

1 Comparing methodologies for classification of zebra finch distance calls

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6 Running title: Multiple methods for distance call classification

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24 **ABSTRACT**

25 Bioacoustic analysis have been used for a variety of purposes including classifying vocalizations
26 for biodiversity monitoring and understanding mechanisms of cognitive processes. A wide range
27 of statistical methods, including various automated methods, have been used to successfully
28 classify vocalizations based on species, sex, geography, and individual. A comprehensive
29 approach focusing on identifying acoustic features putatively involved in classification is
30 required for the prediction of features necessary for discrimination in the real world. Here, we
31 used several classification techniques, namely Discriminant Function Analyses (DFAs), Support
32 Vector Machines (SVMs), and Artificial Neural Networks (ANNs), for sex-based classification
33 of zebra finch (*Taeniopygia guttata*) distance calls using acoustic features measured from
34 spectrograms. We found that all three methods (DFAs, SVM, and ANNs) correctly classified the
35 calls to respective sex-based categories with high accuracy between 92 and 96%. Frequency
36 modulation of ascending frequency, total duration, and end frequency of the distance call were
37 the most predictive features underlying this classification in all of our models. Our results
38 corroborate evidence of the importance of total call duration and frequency modulation in the
39 classification of male and female distance calls. Moreover, we provide a methodological
40 approach for bioacoustic classification problems using multiple statistical analyses.

41 I. INTRODUCTION

42 Acoustic communication is used throughout the animal kingdom in the contexts of mate
43 attraction, territorial defense, raising alarm, and recognition of species, group, and individuals
44 (Bradbury and Vehrencamp, 2011). Understanding the context in which animal vocalizations are
45 used plays a key role in understanding biological function and evolution of animal
46 communication, as well as the underlying mechanisms of vocal communication in the animals
47 producing the vocalizations under study (Bradbury and Vehrencamp, 2011). Research in
48 bioacoustics focuses primarily on the mechanisms of production, transmission, and reception of
49 acoustic signals (Erbe, 2016; Hopp et al., 1998). One approach to bioacoustics research involves
50 describing and then classifying animal vocalizations into categories. This approach helps to
51 reduce naturally-occurring complexity among signal classes by forming categories of signals
52 based on acoustic similarity (Garcia and Favaro, 2017). The categories can be vocal repertoires
53 of different species (Ficken et al., 1978; Salmi et al., 2013), based on the sex of the vocalizer
54 (Campbell et al., 2016), based on geographical locations (Hahn et al., 2013a; Tuncer, 2013),
55 based on ecological habitats (Anderson et al., 2008; Gómez et al., 2018) or based on the
56 individuals (Elie and Theunissen, 2016; Hahn et al., 2013b; Laiolo et al., 2000; (Montenegro et
57 al., 2021, Průchová et al., 2017). The application of this approach varies widely from biological
58 scales (Gentry et al., 2020) to wildlife management and conservation (Laiolo et al., 2008;
59 Teixeira et al., 2019) to animal welfare (Manteuffel et al., 2004; Röttgen et al., 2020; Schön et
60 al., 2004) to life history, and evolutionary biology (Warwick et al., 2015; Xu and Shaw, 2019).

61 Bioacoustics methods, especially vocalization classification, play an important role in
62 investigations of cognitive processes such as perception, memory, and decision making
63 (Shettleworth, 2009). Thorough description and classification of vocalizations are an integral

64 part of understanding the mechanisms involved in biologically relevant processes like mate
65 selection (Delgado, 2006; Hernandez et al., 2016; Vignal et al., 2008), predator interaction (Bee
66 et al., 2016; Congdon et al., 2020), territoriality (Walcott et al., 2006), social interaction
67 (Slocombe and Zuberbühler, 2005), and individual recognition (D'Amelio et al., 2017; Elie and
68 Theunissen, 2018). Classification of vocalizations into specific classes as a tool of bioacoustic
69 analyses dates to the early history of bioacoustics in the 1950s and 60s where scientists used
70 sound spectrograms to describe the prominent features of vocalization types in domestic fowl
71 and weaverbird (Collias, 1963; Collias and Joos, 1953). Since then, the field of bioacoustics has
72 come a long way introducing new concepts, powerful analysis techniques (Herbst et al., 2013;
73 Kershenbaum et al., 2016; Tallet et al., 2013; Wadewitz et al., 2015), and moving towards data-
74 driven and automated classification (Bravo Sanchez et al., 2021; Brooker et al., 2020; Elie and
75 Theunissen, 2016; Mcloughlin et al., 2019; Priyadarshani et al., 2018; Salamon et al., 2016).

76 A multitude of statistical methods, including automated methods, have been used for
77 classification of vocalizations for biodiversity monitoring (Caycedo-Rosales et al., 2013;
78 Priyadarshani et al., 2018), constructing vocal repertoires (Elie and Theunissen, 2016; Wadewitz
79 et al., 2015), and classifying based on sex (Campbell et al., 2016), geography (Hahn et al.,
80 2013a; Tuncer, 2013), and individuals (Elie and Theunissen, 2018; Průchová et al., 2017). These
81 methods mainly include random forest, decision trees, Hidden Markov models, spectrogram
82 cross-correlation, support vector machines (SVMs), and artificial neural networks (ANNs)
83 (Knight et al., 2017). These automated methods are useful for classification, especially for large
84 data sets, although human, and possibly non-human animal involvement, is required to verify the
85 reliability and validity of such analyses. Thus, semi-automated methods with human involvement
86 work best. Uncovering the acoustic features primarily responsible for the classification into types

87 based on species, sex, or individual can reveal the locus of biologically significant stimulus
88 control involved in animal communication signals. An integrated approach is required for
89 classification, from identifying acoustic units to choosing methods of analyses for identifying
90 features responsible for classification (Kershenbaum et al., 2016).

91 Zebra finches are flocking songbirds native to Australia that are highly sexually
92 dimorphic in a number of important aspects. Only male zebra finches produce songs, though
93 both sexes produce a variety of calls (Elie and Theunissen, 2016; Zann, 1996). Distance calls or
94 “long calls” are the most characteristic, species-typical calls produced and are used in a variety
95 of contexts, especially when birds are visually isolated from their mates or conspecifics (Zann,
96 1996). Distance calls are sexually dimorphic: males produce shorter, more acoustically complex
97 calls and females produce longer, relatively unmodulated calls (Zann, 1996). The male distance
98 call is composed of a downsweep frequency modulation with a fundamental frequency of
99 approximately 600-1000 Hz (Figure 1). The female distance call is composed of a harmonic
100 series of unmodulated frequencies with fundamental frequency of 350-550 Hz (Vicario et al.,
101 2001; Zann, 1996). Zebra finches are capable of discriminating mates from others (Vignal et al.,
102 2008) and of recognizing conspecifics (Vignal et al., 2004) using distance calls. The differences
103 in the acoustic structure between male and female versions of these calls allow this
104 discrimination (Vignal and Mathevon, 2011). Call duration, fundamental frequency, and rapid
105 frequency modulation seem to play an important role in eliciting differential behavioural
106 response to male and female distance calls (Vicario et al., 2001; Vignal and Mathevon, 2011).

107 In the past, zebra finch distance calls have been investigated in a number of different
108 manners using a variety of bioacoustically-based classification approaches. A previous study,
109 where the primary aim was to classify vocalizations into types (e.g., song, distance call etc.)

110 based on sexually-dimorphic acoustic features, quantified the potential acoustic features in the
111 distance calls in comparison to other vocalization classes (Elie and Theunissen, 2016). This
112 analysis showed that females produced longer, and lower pitched distance calls compared to
113 male distance calls (Elie and Theunissen, 2016). A subsequent study (Mouterde et al., 2014) used
114 DFA with spectral envelopes, temporal envelopes, and spectrogram features of distance calls, to
115 classify distance calls based on the distance of the emitter of the calls (i.e., from 2m, 16m, 64m,
116 128m, and 256m) from the microphone. In the Mouterde et al. (2014) study, several density
117 functions (mean, standard deviation, skewness, kurtosis, and entropy) of spectral and temporal
118 envelopes and spectrogram principal component parameters were used successfully to classify
119 distance calls at various propagation distances via the individual acoustic signature of the birds.
120 However, the complex acoustic features of distance calls used in the bioacoustic analyses of the
121 two studies just discussed are not straightforward to either measure or manipulate in an
122 experimental context. The acoustic features described in these two studies are problematic for
123 use in an experimental context as they are complex to either measure or manipulate by an
124 experimenter.

125 In this study, we used three statistical methods (DFA, SVM and, ANN) to classify Zebra
126 finch distance calls by sex of the emitter based on bioacoustic features, some of which are known
127 to differ between sexes. We used 10 acoustic measurements in our analyses including both
128 temporal and spectral measures. We predict similar classification performance in all three
129 classification methods given the past successes using these methods for similar tasks (Mouterde
130 et al., 2014, Elie and Theunissen, 2016). Furthermore, we predict that total call duration and
131 frequency modulation will be the predominant features used to classify the calls, as these
132 features are visually distinct in the spectrograms of these calls and previous studies (Elie and

133 Theunissen, 2016; Vignal and Mathevon, 2011) suggest that these are the key features
134 facilitating sex-based call discrimination.

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136 **II. METHODS AND RESULTS**

137 **A. Recordings**

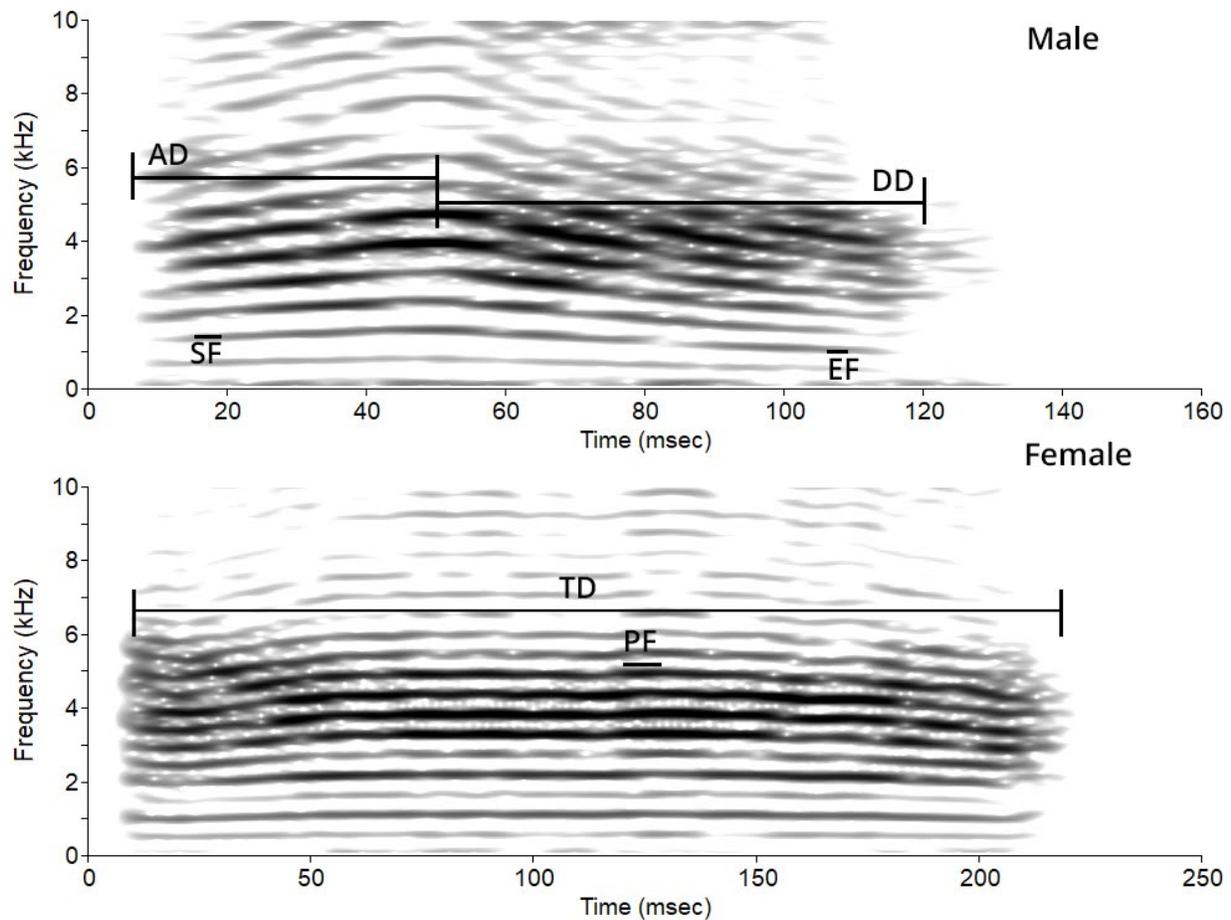
138 In total, 83 zebra finch distance calls were obtained from the data sets of D'Amelio et al.
139 (2017), Elie and Theunissen (2016), and from adult zebra finches recorded by members of the
140 Phillmore lab at Dalhousie University, Halifax, NS Canada. The set consisted of 38 female and
141 45 male distance calls produced by 21 females and 26 males, with 1-2 vocalizations per
142 individual. There were 12 male calls and 12 female calls obtained from 12 male individuals and
143 12 female individuals respectively from D'Amelio et al. (2017), 18 male calls and 20 female
144 calls from 9 male individuals and 10 female individuals respectively from Elie and Theunissen
145 (2016), and 15 male calls and 6 female calls from 8 male individuals and 4 female individuals
146 respectively from the Phillmore lab. All the recorded calls were recorded in the laboratory with
147 digital recorders and microphones having frequency response ranges from 200 Hz to 10,000 Hz.
148 The calls from D'Amelio et al. (2017) and Elie and Theunissen (2016) were recorded at a
149 sampling rate of 44,100 Hz. The calls obtained from Phillmore lab were recorded at a sampling
150 rate of 48,000 Hz. For all sources, calls were recorded at a distance between 0 and 100 cm from
151 the birds. Thus, these recordings provided us with a diverse dataset of high-quality recordings of
152 distance calls. Because calls were recorded with different sampling rates, all distance calls were
153 resampled using SIGNAL software version 5.16.11 (Beeman, 2017) at 44,100 Hz before further
154 analyses.

155

156 **B. Acoustic measurements**

157 The following acoustic analyses and measurements were conducted in SIGNAL and
158 performed by the first author (PS). For each sound file, 5 ms of silence was added to the
159 beginning and end of the vocalization and tapered to remove transients; peak RMS amplitude
160 was equalized to 1. Spectrograms were created with a Hanning window and 256 pts transform,
161 frequency resolution of 172.3 Hz and 5.8 ms time resolution. Power spectra were produced with
162 an FFT window of 16,384 points and 88 Hz smoothing for amplitude measurement. The
163 following acoustic features were measured manually from the spectrograms of individual calls:
164 (a) Total duration (TD), measured from the start to the end of the highest amplitude harmonic
165 band, (b) Start frequency (SF), measured at the start of the first clearly visible and continuous
166 harmonic band, in this case, the second frequency band in the spectrogram, (c) End frequency
167 (EF), measured at the end of the first clearly visible and continuous harmonic band, in this case,
168 the second frequency band in the spectrogram (d) Peak frequency (PF), measured at the highest
169 frequency observed of the highest amplitude harmonic band, (e) Ascending duration (AD),
170 measured from the start to the peak frequency of the highest amplitude harmonic band, (f)
171 Descending duration (DD), measured from the peak to the end of the highest amplitude harmonic
172 band, (g) Frequency modulation of ascending frequency (F_{masc}; Peak frequency-Start
173 frequency/Ascending duration), (h) Frequency modulation of descending frequency (F_{mdsc}; End
174 frequency-Peak frequency/Descending duration), (i) Frequency at highest amplitude (F_{max}),
175 measured at the peak frequency of the highest amplitude harmonic band from the power spectra
176 and, (j) Fundamental frequency (F₀) (Campbell et al., 2016; Nowicki and Nelson, 1990). The
177 fundamental frequency was measured in Praat 6.1.38 (Boersma and van Heuven, 2001;

178 Goldstein, 2021) Figure 1 shows the measured acoustic features from the spectrograms of male
179 and female zebra finch distance calls.



180 **Figure 1.** Measured acoustic features from the spectrogram showing Total duration (TD), Start
181 frequency (SF), End frequency (EF), Peak frequency (PF), Ascending duration (AD),
182 Descending duration (DD) with male distance call at top and female distance call at bottom. F0
183 was measured in Praat (not pictured here).

184

185 C. Statistical analyses

186 All analyses were conducted in R 3.6.2 (R Core Team, 2019). The linear discriminant
187 analysis (LDA) and discriminant function analysis (DFA) were conducted using the *MASS*

188 (Venables and Ripley, 2002) and *klaR* (Weihs et al., 2005) packages, the SVM was conducted
189 using the *e1071* package (Meyer et al., 2019), and the ANN was conducted using the *neuralnet*
190 package (Günther and Fritsch, 2010). Mathews correlation coefficient (MCC) was calculated
191 using *mltools* (Gorman, 2018). For LDA, standardized coefficients were obtained using
192 canonical discriminant analysis from the *candisc* package (Friendly and Fox, 2021). The relative
193 importance of variables or weights for SVM were calculated using the weight vectors (Meyer et
194 al., 2019). The relative importance of input variables for ANN were calculated using the *olden*
195 function of *NeuralNetTools* (Beck, 2018).

196 All measured acoustic features were scaled by z-standardization, using the *scale* function
197 in R to account for and standardize across multiple units of measurement. This allowed us to
198 compare between measures, even when those measures differed in units. The z-standardization
199 of an individual acoustic feature involves subtracting the mean of the specific acoustic feature
200 from the individual measurement and dividing by its standard deviation. We conducted
201 correlation analyses to identify and omit redundant and highly correlated acoustic features. The
202 Ascending duration (AD) and Descending duration (DD) were highly correlated with each other
203 (*Pearson's r* = 0.75, $p < 0.001$) and with Frequency modulation of ascending frequency, *Fmasc*
204 (AD and *Fmasc*: *Pearson's r* = -0.85, $p < 0.001$) and Frequency modulation of descending
205 frequency, *Fmdsc* (DD and *Fmdsc*: *Pearson's r* = - 0.83, $p < 0.001$). Thus, AD and DD were not
206 included in further analyses. TABLE 1 shows correlation across the measured acoustic features.
207 Below, we review how each technique operates, what kinds of results they return, what results
208 we obtained using each technique and finally provide a comparison among the techniques.

209

210 TABLE I. Table showing Pearson's correlation coefficients across acoustic features. * represents
 211 significant correlation

	TD	SF	EF	PF	AD	DD	Fmasc	Fmdsc	Fmax	Fo
TD										
SF	-0.06									
EF	0.26*	0.48*								
PF	-0.01	-0.16	-0.22*							
AD	0.56*	0.04	0.43*	-0.26*						
DD	0.61*	0.03	0.30*	-0.1	0.75*					
Fmasc	-0.39	-0.19	-0.46*	0.52*	-0.85*	-0.54				
Fmdsc	0.43*	0.15	0.49*	-0.42*	0.72*	0.83*	-0.71*			
Fmax	0.05	-0.14	-0.08	0.51*	-0.03	0.02	0.27*	-0.2		
Fo	0.22	0.03	0.14	-0.17	0.42*	0.23*	-0.50*	0.31*	-0.16	

212

213

214 ***1. DFA, pDFA, and LDA.***

215 Discriminant function analysis (DFA) is used for classification of exemplars into
 216 groups based on a linear combination of features which separate the groups. In bioacoustics
 217 analyses, DFA can be used to classify vocalizations into types (Jaiswara et al., 2013) or across
 218 individuals (Chen and Goldberg, 2020; Mundry and Sommer, 2007). For example, DFA has
 219 been used to classify vocalizations of mountain chickadees (*Poecile gambeli*) based on elevation
 220 gradient (Branch and Pravosudov, 2015, 2019). DFA has also been used to classify black-capped
 221 chickadee (*Poecile atricapillus*) vocalizations based on geography (British Columbia and
 222 Ontario; Hahn et al., 2013a) and sex (Campbell et al., 2016).

223 We used a stepwise DFA with the leave-one-out method of cross-validation for
 224 classifying distance calls based on sex. In this process, a single vocalization is withheld while the

225 rest of the vocalizations are used to obtain the discriminant functions. The accuracy of the
226 discrimination functions can then be obtained by comparing the predicted group, male or female,
227 of the withheld vocalization to the original class of that vocalization (i.e., was the function able
228 to classify a male call as male, and a female call as female.). This method was repeated until all
229 the vocalizations were classified, thus giving us overall percent correct classification (Betz,
230 1987; Mundry and Sommer, 2007).

231 When multiple vocalizations from the same individuals are used for DFA, there is the
232 possibility of pseudoreplication. Pseudoreplication occurs when non-independent data points
233 from the same subject (e.g., multiple vocalizations from one individual) are analyzed as
234 independent replicates (Mundry and Sommer, 2007). A permuted DFA (pDFA) can be used to
235 account for pseudoreplication (Mundry and Sommer, 2007). With pDFA, we compared the
236 percent correct classifications by DFA from the original distance call distribution to percent
237 correct classifications obtained from null distributions. The null distributions of distance calls are
238 constructed by randomly assigning individual calls as male or female. One thousand such null
239 distributions were constructed, and percent correct classifications were obtained by leave-one out
240 method of DFA as mentioned above. The proportion of times percent correct classification by
241 pDFA were equal to or greater than correct classification by original DFA was obtained and was
242 noted as p-value as described by (Mundry and Sommer, 2007).

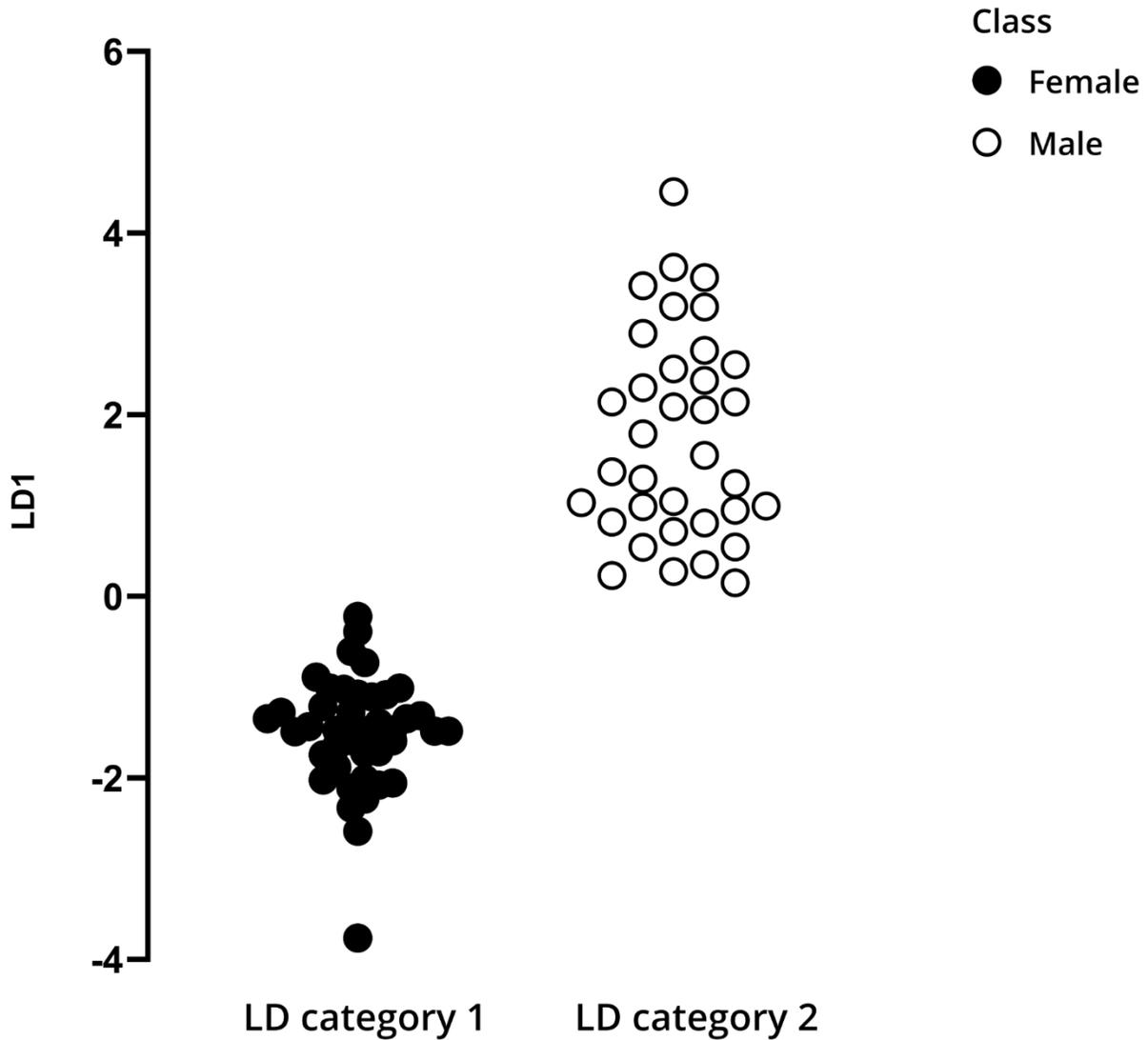
243 The stepwise DFA accurately classified distance calls based on sex using all eight of the
244 remaining measured acoustic features. In the forward stepwise DFA method for variable
245 selection for classification, where each feature is entered individually, one by one (rather than all
246 features entered all at once) with total duration (TD) as starting variable, Total duration (TD),
247 End frequency (EF), Frequency modulation of ascending frequency (Fmasc), Frequency

248 modulation of descending frequency (Fmdsc), and Frequency at highest amplitude (Fmax), were
249 all used together for the sorting of distance calls into the respective sex category that produced
250 the calls. The forward stepwise DFA classified the distance calls into the correct category with
251 96.3% accuracy. We then used a pDFA to test the validity of the stepwise DFA; conducting the
252 pDFA involved constructing null distributions of distance calls, where sex identity of each call
253 was randomized. The mean correct classification for all 1,000 null distributions was $50.2\% \pm 6.6$
254 (mean \pm sd), meaning pDFA could only classify the null distributions with ~50% accuracy. None
255 of the pDFAs produced correct percent classification greater than the stepwise DFA
256 classification percentage, thus giving a p-value of 0 for the pDFA null model, indicating that the
257 stepwise DFA accurately classified calls by sex of producer.

258 We also used a supervised linear discriminant analysis (LDA) with the hold out method
259 of cross-validation to classify distance calls based on sex for a direct comparison with support
260 vector machine (SVM) and artificial neural network analysis (ANN). In the hold out method of
261 cross-validation, the data set is separated into two sets: training and testing. The function uses the
262 training set to build a model to predict the output of the testing set. In supervised LDA, 75% of
263 the vocalizations were chosen randomly for training and then the remaining 25% are used in a
264 test to validate the accuracy of the same testing dataset. This procedure was repeated 1,000 times
265 and mean percent accuracy was calculated (Engler et al., 2014; Ligout et al., 2016).

266 In the supervised LDA, all the eight acoustic features were used to calculate the
267 discriminant functions and predict classification for testing datasets. This process was repeated
268 and cross-validated 1,000 times to obtain the mean correct percent classification. Using all the
269 eight features, the LDA classified distance calls with $96.3\% \pm 3.4$ accuracy. The mean MCC for
270 the LDA was 0.96 (range: 0.63-1.00) from 1,000 testing datasets which indicates high

271 classification performance, meaning there was no significant effect of unbalanced datasets with
272 unequal numbers of samples in the two groups. This can potentially pose a problem resulting in a
273 larger dataset overestimating the classifier. Figure 2 shows the distribution of the individual
274 distance calls according to the first discriminant function, LD1, male and female calls are well
275 separated.



276
277 **Figure 2.** Distribution of the first discriminant function (LD1) for all male and female distance
278 calls.

279

280 DFA is a robust and stable classification technique when the classes are well separated,
281 thus giving accurate parameter estimates that separate the classes. However, when the dataset is
282 limited with high dimensionality (has more features than samples), there is risk of over-fitting.
283 This over-fitting might reduce the cross-validation performance of classifiers (James et al., 2013;
284 Tachibana et al., 2014). Our overall dataset is not high dimensional (i.e., we have more samples,
285 $n=83$ than features, $n=8$), but data are from various sources produced from different individuals.
286 We used support vector machine (SVM) algorithms, as SVM avoids the problem of overfitting, a
287 potential issue with DFA. SVM also helps reduce human effort involved in other classification
288 methods, as SVM works with a relatively small instruction (training) dataset in comparison to
289 other methods.

290 **2. SVM**

291 Support vector machines (SVMs) are supervised learning algorithms used for mainly
292 two-group classification problems (Cortes and Vapnik, 1995). SVMs use all the eight measured
293 acoustic features as input variables, similar to a DFA, and then build a prediction model.
294 However, when data are not linear, there is a possibility for interaction among variables, which
295 can happen when classifying using a DFA. SVMs solve this problem by using a kernel approach.
296 Kernels are various functions (e.g., linear, polynomial, radial, and sigmoid) that can be applied to
297 input data so that data are separated linearly in the feature space. Here, we used a linear kernel
298 for both training and prediction. SVMs have been widely used for classification of songbirds to
299 their species by their songs, for example, using song syllables of 7 bird species (Dufour et al.,
300 2014) or using flight calls of 11 species of birds (Tung et al., 2003), and more recently, using
301 additional automated methods such as hybrid model of deep convolutional neural networks and
302 hidden Markov models for classification of birdsong using song notes and syllable elements

303 (Koumura and Okanoya, 2016; Tachibana et al., 2014). In this study, we used a linear SVM
304 where classification boundaries are determined by maximizing margins between the nearest
305 samples and boundary hyperplane for distance call classification. In this supervised semi-
306 automated method, we randomly divided all 83 vocalizations using a 3:1 ratio to serve as training
307 and testing datasets, respectively. The validity of the model based on the training dataset was
308 measured against a testing dataset.

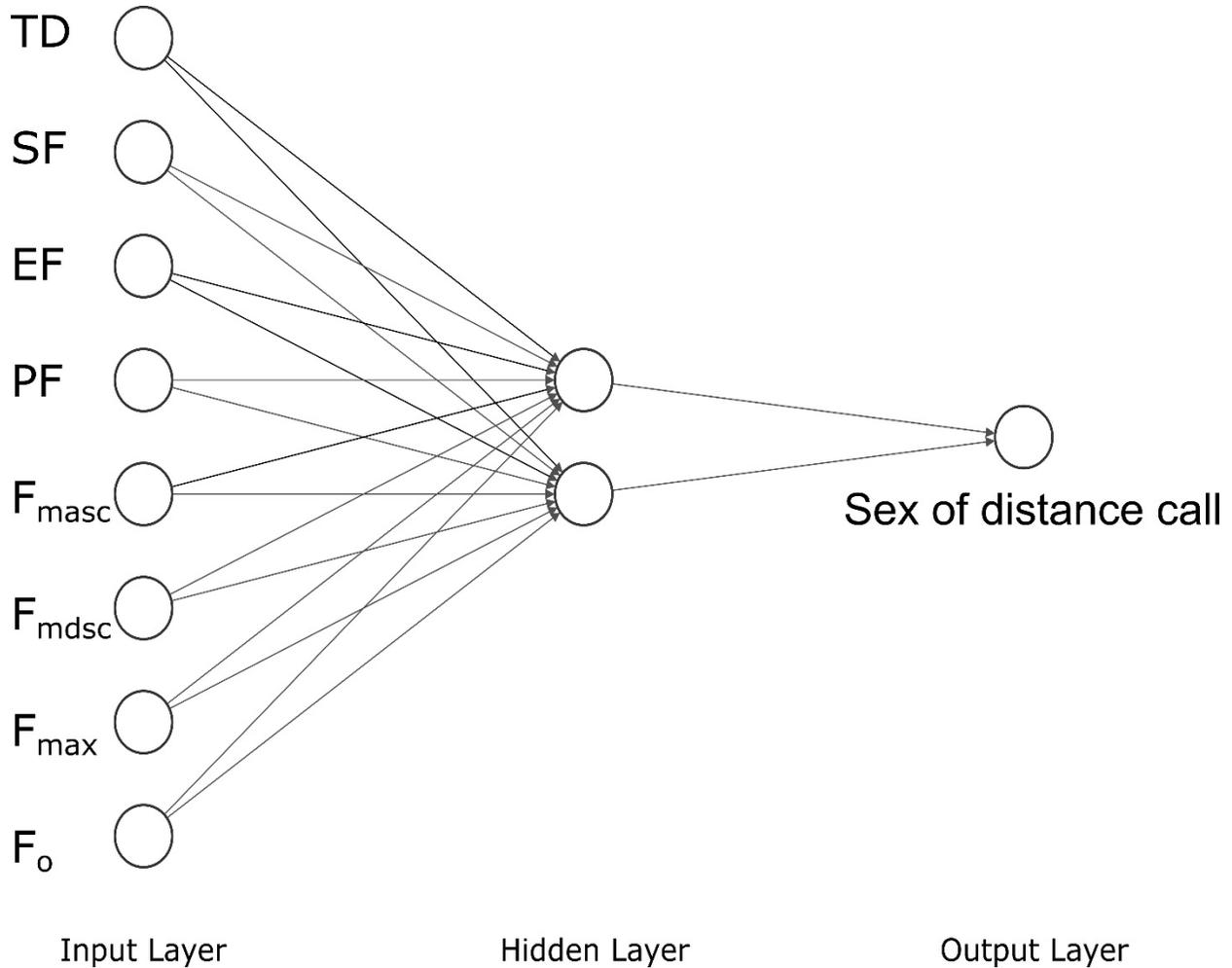
309 We cross validated performance SVM with the testing datasets. This process of cross
310 validation was repeated 1,000 times with randomly chosen testing dataset for mean correct
311 classification percentage. SVM classified distance calls with a mean of 94% \pm 5.1 correct. The
312 mean MCC for SVM was 0.88 (range: 0.42-1.00). Next, we built ANNs to compare ANN
313 classification accuracy with the accuracy obtained from discriminant analyses and SVM.

314 **3. ANN**

315 Artificial neural networks (ANN) consist of connected input nodes and edges in multiple
316 layers; acoustic features can be used as input to produce a predicted category as target output
317 (Izenman, 2008). In bioacoustic analyses, neural networks have been used in the context of
318 species classification using acoustic features, ranging from whole vocalizations to individual
319 song and call notes, to sort vocalizations by species (Chou and Liu, 2009, 2009; Piczak, 2016) or
320 to sort notes into note types (Dawson et al., 2006). ANNs for binary classification are very
321 similar to SVM, apart from the training algorithms that are used for calculation of classification
322 functions: ANNs use backpropagation whereas SVM uses hyperplane to make predictions. In
323 backpropagation, the weights of a neural net are fine-tuned according to error rate or loss
324 function of previous epochs or iterations in training while in hyperplane, observations are
325 separated into two classes by a threshold hyperplane, calculated from linear combination of the

326 dependent variables (Izenman, 2008; James et al., 2013). ANNs can account for potentially
327 complex relationships among input features without compromising classification performance
328 (Collobert and Bengio, 2004; Jakkula, 2011).

329 We used a supervised ANN, which used the eight measured acoustic features from
330 distance calls as input, to classify the calls. We built an artificial neural network using the
331 *neuralnet* package in R with the default logistic activation function (Günther and Fritsch, 2010).
332 The neural network consisted of eight acoustic input features with a single hidden layer
333 consisting of two neurons and one output unit to predict sex of the producer of the distance call.
334 The input features were multiplied by a random set of weights prior to the training. The logistic
335 activation function applied to the multiplied numbers and output as neurons in the hidden layer.
336 The neurons in the hidden layer were again multiplied by a random set of weights, and the
337 activation function was applied to these numbers to produce a single output. The prediction
338 output (lies between 0 and 1) was compared with the true output. The loss or error was then
339 calculated with a cross-entropy function to know how far off our prediction from true output
340 (Izenman, 2008). We used resilient backpropagation algorithms to get the gradients for each
341 weight from the initial random weights. During epochs of training, the error got smaller, and
342 weights got optimized for best prediction of output (Günther and Fritsch, 2010). A schematic of
343 the neural network is shown in Figure 3.



344

345 **Figure 3.** Schematics of the neural network showing acoustic features as input, two neurons in
 346 hidden layer and output layer where TD: Total duration, SF: Start frequency, EF: End frequency,
 347 PF: Peak frequency, F_{masc}: Frequency modulation of ascending frequency, F_{mdsc}: Frequency
 348 modulation of descending frequency, F_{max}: Frequency at highest amplitude, and F_o:
 349 Fundamental frequency.

350

351 Seventy-five percent of the total pool of vocalizations were chosen randomly to be used
 352 as a training set for supervised learning, while the remaining 25% of the vocalizations were
 353 withheld and used to validate the accuracy of the training model. This training and validation

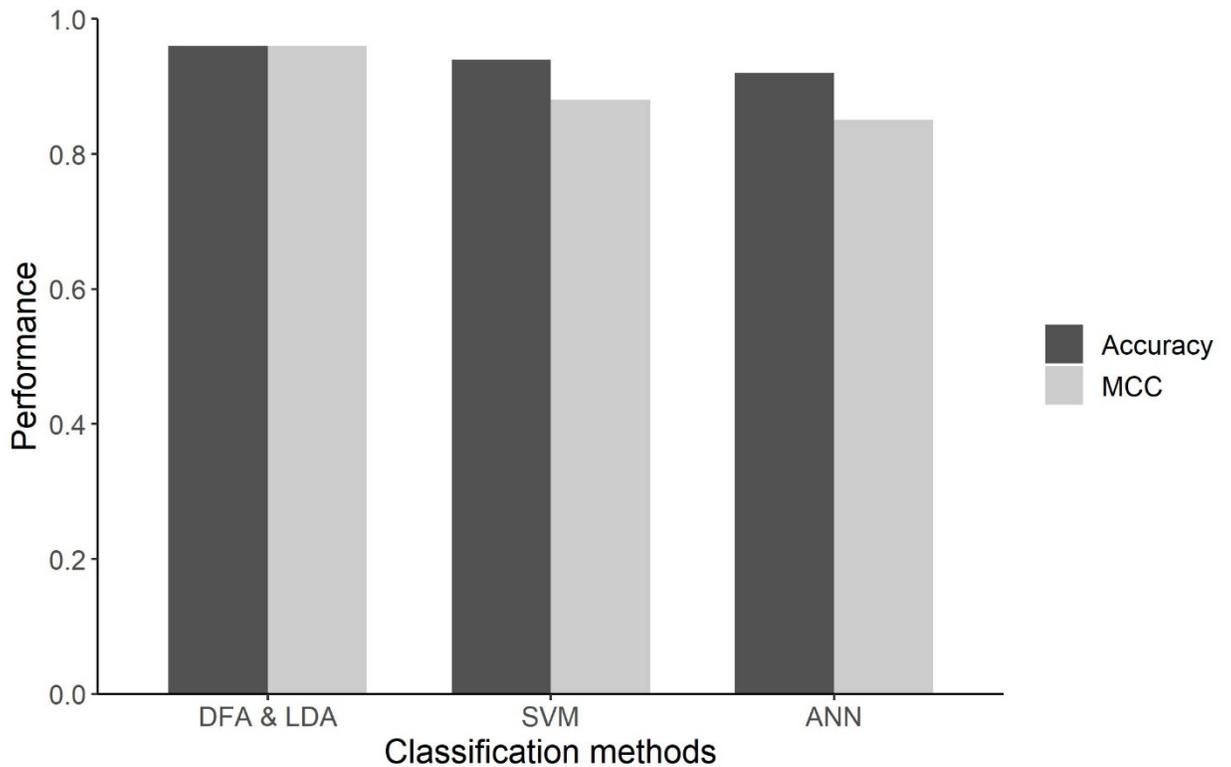
354 method was repeated 1,000 times. We trained the ANN until all absolute partial derivatives of
355 the error function were smaller than 0.01 meaning we achieved asymptotic performance, a
356 standard stopping point for confirming validity of ANNs (Günther and Fritsch, 2010).

357 The neural network classified the distance calls to the respective sex of producer with a
358 mean accuracy of $92.5\% \pm 5.4$ correct. The mean MCC for neural networks was 0.85 (range: 0.46-
359 1.00), consistent with the MCC for both LDA and SVM.

360 ***4. Model comparison***

361 The use of multiple methods of classification of distance calls will give us an overview of
362 classification using a variety of methods while constructing a base for future classifications of
363 similar problems. All methods used (DFA, LDA, SVM and ANN) classified calls into the correct
364 sex of the produce with high accuracy (DFA and LDA: 96 %, SVM: 94 %, ANN: 92 %). For the
365 stepwise DFA, pDFA validated the classification. To evaluate the relative classification
366 performance for the rest of the methods (LDA, SVM and ANN), we calculated and compared
367 MCC and the classification accuracy of each. MCC is a measure of quality of two-class
368 classification used in various fields of research including songbird vocalization classification
369 (Chicco and Jurman, 2020; Matthews, 1975; Wellock and Reeke, 2012). The MCC for all the
370 methods (LDA: 0.96, SVM: 0.88, ANN: 0.85) were high and consistent with each other. Figure 4
371 shows a comparison of classification performance with accuracy and MCC. Further, we assessed
372 the relative importance of specific acoustic features in classification across stepwise DFA, LDA,
373 SVM and ANN methods. Comparing the relative importance across various methods will inform
374 us as to whether the same acoustic features were used preferentially for each method for
375 classification. Such methodological comparisons will further allow researchers to make informed

376 decisions when selecting which methodological tools they will employ for their particular set of
377 circumstances.



378
379 **Figure 4.** A bar graph showing classification accuracy (scaled to 1) and MCC (Matthews
380 correlation coefficient) values for all classification methods; pDFA & LDA, SVM, and ANN.
381 Higher value is better.

382
383 In all four methods (stepwise DFA, LDA, SVM, and ANN), Frequency modulation of
384 ascending frequency (F_{masc}), Total duration (TD), and End frequency (EF) were three top
385 features used for classifying distance calls according to the sex of the producer (see TABLE II
386 for full list). Although the methods used apply different algorithms for classification, they
387 produced similar classification results with a similar relative importance for the input features. In
388 sum, all methods tested successfully classified zebra finch calls by sex of the producer.

389 TABLE II. A table showing acoustic features used and their relative importance^a (in descending
 390 order with relative proportion in the brackets) used for classification by Linear discriminant
 391 analysis (LDA), Support vector machines (SVM), and Artificial neural networks (ANN). For
 392 stepwise DFA, the DFA column shows features used for obtaining a classification accuracy of
 393 96%.
 394

Order of Importance	DFA	LDA	SVM	ANN
1	TD	Fmasc (0.43)	Fmasc (0.41)	Fmasc (0.42)
2	EF	TD (0.15)	EF (0.2)	EF (0.25)
3	Fmasc	EF (0.11)	TD (0.12)	TD (0.12)
4	Fmdsc	PF (0.1)	Fmdsc (0.08)	Fmdsc (0.07)
5	Fmax	Fo (0.08)	PF (0.07)	Fo (0.04)
6		Fmdsc (0.01)	SF (0.06)	PF (0.04)
7		SF (0.01)	Fo (0.04)	SF (0.04)
8		Fmax (0.004)	Fmax (0.0001)	Fmax (0.01)

395
 396 ^a Refer to Statistical analysis section for calculation of relative importance of variables
 397 for each method.

398
 399

400 **III. DISCUSSION**

401 All the methods (DFA, LDA, SVM, and ANN) were highly accurate at classifying
402 distance calls into male and female; each had a classification accuracy greater than 92%. Three
403 methods (LDA, SVM and ANN) had MCC values greater than 0.85, indicating highly correct
404 predictions for both male and female calls independent of their potentially problematic unequal
405 sample size in the dataset. The results from DFA, LDA, SVM, and ANN consistently and
406 accurately classified male and female distance calls. Both the leave-one-out method and holdout
407 method of cross-validation produced similarly excellent classification performance. This
408 suggests that there are acoustic features that differ between male and female distance calls such
409 that they can be used to effectively classify them with all four of these methods. Frequency
410 modulation of ascending frequency (F_{masc}), end frequency (EF), and total call duration (TD) of
411 the distance call were the top ranked acoustic features used by stepwise DFA, LDA, SVM, and
412 ANN. The LDA, SVM, and ANN all ranked F_{masc} as the most important acoustic feature. SVM
413 and ANN ranked EF and TD as the second and third most important features whereas LDA
414 ranked TD and EF as second and third most important features. The order change may be due to
415 different algorithms used for classification and for relative importance nevertheless frequency
416 modulation was the most crucial feature used for classification. The stepwise DFA approach is
417 useful and efficient for investigating and pruning variables when there are a large number of
418 input variables involved; all variables can be entered in one step and the DFA outputs the
419 variables used in the classification. SVM, on the other hand, works best for binary classifications
420 with the use of maximum margin linear classifiers and for high dimension data, relatively large
421 datasets, with the help of various available kernel functions. ANN is useful with multi-class
422 classification with large datasets. Ideally, we recommend the use of a combination of these

423 methods to account for stochasticity of real-world data. Pragmatically, if we were to choose one
424 method, SVM would be our recommendation for the current question of sex-based call
425 classification, due to its simplicity and ease of use for binary classification problems. Our study
426 adds to the literature of methodological comparisons of vocalization classification (Bat
427 echolocation: Armitage and Ober, 2010; Mouse ultrasonic: Ivanenko et al., 2020).

428 The distance calls used here were from several sources: birds were from the colonies in
429 the USA, Germany, and Canada (D'Amelio et al., 2017; Elie and Theunissen, 2016). Thus, the
430 study involved calls from a diverse sampling space which extends the external validity of the
431 study. The acoustic features we measured and entered into the algorithms resulted in successful
432 classification by DFA, SVM, and ANNs; all approaches were able to classify the distance calls
433 with high accuracy of over 92%. It would be ideal to test vocalizations from other captive
434 colonies and to wild birds to determine whether accuracy remains high with vocalizations from
435 other groups of finches, including non-domesticated birds. Distance calls are sexually dimorphic,
436 making the classification task relatively easy. It would be interesting to expand this study to test
437 the performance of the classification methods with other zebra finch calls such as stacks and tets
438 which contain individuals' sex identity.

439 Because all measurements for acoustic features were collected manually, there is a degree
440 of subjectivity in the data that could have resulted in some potential for increased variability in
441 the measurements collected. In the future, one refinement might be using an automated process
442 to measure acoustic features with more consistency and less chance of bias. However, even
443 automated or semi-automated measurement techniques require some level of human
444 involvement, either for establishing the method or verifying the accuracy of the chosen method
445 (Priyadarshani et al., 2018). We did not use additional acoustic measures that were difficult to

446 obtain (e.g., Mel-Frequency Cepstral Coefficient or moments of spectral density functions like
447 skewness, kurtosis, entropy etc.) for classification. Thus, variables used for the classification
448 may have been oversimplified and as a result some important acoustic features potentially used
449 by zebra finches for discrimination may not have been detected. It would be interesting to
450 compare classification performance with the methods discussed here when using predefined
451 acoustic features (e.g., intensity measures, pitch, frequency measures) vs a complete
452 representation (e.g., Modulation power spectrum, full spectrogram, Mel frequency cepstral
453 coefficients) of the acoustic stimuli (Elie and Theunissen, 2016). In future, larger samples from
454 many individuals may be helpful to alleviate this issue by being able to assess interrater
455 reliability of acoustic measurements in cases where more than one individual measured calls.

456 Distance calls contain information about individual identity of the caller. Studies could
457 compare the classification performance of these methods for the classification of distance call
458 based on individual identity which would require large number of calls from each individual.
459 The features used here for distance call classification based on sex of caller can be used as a
460 starting point to design future experiments to validate the acoustic measures used in the present
461 study, such as an operant conditioning study to directly test the birds' ability to discriminate the
462 manipulated on those features measured here. Such an operant study would add to the literature
463 combining detailed bioacoustics analysis with perceptual studies by assisting in identifying and
464 then manipulating simple spectrogram features to create experimental stimuli. That is to say;
465 studies could test if only duration or frequency cues from the calls are discriminable. We would
466 expect the acoustic features identified here would be relatively easily discriminable based on
467 previous research works (Lohr et al., 2006, Lohr et al., 2003, Prior et al., 2018). Apart from
468 distance calls, other zebra finch calls like stacks and tets also contain sex identity of the caller.

469 We predict that a single acoustic feature from distance call will not be enough to convey
470 information about sex. Our study adds further evidence about the importance of these acoustic
471 features and methodologies for classification and these methodologies can be used for
472 classification of other call types.

473 The relative importance of variables in classification models provide information about
474 what acoustic features animals may attend to preferentially when listening to and making
475 decisions about responding to conspecific vocalizations. Previous studies focusing classification
476 of vocalizations have primarily used Canonical loadings from DFA (Khan and Qureshi, 2017;
477 Tooze et al., 1990), Gini index, or mean decrease accuracy for Random Forest algorithm
478 (Armitage and Ober, 2010; Elie and Theunissen, 2016; Henderson et al., 2011; Robakis et al.,
479 2018; Valletta et al., 2017) to determine relative importance of input variables due to their
480 successful use in various contexts and ease of implementation in statistical software. We used
481 similar measures for variable importance and expanded with the connection weight algorithm
482 (Olden and Jackson, 2002) for variable importance in ANN. Future studies could use the above
483 variables of importance and possibly improve with other methods for assessing the relative
484 importance of input variables for ANN (Ibrahim, 2013).

485 In conclusion, we show that discriminant functions, support vectors, and neural networks
486 were consistent with each other in accurately classifying zebra finch distance calls by sex of
487 caller. Zebra finch distance calls can be accurately classified by sex using primarily three
488 acoustic features: total duration, end frequency and frequency modulation ascending frequency.
489 Highly similar patterns of acoustic feature rankings were observed for classification for all the
490 methods. We believe our framework used in this bioacoustic analysis and subsequent

491 classification of distance calls can be used as a starting point for researchers wanting to conduct
492 similar bioacoustics studies in the future.

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