociability, more than emotional arousal per se, influenced sheep vocalisations structure in our study, especially in the context of an isolation test where such trait is strongly expressed. This may reveal that specific acoustic markers are co-selected when selecting for sociable animals. Last, the results showed that the SSI negatively impacted the encoding of individual’s identity in isolation bleats: highly sociable animals tended to produce isolation bleats with lower probability of being correctly attributed to the caller. Overall, this suggests that tem-

perament traits such as sociability strongly impact on vocalisations of farm animals such as sheep, prevailing in some contexts over the effect of emotional arousal and impacting on vocal individuality.

Context specific divergence of acoustic structure of bleats in sociable and less sociable sheep lines

The results from the acoustic analysis showed that common acoustic parameters were involved in the discrimination of high bleats produced by female lambs belonging to two divergent genetic lines based on social traits (S+, highly sociable and S-, less sociable): mean fundamental frequency, median of the mean spectrum, mean value of second formant and its relative amplitude. Based on the source-filter theory of vocal production, vocal signals result from a two-stage production, with the glottal wave generated in the larynx (the source) being subsequently filtered in the supralaryngeal vocal tract (the filter) [38]. Here, we show that both source (fundamental frequency, median of the mean spectrum) and filter (formants) parameters may participate in the discrimination of the divergent lines consistently in both contexts. It is likely that the anatomy of the larynx and vocal tract may be influenced by genetic characteristics (heritability of structures of the vocal apparatus), leading to diverging vocal signature between sheep of the two lines. If this was the only mechanism underlying the acoustic difference we found between genetic lines, we would have expected a correct classification of bleats according to the line regardless of the recording contexts. On the contrary, only isolation bleats could be correctly classified according to the line and not the pre-feeding bleats. The ‘line acoustic signature’ is hence context-specific, suggesting that selection (in general) and heritability of structures of the vocal apparatus cannot, alone, explain the acoustic difference in bleat structure revealed in our study

This conclusion may find support in the fact that other acoustic parameters discriminated between the two lines in one context but not the other, showing some flexibility. Indeed, dominant frequency mean and its standard deviation, relative amplitude of third formant and duration were important parameters for distinguishing S+ from S- in isolation bleats, but not for pre-feeding bleats. As sheep are highly gregarious, isolation from conspecifics was assumed to be a negative situation of high arousal (provoking high bodily activation [46]). In several species, stress involves the production of calls with longer durations and higher frequencies [3–5]. Here, S+ lamb, which expressed a higher behavioral reaction to isolation (see also [29]), also produced high bleats with higher dominant frequency and longer duration. Hence, our acoustic results are in line with the hypothesis that S+ lambs may be more stressed in social isolation than S- lambs. However, specific changes in acoustic structure of bleats as a function of the

emotional valence and arousal of the producer are still lacking for sheep, and this should be further validated with standard tests [5].

Different parameters played an important role in distinguishing high bleats from S+ and S- lamb in pre-feeding context: quartiles of the mean spectrum and its the inter-quartile range as well as the spectral flatness (noisiness), but the classification of these calls according to the line was not above chance level. The pre-feeding context may represent an emotional state of intermediate arousal (walking or running), but characterized by a positive valence (pleasant), and not related to social behaviour like in the case of the social isolation [35,36]. In fact, it is possible that the selection process, based on sociability, leads to a stronger selective pressure on isolation bleats than on pre-feeding bleats, explaining why the difference emerges in isolation bleats, especially in calls from highly sociable lambs.

Beyond social stress: vocal encoding of heritable sociability in isolation bleats

To investigate whether the above-described ‘line acoustic signature’ is driven by emotional arousal or other factors, our model tested predictors related to the social selection process, the social behaviour expressed during the test and emotional arousal, while controlling for the weight of the animal. This allowed us to potentially disentangle between two hypothesis that could explain difference in bleat acoustic structure between our two lines: the emotional hypothesis and the socio-acoustic co-selection hypothesis. Results showed that, although the emotional arousal during isolation was clearly different between the two lines, with S+ being more active, there was no evidence of a relationship between our arousal proxy and the acoustic score describing isolation bleats structure. Instead, the SSI and spatial proximity to conspecifics during the reunion that immediately followed the isolation were the main predictors of the acoustic score describing the isolation bleat structure. Our results are therefore in favor of the socio-acoustic co-selection. In other words, selecting for sociability, based on behavioural reaction to isolation, likely also selected for individuals more motivated to be closer to conspecifics and for individuals producing isolation bleats having a typical vocal signature or expressing emotional arousal/motivation to reunite with conspecifics to a higher extent than predicted by their behaviour. Indeed, temperament has been shown to affect expression of emotions in other ungulates such as horses, in which proactive horses appear more stressed based on their behaviour compared to their physiology [8]. In genetic selections, selection for a trait might co-select another one, with several examples in poultry reviewed by Jones and Hocking (1999) [47]. We show here a possible vocal encod-

ing of sociability, a heritable temperament trait linked to emotional reactivity during social separation and to the motivation to associate with others.

Implications of a socio-acoustic co-selection for social cognition

Our results confirmed that an individual vocal signature is encoded in high bleats, in accordance with previous studies [24,25]. Interestingly, based on the acoustic parameters used here, the classification of calls to the correct caller was slightly impacted by the SSI: the higher the index, and thus the higher the sociability, the lower the ratio of isolation bleats correctly classified to the caller. Several hypotheses have been proposed to explain individual distinctiveness in calls, summarized in [48]. Based on the 'distance communication hypothesis', individually in long distance calls should be higher than in short range ones, for which identity of the caller may be encoded by other modalities (e.g. visual or olfactory [49]). In our study species, high bleats may be considered as rather long-distance calls: contrary to low bleats, they are produced at higher amplitude and when an individual loses contact with conspecifics (however, short separation distance may also trigger the production of high bleats in experimental settings) [25]. In accordance to the distance communication hypothesis, high bleats encode more individuality than low bleats, at least in adult female sheep [25]. Our results revealed a rather high individuality in the high bleats, as shown by high cross classification scores in general. In addition, higher scores were found in isolation bleats than pre-feeding bleats, which may be in line with the distance communication hypothesis, since during isolation, sheep were at further distance from conspecifics. Secondly, based on the 'social hypothesis', individuality in call structure should be driven either by the context or the receiver the call is intended to: calls addressed to a specific individual are predicted to display higher individuality than calls directed to a group or produced in a general context (food, alarm calls, distress) [50,51]. In baby cries for example, individual signatures in pain cries are less reliable than in discomfort cries and it was hypothesized that low individuality in distress calls produced within a social group function to trigger the attention of the entire group rather than to the caretaker only [30]. Recently, using a database of contact calls from seven ungulate species, Osiecka et al. showed that individual distinctiveness in calls was impaired in negative contexts compared to positive ones of similar arousal [31], and hypothesized that calls produced in negative contexts may be addressed to a broader audience than those produced in affiliative contexts. Our results show here that, within the same negative context (social isolation) and beyond social stress, encoding of sociability also impairs individuality in high bleats: calls from more sociable animals carry less individuality. Calls produced by more sociable animals in a context of social separation, where they are highly motivated to reunite with con-

specifics, may thus be perceived by multiple receivers and increase the probability of triggering a response from at least one group member, independently from the identity of the caller. If this hypothesis is true, it could have implications for social cognition, as it might impair individual recognition (mother-lamb or social partners) of more sociable sheep based on their high bleats. More research is needed to investigate the consequences of vocal encoding of sociability for communication and its interplay with vocal perception of emotions.

Conclusion

To our knowledge, our study provides the first evidence of the consequences of genetic selection for sociability traits on vocal communication, using sheep as a model species. Our findings lead us to suggest a socio-acoustic co-selection: selecting for sociable animals leads to exaggerated vocal expressions of emotional arousal during social isolation and impairs vocal signatures at the group and individual level. The phenomenon we quantified opens the question of the long-term consequences of such selection on social cognition. The divergent lines we describe here also provide a model to study the co-evolution between social traits and social and emotional communication in a gregarious species and explore welfare consequences.

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Authors' contribution

ASV designed the study, collected the acoustic data, ran the analyses and drafted the manuscript, AB designed the behavioural tests, discussed the design of the study and revised the manuscript, GB, CD and SD carried out the behavioural tests, MMM discussed the design of the study and revised the manuscript, PRG collected the acoustic data, ran the analyses and revised the manuscript, DH designed and computed the genetic selections and revised the manuscript, EFB designed the study, revised the manuscript and funded the study.

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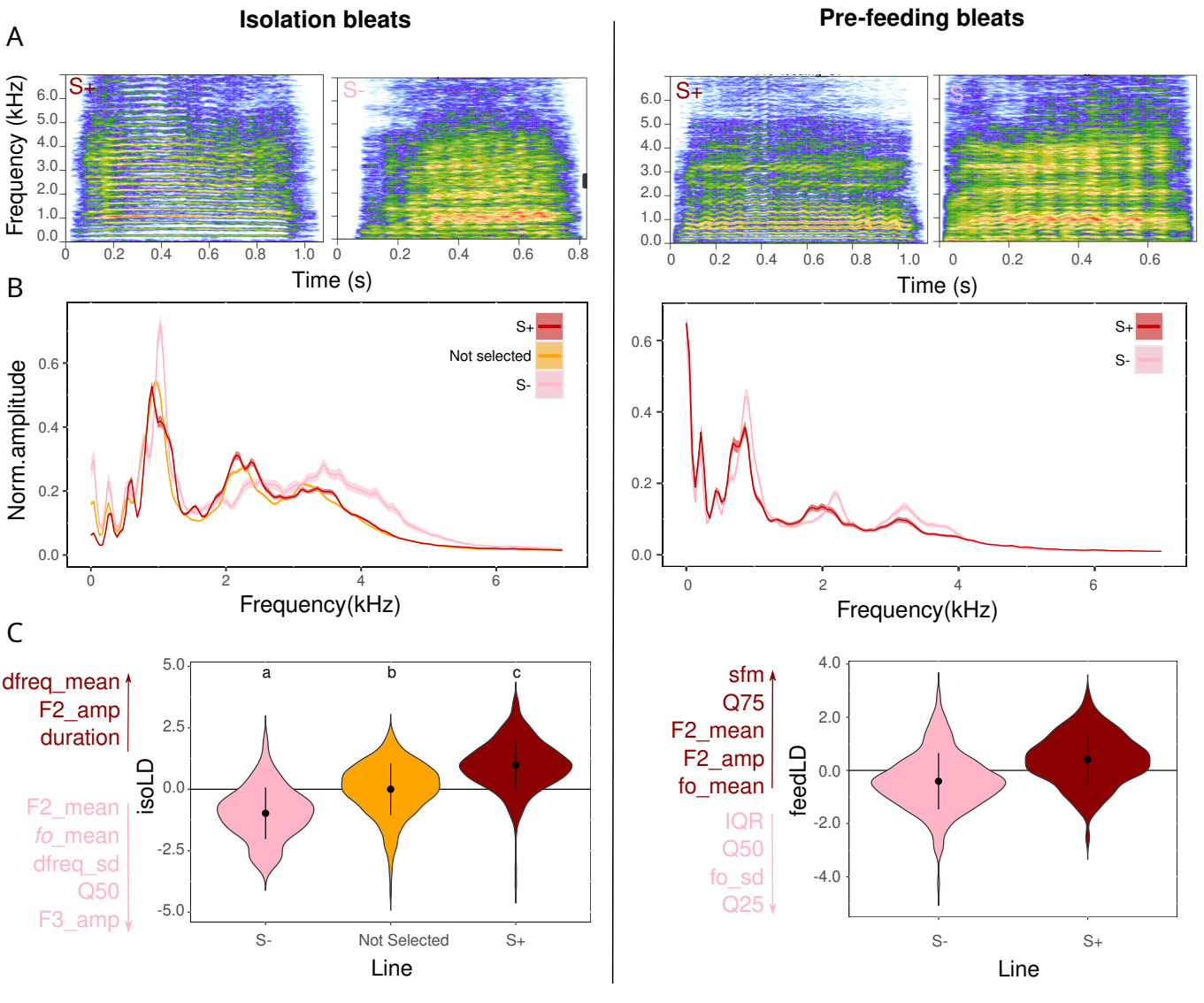
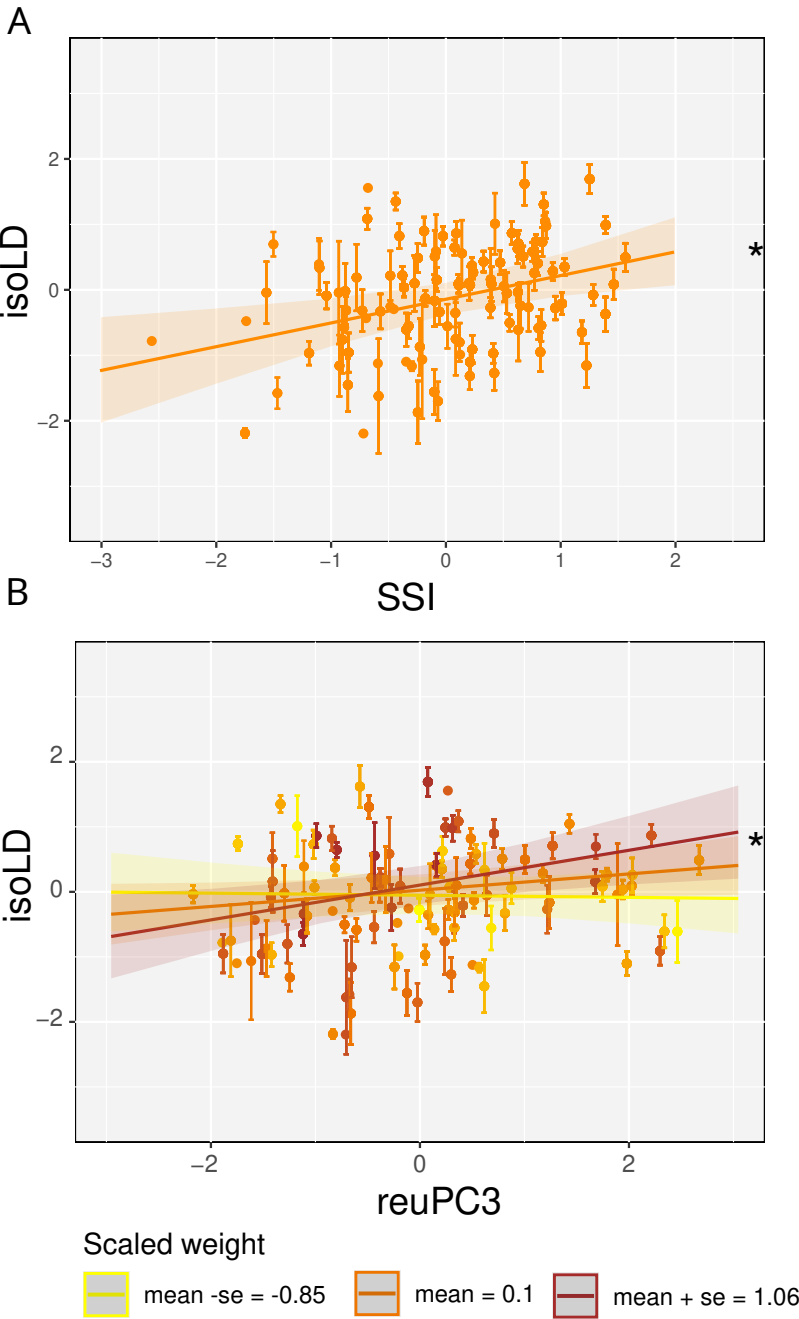
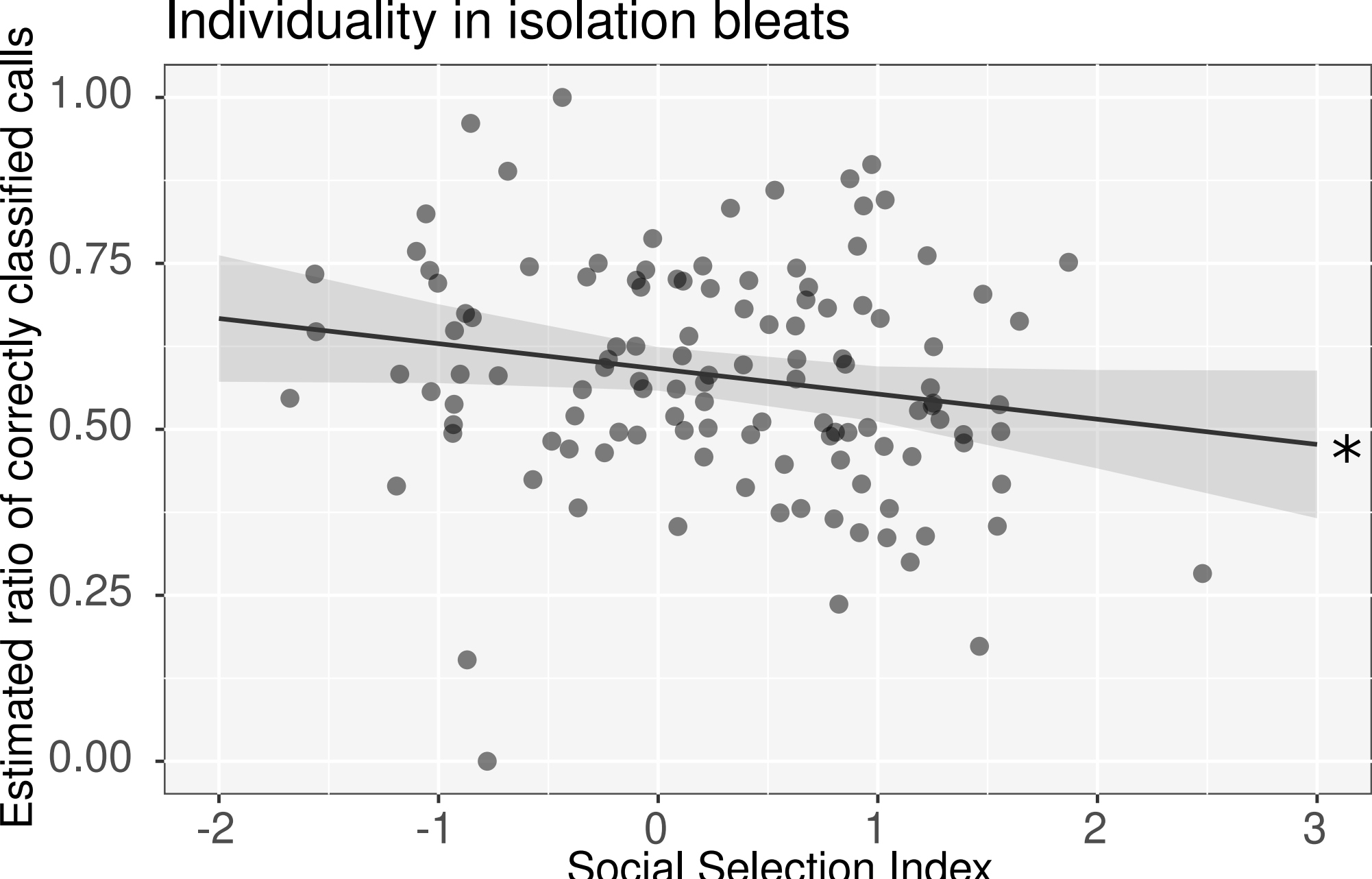

Isolation bleats
Pre-feeding bleats
A
S+
S-
Frequency (kHz)
Time (s)
B
Norm.amplitude
Frequency(kHz)
S+
Not selected
S-
C
dfreq_mean
F2_amp
duration
F2_mean
fo_mean
dfreq_sd
Q50
F3_amp
isoLD
a
b
c
S-
Not Selected
S+
Line
sfm
Q75
F2_mean
F2_amp
fo_mean
IQR
Q50
fo_sd
Q25
feedLD
S-
S+
Line


A
isoLD
2
0
−2
*
−3
−2
−1
0
1
2
SSI
B
isoLD
2
0
−2
*
−2
0
2
reuPC3
Scaled weight
mean -se = -0.85
mean = 0.1
mean + se = 1.06


Individuality in isolation bleats
Estimated ratio of correctly classified calls
1.00
0.75
0.50
0.25
0.00
-2
-1
0
1
2
3
Social Selection Index
*