Males use time whereas females prefer harmony: individual call recognition in the dimorphic blue-footed booby

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In breeding birds, acoustic signalling is often an important cue for reunion between sexual partners. However, in spite of its potential interest, mate recognition has rarely been examined by comparing the two sexes. We studied the blue-footed booby, Sula nebouxii, a socially monogamous seabird, with a dramatic call sexual dimorphism suggesting two different strategies for identity coding: the female call is an amplitude-modulated sound with a harmonic series slowly modulated in frequency, while the male call is a noisy whistle strongly modulated in frequency. To compare acoustic strategies between the sexes, we (1) recorded calls of both males and females, (2) searched for an individual signature and characterized it, and (3) tested, using a playback experiment, whether the calls of males and females were equally efficient for mate recognition. Results showed that an individual signature was present in the calls of both sexes. However, the acoustic parameters involved differed: female individual identification was principally achieved by a spectral analysis of the call whereas males’ identity relied mostly on temporal cues. More than 70% of both females and males tested in playback experiments successfully recognized their mate. This suggests that the coding strategies are equally efficient in terms of individual recognition between mates. From a broader point of view, our results underline the importance of assessing both males and females within the same investigative framework.

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Most animal behaviour studies consider males and females differently, by attributing them conventional roles (Karlsson Green & Madjidian 2011). As a consequence, scientific questions and approaches are usually sex-biased, with questions and experimental paradigms differing between the sexes. Although a differential approach between males and females may be appropriate in some cases (e.g. when considering sexual selection questions or behaviours that are only expressed in one sex), this bias is also found in studies in which there is no a priori reason to expect male and female behaviours to differ. This is problematic since true differences between male and female behaviours are of primary interest from an evolutionary point of view. However, demonstrating behavioural differences between the sexes generally requires that male and female be treated equally, with identical and reciprocal experimental approaches for both sexes. Unfortunately, this approach is still rarely taken (Karlsson Green & Madjidian 2011).

One field in which studies of female and male behaviours are biased by a common view of sex roles is animal communication, in which the role of the ‘receiver’ is often attributed to females (Tibbetts & Dale 2007; Karlsson Green & Madjidian 2011). This is probably because of the abundance of animal species showing a pronounced sexual dimorphism in the type of communication signals used (e.g. species in which only males sing) or in the roles played in social interactions (e.g. courtship and expulsion of rivals).

In birds, as male songs are generally used for mate attraction, females are often tested with male vocalizations to investigate their mate choice process. Besides their partner attraction role, acoustic signals in monogamous bird species are important for partner reunions and pair bond maintenance (Aubin & Jouventin 2002; Elie et al. 2010). Seabirds usually form long-lasting pair bonds and reciprocal mate recognition is mandatory for successful reproduction, especially in species that gather in dense colonies with a high risk of confusion between individuals. In this behavioural context, there is no reason to consider that males and females should differ in their ability to discriminate between their mate and a stranger individual (e.g. Seddon & Tobias 2010; Berg et al. 2011). However, individual recognition of mates has rarely been examined by comparing the sexes using the same experimental paradigm.

Until now, most studies that have considered both sexes have not provided a comparison between males’ and females’ calls (e.g.
Aubin & Jouventin 2002), or only presented qualitative sexual differences, with insufficient sample size to allow statistical comparison (Mulard et al. 2008; Cure et al. 2011). To our knowledge, only one study, on the Mediterranean Cory’s shearwater, Calonectris diomedea diomedea, has demonstrated reciprocal mate vocal recognition (Cure et al. 2009). Sexually dimorphic calls have been reported in this species (Bretagnolle & Lequert 1990) as well as in various colonial seabirds (e.g. Puffinus gravis: Brooke 1988; Pachyptila belcheri: Bretagnolle et al. 1998; Rissa tridactyla: Aubin et al. 2007; Mulard et al. 2008; Puffinus yelkouan: Cure et al. 2009), in which differences between males’ and females’ vocalizations can sometimes be extreme (e.g. Sula spp.: Nelson 1978). As the potential of coding information about individual identity depends on acoustic signal structure (Aubin & Jouventin 2002), testing mate vocal recognition in a species with a dramatic vocal dimorphism between sexes represents a situation in which sex differences in vocal discrimination should have the greatest chance to appear.

In boobies (Sula spp.) the greeting calls used during courtship and partners’ reunions are high-pitched whistles in males and loud trumpet-like honks in females (Nelson 1978). This strong sexual dimorphism has been used in the field to sex individuals of Nazca, Sula grants, masked, Sula dactylatra, blue-footed, Sula nebouxii, and Peruvian, Sula variegata, boobies and has been reported for the brown booby, Sula leucogaster (Nelson 1978; Anderson 1993; Velando & Alonso-Alvarez 2003; Zavalaga et al. 2009). Given that acoustic signal structure differs strongly between female and male, the coding strategy of individual identity may vary accordingly and so may the efficiency of both sexes to recognize their partner.

Blue-footed boobies are long lived (around 20 years old) marine birds and colonial breeders with biparental care (Nelson 1978). They are monogamous during the breeding period and use feet colour for mate selection and maternal investment as evidenced by various experimental manipulations (Torres & Velando 2003; Velando et al. 2006; Dentressangle et al. 2008). These studies highlight the importance of visual cues in this species, in which integument coloration (Velando et al. 2006; Morales et al. 2009) seems to be an efficient communication channel, which enables mates to be informed continuously about the quality and health status of their partner. Nevertheless, as feet colour can change in less than 48 h if no food is provided (Velando et al. 2006), such a highly dynamic signal may not be a reliable mate recognition cue. Blue-footed boobies show a strong sexual dimorphism in size, with females generally 32% heavier than males (Torres & Drummond 1997), as well as in vocalizations as in other booby species, with males producing long drawn-out beeseching whistles and females nasal honks (Nelson 1978). Greeting calls are part of the courtship in this species accompanying the typical sky-pointing display and are also used in mate interactions during nest shifts (saluting) and in aggressive interactions (yes-headshaking) with other birds (Nelson 1978).

In this study, we analysed the structure of signals produced by male and female blue-footed boobies and we compared the ability of both sexes to recognize their partner’s call in a reciprocal playback paradigm. To our knowledge, this is the first study investigating how highly sexually dimorphic signals within a species encode for individual identity and whether or not both signals achieve similar efficiency in terms of mate recognition. The pronounced audible difference between male and female calls suggests that the two sexes may use a different acoustic code for individual identity, leading to potential differences in mate recognition abilities. Additionally, given the importance of visual cues for mate selection in this species, it is important to determine whether or not the acoustic channel is used for mate recognition.

METHODS

Field Study

Recordings and playback experiments were carried out during March–April 2006 in the breeding colony of blue-footed boobies located at Isla Isabel, off the Pacific coast of Mexico (25°52′N, 105°54′W). We selected 20 pairs (at least 15 m apart) with one or two chicks from 7 to 25 days old distributed along the coast of the island on a 400 m transect. All nests were individually marked with numbered flags and chicks were banded with numbered plastic colour bands to confirm nest identity. For each focal pair, we recorded a series of calls of adult males and females using a Sennheiser ME64 microphone fixed on a 4 m long pole and connected to a Marantz PMD 670 recorder (sampling frequency = 44100 Hz; frequency response: 50–15 000 Hz ± 2.5 dB frequency range). The experiment complied with the current laws of Mexico; permissions were granted by SEMARNAT (SGPA/DGVS/01583/06) and the Parque Nacional Isla Isabel.

Sound Analysis

To test for the presence of a vocal signature in calls, we measured and analysed 11 features in females’ calls and 10 in males’ calls (Fig. 1a, b). Call features were measured in both temporal and frequency domains using a customized routine analysis built with the Seewave R package (Sueur et al. 2008; see Fig. 1 for details of measured variables). The fundamental frequency was tracked through a short-term cepstral transform (Fig. 1c, d, Oppeheim & Schafer 2004). From the measurements of the start frequency (Fundstart), the maximal frequency (Fundmax), the end frequency (Fundfin), the duration between the temporal positions of Fundmax and Fundstart and the duration between the temporal positions of Fundfin and Fundmax, we calculated two slopes representing the modulation of the fundamental frequency (Slope FM1 and Slope FM2). The mean of the fundamental frequency (Fund mean) and its SD (Fund SD) were also calculated. The sound duration (Duration, Fig. 1e, f) was measured from the amplitude envelope (amplitude threshold for signal detection = 10%). By smoothing the envelope (sliding window = 40 points) and using an amplitude threshold for signal detection of 20%, we measured the mean time period of the main amplitude modulation of female calls (AM envelope). Because of the absence of a pronounced and regular amplitude modulation in male calls, this parameter was not measured for males. The call frequency spectrum (Fig. 1g, h, window length = 1024, overlap = 99%) was characterized by its mean frequency (Freq mean) and its SD (Freq SD), two parameters reporting the distribution of energy (frequency at the first quartile, Q25, and frequency at the third quartile, Q75), and the spectral entropy (sh) calculated as $S = -\sum \frac{y \log y}{\log (N)}$ with $y$ = relative amplitude of the $i$ frequency with $\sum (y) = 1$ and $N$ = number of frequencies (the spectral entropy of a noisy signal will tend towards 1 whereas the spectral entropy of a pure tone signal will tend towards 0, Sueur et al. 2008).

Our sample for sound analysis was composed of a total of 721 and 603 calls from 17 females and 16 males, respectively (21–80 calls/individual). To avoid different weightings in the analysis as extracted variables have different units (e.g. fundamental frequency in Hz, sound duration in s), they were transformed into z scores (centred and normalized).

Statistical Analysis

We used a multivariate approach to test whether calls produced by each individual could be reliably classified. As male and female
calls were clearly distinct, we performed a separate analysis for each sex. We used a cross-validated and permuted discriminant function analysis (pDFA) using a customized R routine, following the protocol proposed by Mundry & Sommer (2007). The DFA is composed of two steps. First, a set of discriminant functions is obtained from a training data set; second, these functions are used to test the classification on a validation set. In the first step of our analysis, a training data set comprising two-thirds of the total calls recorded for each individual was used to generate linear discriminant functions (LD). For the second step, a validation data set comprising the remaining calls from each individual was used to assay the number of correctly classified calls using the discriminant functions obtained in the first step of the DFA. This cross-validation step gives a measure of the effect size (the percentage of correctly assigned calls) and enables us to extract a confusion matrix informing about the conditional probability of identifying that a call produced by individual i was in fact produced by j: confusion(i, j) = pij. For each individual bird 100 random selections of both training and validation data sets were run. The mean effect size was calculated from these 100 iterations. By performing the cross-validation step, not only does one obtain a desirable measure of effect size (the percentage correct) but also the assumption of normality is relaxed.

To obtain the statistical significance of the effect size calculated by the cross-validated step, we created data sets in which the identity of calls was randomly permuted between individuals (using a pDFA procedure). For each of these randomized sets, we followed the same steps, training and validation, as with the non-randomized sets. After 1000 iterations, we calculated the proportion of randomized validation data sets revealing a number of correctly classified calls being at least as large as the effect size obtained with the nonrandomized validation data set. This proportion gives the significance of the level of discrimination and is equivalent to a P value (Mundry & Sommer 2007).

Initially AM envelope (only measured in females) was included in the DFA analysis but it did not improve the classification rate; hence, to obtain a comparable analysis between males and females, this variable was excluded.

Potential for Individual Coding Calculation

To allow individual recognition, vocalizations have to show a highly individualized acoustic structure, meaning that acoustic parameters (spectral and/or temporal) coding for the individual signature must show less variation within than between individuals (potential for individual coding, PIC, Scherrer 1984). To complete the multivariate approach used in the DFA, we thus calculated the PIC value for each acoustic parameter and for each sex. This approach consists of calculating the within-individual (CVi) and interindividual (CVb) coefficient of variation for each variable using the formula: $CV = 100 \times \sqrt[4]{\frac{1}{N}} \times \frac{SD}{mean}$, where SD is the standard deviation, mean is the mean of the sample and N is the sample size (Scherrer 1984). To assess the PIC for each acoustic parameter, we calculated the ratio: $PIC = CVb/mean CVi$, where mean CVi is the mean value of the CVi for all the individuals. Parameters with a PIC value greater than 1 are considered as individual specific because their within-individual variation is smaller than the interindividual variation (Robinson et al. 1993).

Playback Experiment

To test whether male and female boobies were able to recognize their partner using acoustic cues, adult birds were tested once with two playback sessions separated by an interval of at least 15 min. During each of the two playbacks, a series of calls recorded either from the partner or from a stranger (a male if the tested bird was a female and vice versa) was broadcast. To avoid any playback order effect, half of the birds were tested first with their partner’s calls and then with calls of a stranger and the other half in the opposite way. Stranger calls were previously recorded at a nest at least 50 m from the tested nest to avoid any familiarity effect among neighbours. Calls from every bird recorded in the study were used once as partner calls and once as stranger calls. Among the initial set of 20 pairs and for various logistic reasons (insufficient number of recorded signals, field opportunities to test the birds), we were able to test 15 males and 14 females.

Playback sequences were constructed from the recorded calls and broadcast at a distance of 10 m from the focal nest on an Audax loudspeaker (frequency response: 100–8000 Hz ± 2.5 dB) using a Marantz PM680. Signals were amplified using a 10 W customized amplifier and broadcast at natural sound pressure (mean ± SD = 85 ± 5 dB, measured at 1 m with a 2235 Bruel and Kjaer decibel-meter set, microphone type 4176, linear setting). Each playback sequence (i.e. one for the partner and one for the stranger) counted around 30 calls (32 ± 5) and lasted 15 s (± 1 s). We waited at least 15 min (or until the bird ceased vigilance activity) after setting up the equipment to begin the playback and never broadcast calls when the focal bird was engaged in any type of interactions with its neighbours or chicks. All playbacks were done in the absence of the partner. A second observer, sitting 20 m from the nest and who was blind to the type of calls (partner/stranger) being broadcast, scored the behavioural response.

Blue-footed boobies typically arrive at their nest flying and vocalize on the approach. Their partner waiting at the nest generally screens the sky searching for its mate and can also vocalize. Hence, we used these behaviours to evaluate the bird’s response to the playback and compared the intensity of the response to the partner and to the stranger calls. The intensity of the scanning behaviour was assessed using two types of behaviours: (1) the focal bird looked in the direction of the loudspeaker (the bill crossed the imaginary straight line between the loudspeaker and the nest) and (2) the focal bird screened the sky (the bill was at least 45° above the horizontal). These two behaviours (look at the loudspeaker, screen the sky) and the focal bird’s vocal response were each given an intensity score on a one to four scale as follows. Level 1 ‘absent’ was given when the behaviour was not expressed at all while level 2 corresponded to a response of ‘weak intensity’, level 3 to a response of ‘average intensity’ and level 4 to a response of ‘high intensity’. For each of the three behaviours, we compared the response of the focal bird between the two playback sessions (partner calls versus stranger calls) and assigned a score of +1, 0 or −1 to each behaviour depending on whether the focal individual’s response to its partner’s calls was, respectively, superior, equal or inferior to its response to the stranger’s calls. The total differential score for a tested individual was then calculated by summing the single score of the three behaviours (possible total scores: −3, −2, −1, 0, +1, +2, +3). Thus, a total score of +3 was obtained if partner calls elicited a greater response than stranger calls for each of the three behaviours and a total score of −3 if the opposite was true (i.e. stronger response to stranger for all three behaviours). In general, a positive total score corresponded to a preferential response to partner calls whereas a null or negative score meant an absence of preference towards partner calls. For each sex, we used a Wilcoxon signed-ranks test to evaluate the preference for the partner versus the stranger calls. To test for a possible sex effect in the recognition process, we compared results of both males and females with a chi-square test.

RESULTS

Sexual Dimorphism in Calls

Calls from blue-footed booby males and females sound very different owing to their strong dimorphic acoustic structure...
(Fig. 1a, b, Supplementary sound files S1 and S2). The female call is a complex sound, showing harmonic series slowly modulated in frequency (low value of slope FM1 and slope FM2, Table 1, Fig.1a, c), with a heterogeneous distribution of energy among the spectrum and strong amplitude modulations (AM envelope = 219.98 ± 48.01 Hz).

The male call structure is also complex but displays neither harmonics nor rhythmic AM and sounds like a noisy whistle strongly modulated in frequency (high value of slope FM1 and slope FM2, Table 1, Fig. 1b, d). Compared to females, values of the mean fundamental frequency were higher (Table 1, Fig. 1c, d) and sound duration longer in males (Table 1, Fig. 1e, f). Mean fundamental frequency ranged from 0.154 to 0.319 kHz in females and from 2.205 to 4.960 kHz in males and was more variable in males than in females (fund SD, Table 1).

For both sexes the value of the spectral entropy was high (i.e. close to 1, Table 1), meaning that both call types are noisy signals. The distribution of energy among the spectrum differed slightly between males and females, with females showing lower Q25 values and less variable Q75 values than males (males’ SD of Q75 was more than twice that observed in females, Table 1, Fig. 1g, h).

Parameters Coding Individual Identity

From the pDFA analysis, we were able to identify the presence of a vocal signature in both sexes (Fig. 2, Table 2). The cumulative percentage of the first three functions (LD) explained 56.64% and 56.70% of the total variance for females and for males, respectively (Table 2). Information about individual identity was coded by different parameters in the calls produced by each sex (Table 2). For females, individuals were separated principally on the basis of spectral cues whereas in males both temporal and spectral cues were necessary to separate individuals (Table 2). Two of the three parameters that had the largest coefficients in the first discriminant function differed strongly between males and females. For female calls, the mean frequency (Mean Freq, a spectral parameter) was the most important parameter whereas it was the SD of the mean frequency (Freq SD, a temporal parameter) for male calls. Two of the first three parameters in the first discriminant function were related to the spectral domain in females whereas they were related to temporal cues for male calls. The second function also differed between the sexes: in females, the most influential variable was related to the partitioning of energy among the spectrum (Q75) whereas in males it was the SD of the mean frequency (Freq SD). For the third function, all parameters were related to the spectral domain in females, principally on the energy partitioning (Q25 and Q75) whereas in males the SD of the mean frequency remained an influential parameter. Hence, our results suggest that males and females coded their identity in a different way; female identity was achieved first by a spectral analysis of the calls and in a smaller proportion by a temporal analysis. In contrast, male identity was first based on a temporal analysis of the spectrum and second by a spectral analysis of the call. These results were confirmed by the PIC analysis, in which the parameter showing the highest value also differed between the sexes. The mean frequency was the most likely candidate variable for identity coding in females, whereas it was the sound duration in males (Table 2).

The cross-validation step showed that 41.8% of the female calls and 51.60% of the male calls were correctly identified (P = 0.001). Although perfect individual identification was not achieved, these rates of success were well above chance levels (chance = around 6% for each sex, 1/17 in females and 1/16 in males). The confusion matrix that shows the joint probability of the actual and predicted individuals (Fig. 2a, b) illustrates the great variation in the classification success rate among individuals (females: 15–95%; males: 15–86%). Thus, some individuals appear to produce very distinctive calls (e.g. female Ind. 5, male Ind. 10) while others are easily confounded with other individuals (e.g. female Ind. 7, male Ind. 15). The mutual information calculated from the confusion matrix was 1.9 bits for both sexes (well below the ceiling value or log2 (17) = 4.09 for females and log2 (16) = 4 for males; see Mathewson et al. 2010 for a discussion about bias in mutual information).

Partner Recognition

Male and female blue-footed boobies both responded preferentially to their partner’s calls (14 females: W = 82, P = 0.009; 15 males: W = 78, P = 0.002) and there was no difference in the proportion of partner recognition between males and females (x² = 0.11, P = 0.74). Females reacted more to their partner’s calls than to a strange male: 11 of 14 tested females expressed a clear behavioural response in favour of their partner’s calls, while 15 of 17 males showed a similar response (x² = 2.205, P = 0.14). This suggests that males are more tolerant of unfamiliar individuals, as indicated by the lower numbers of calls they produce when a stranger is present (Figs. 1a, b). We obtained comparable results with males: 11 of 15 tested males showed a preferential response to their partner’s call, three did not show any preference for their partner’s call versus a stranger’s call, and the two remaining individuals responded with a slight preference for the stranger’s call (Fig. 3a). We obtained comparable results with males: 11 of 15 tested males showed a preferential response to their partner’s call, three did not show any preference for their partner’s call versus a stranger’s call, and one individual responded preferentially to the stranger’s call (Fig. 3b).

DISCUSSION

This study demonstrates the presence of an individual vocal signature in blue-footed booby calls, and shows that both females and males have comparable ability to discriminate their partner from a stranger in spite of a pronounced call sexual dimorphism. From a broader point of view, our results underline the interest of assessing both males and females within the same investigative framework, including for traits or behaviour showing extreme sexual differences.

The sexually dimorphic greeting call of blue-footed boobies encodes identity information in a different way for males and females but both coding systems appeared equally efficient. Females’ calls show a series of harmonics that are not found in males’ calls, and have a lower fundamental frequency value compared to males’ calls. Male calls are strongly modulated in frequency. Both sexes use two different coding processes for individual identity that are generally described between different species. Females’ identity is principally based on spectral cues and the distribution of energy among the spectrum, a coding process close to that described in nesting penguins such as the Adelie, Pygoscelis adeliae, macaroni, Eudyptes chrysophalus, or the gentoo, Pygoscelis papua, penguins (based on frequency band analysis or

Table 1

<table>
<thead>
<tr>
<th>Acoustic variables measured</th>
<th>Females (N=721 calls) Mean</th>
<th>Females (N=721 calls) SD</th>
<th>Males (N=603 calls) Mean</th>
<th>Males (N=603 calls) SD</th>
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<tbody>
<tr>
<td>Sound duration (s)</td>
<td>0.129</td>
<td>0.043</td>
<td>0.190</td>
<td>0.083</td>
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<tr>
<td>Fundamental mean (Hz)</td>
<td>220.172</td>
<td>35.421</td>
<td>353.064</td>
<td>459.376</td>
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<td>SD of fundamental (Hz)</td>
<td>27.005</td>
<td>11.208</td>
<td>426.974</td>
<td>130.605</td>
</tr>
<tr>
<td>Slope FM1</td>
<td>1.230</td>
<td>1.431</td>
<td>18.087</td>
<td>8.225</td>
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<tr>
<td>Slope FM2</td>
<td>-1.267</td>
<td>2.122</td>
<td>-16.533</td>
<td>8.143</td>
</tr>
<tr>
<td>Mean frequency (Hz)</td>
<td>4324.665</td>
<td>563.596</td>
<td>4756.251</td>
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<tr>
<td>SD of mean frequency (Hz)</td>
<td>2549.118</td>
<td>389.024</td>
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<td>536.326</td>
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<tr>
<td>Q25 (Hz)</td>
<td>2543.593</td>
<td>584.320</td>
<td>3047.186</td>
<td>413.613</td>
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<tr>
<td>Q75 (Hz)</td>
<td>5390.962</td>
<td>693.976</td>
<td>5678.277</td>
<td>1462.820</td>
</tr>
<tr>
<td>Spectral entropy</td>
<td>0.882</td>
<td>0.032</td>
<td>0.846</td>
<td>0.051</td>
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</table>
timbre analysis, Aubin & Jouventin 2002). However, the males’ coding process seems closer to the strategy used by non-nesting penguins, in which individual recognition is based on a frequency–time analysis (frequency modulation and sound duration). Females' calls are also modulated in amplitude and even if this parameter can potentially be involved in the vocal signature...
as found in the emperor penguin, Aptenodytes forsteri (Aubin & Jouventin 2002), it does not seem to be relevant in female blue-footed boobies. When this variable was excluded from the DFA analysis, our results were unchanged. This finding was also confirmed by a low value (close to 1) of the PIC for this variable, reflecting a poor ability in coding for identity.

For a given species, the coding system is often described as an adaptation to face environmental constraints. In the blue-footed booby, males and females are subject to the same constraints (background noise generated by the colony) and the sexual dimorphism present in calls is unlikely to be explained by environmental constraints. As female blue-footed boobies are on average 30% heavier than males, the dimorphic call could instead be a by-product of the sexual size dimorphism reported in Sula spp. (Nelson 1978; Wallslager 1980; Fletcher 2004; but see Aubin et al. 2007) and both coding strategies could have been maintained because they are equally efficient and enable a sufficient level of recognition. Indeed, in both sexes, several acoustic variables are involved in the coding system of the individual signature, resulting in a multiparametric signature that is likely to resist environmental degradation (Aubin & Jouventin 2002; Cure et al. 2009).

Blue-footed and brown boobies show the highest sexual dimorphism among the five booby species and have a marked vocal dimorphism in contrast to the less dimorphic red-footed booby (Lewis et al. 2005; Zavala et al. 2009). The strong difference observed in the call structure may reflect a dimorphism at the level of the syrinx (Murphy 1936). Testosterone has been identified as a factor influencing the development of the syrinx in the black-headed gull, Larus ridibundus (Groothuis & Meeuwsen 1992) and the grey partridge, Perdix perdix (Beani et al. 1995). Nevertheless, if an anatomical difference in the syrinx exists in the blue-footed booby, it does not seem to be testosterone dependent, as male and female did not differ in plasma concentration of testosterone during for the first 15 days of life (F. Dentressangle & R. Torres, personal communication) or at the adult stage (Wingfield et al. 1999).

The ability of monogamous species with biparental care to recognize their partner within a colony is important for their breeding success and is supported by our playback results (73% of the tested males and 78% of the tested females recognized their partner). In this species, chicks are never left unattended before 8—9 weeks of age, implying the continuous presence of one adult close to the nest while the other is foraging at sea (Nelson 1978).

### Table 2

<table>
<thead>
<tr>
<th>Variables</th>
<th>Females</th>
<th></th>
<th></th>
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<th>Males</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>LD 1</td>
<td>LD 2</td>
<td>LD 3</td>
<td>PIC</td>
<td>LD 1</td>
<td>LD 2</td>
<td>LD 3</td>
<td>PIC</td>
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<tr>
<td><strong>Spectral</strong></td>
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<td>Fund mean</td>
<td>−0.107</td>
<td>−0.038</td>
<td>0.038</td>
<td>1.06</td>
<td>−0.087</td>
<td>−0.108</td>
<td>−0.215</td>
<td>1.15</td>
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<td>Mean Freq</td>
<td>1.507</td>
<td>0.018</td>
<td>0.177</td>
<td>1.45</td>
<td>0.579</td>
<td>0.608</td>
<td>0.553</td>
<td>1.31</td>
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<tr>
<td>Sh</td>
<td>−0.908</td>
<td>−0.234</td>
<td>−0.021</td>
<td>1.31</td>
<td>1.436</td>
<td>0.406</td>
<td>0.082</td>
<td>1.36</td>
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<td>Q25</td>
<td>−0.099</td>
<td>−0.115</td>
<td>−0.065</td>
<td>1.21</td>
<td>−0.638</td>
<td>0.064</td>
<td>0.046</td>
<td>1.14</td>
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<td>0.454</td>
<td>−0.120</td>
<td>1.42</td>
<td>0.428</td>
<td>−0.350</td>
<td>−0.181</td>
<td>1.44</td>
</tr>
<tr>
<td><strong>Temporal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>0.302</td>
<td>−0.037</td>
<td>−0.019</td>
<td>1.09</td>
<td>0.746</td>
<td>−0.272</td>
<td>−0.088</td>
<td>1.46</td>
</tr>
<tr>
<td>Fund SD</td>
<td>−0.025</td>
<td>0.004</td>
<td>−0.003</td>
<td>1.02</td>
<td>−0.607</td>
<td>0.006</td>
<td>−0.007</td>
<td>1.09</td>
</tr>
<tr>
<td>Freq SD</td>
<td>0.679</td>
<td>−0.317</td>
<td>−0.031</td>
<td>1.41</td>
<td>−1.701</td>
<td>−0.728</td>
<td>−0.307</td>
<td>1.39</td>
</tr>
<tr>
<td>Slope FM1</td>
<td>−0.077</td>
<td>−0.024</td>
<td>−0.023</td>
<td>0.07</td>
<td>−0.132</td>
<td>0.040</td>
<td>0.009</td>
<td>1.18</td>
</tr>
<tr>
<td>Slope FM2</td>
<td>−0.056</td>
<td>0.064</td>
<td>−0.003</td>
<td>0.92</td>
<td>0.018</td>
<td>0.003</td>
<td>0.025</td>
<td>1.15</td>
</tr>
<tr>
<td>% Variance explained</td>
<td>21.89</td>
<td>40.63</td>
<td>56.64</td>
<td></td>
<td>22.55</td>
<td>40.97</td>
<td>56.70</td>
<td></td>
</tr>
</tbody>
</table>

PIC: potential for individuality coding calculated for each acoustic parameter. The first three coefficients loading more on each LD and the highest value of the PIC are shown in bold.
During breeding, blue-footed boobies typically make one or two long pelagic foraging trips per day (Anderson & Ricklefs 1992; Castillo-Guerrero & Mellink 2010). When coming back to the breeding colony birds must unequivocally identify their partner to ensure the smooth exchange of birds at the nest and enable the partner to go foraging at sea. Additionally, it may be important for blue-footed boobies to recognize and locate their mate quickly to avoid aggression from neighbours, which defend a small territory around their nest.

Behavioural responses obtained in the playback experiment highlight that calls of both males and females have a similar efficiency in terms of mate recognition. This result suggests that individual recognition is not sex-biased, as may be expected in a species with biparental care, in which both partners are likely to be under similar selection pressures to recognize their partner in order to reproduce successfully. While different acoustic parameters are involved in the coding of individuality, the two sexes may use different decoding processes to identify their partner. Sexual difference in the decoding process to identify species-specific male song has already been reported in blackbirds, Turdus merula (Dabelsteen & Pedersen 1993). The present study shows that male and female can also differ in how they identify individuals within their species.

Although the recognition rate in the playback experiment was relatively high, one could have expected a higher classification rate using the factors generated in the DFA. One possible explanation could be the number of calls’ features (10 for both sexes) used in the analysis. This restricted number of parameters is certainly not sufficient to describe perfectly these complex calls (especially in females in which harmonic series and amplitude modulations are present) but it allowed us to obtain comparable results between the sexes within the same species and provided evidence of different coding strategies. Moreover, the mathematical analysis is not based on the series of calls but on one call at the same time. Hence, some information that could be contained in the succession and/or the rate of calling may be lost. However, another important factor that has to be taken into account is the breeding biology of this species. In the blue-footed booby, males are highly territorial during the breeding season and actively defend a small territory (on average 7.6 m²) located close to their natal site. Both males and females are highly philopatric, and a recent study showed that successive short breeding dispersals tended to maintain males and females at an average of 34.9–39.4 and 36.4–42.2 m, respectively, from their natal sites (Kim et al. 2007). The breeding biology is known to influence the encoding process of individuality as evidenced by the comparison of non-nesting versus nesting species (Aubin & Jouventin 2002; Mathevon et al. 2003). Indeed, for species that use a breeding nest, the risk of confusion between individuals is lower because the probability that a bird produces the right call at the wrong place is small (Aubin & Jouventin 2002). Hence, the meeting point as evidenced in other colonial birds could be an important factor involved in blue-footed booby recognition.

Additionally, it is important to underline that blue-footed boobies have well-developed vision (Reed 1987) and use feet colour for mate selection and maternal investment (Torres & Velando 2003; Velando et al. 2006; Dentressangle et al. 2008). In this study we did not test whether or not blue-footed boobies’ calls reflect individual quality and are subject to variation according to the nutritional status of the individual, as shown for feet colour (Velando et al. 2006). Nevertheless, even if such variation occurs, we should expect a conservation of the individual signature needed by birds to recognize their partner. In species that accurately use visual cues for sexual selection, mate recognition may be multimodal. The transmission of individual identity may be partially encoded by acoustical cues and may need visual information and/or a meeting place to confirm the identity of the caller.

Here, we report the first evidence that male and female blue-footed boobies’ calls contain individual information that is used by mates to recognize each other. Although the vocal signature was not as strong as in other seabird species (e.g. in penguins), it was sufficient to allow mate recognition in more than 70% of the playback tests. Furthermore, the highly dimorphic call of boobies did not influence the efficiency of the recognition process as males and females responded to the playback experiment in a similar way. Acoustic parameters coding for the individual signature differed between the sexes, suggesting a different decoding process for males and females. From a broader point of view, the present study underlines the importance of experimentally investigating the behaviour of both sexes without attributing a priori differences in sex roles.

Acknowledgments

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Supplementary Material

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References


